BRILL
WAGENINGEN
ACADEMIC

JOURNAL OF INSECTS AS FOOD AND FEED (2024) DOI:10.1163/23524588-00001051

Journal of Insects as Food and Feed
brill.com/jiff

RESEARCH ARTICLE

Volatile compounds and quality changes of lesser mealworm larvae: effect of blanching, storage temperature and time

S. Yener[1,2,#], *M. Mishyna*[1,#,*], *L. Zhao*[1], *O.N. Velazco*[1,3], *L. Cadesky*[4,5], *C. Lakemond*[1] and *V. Fogliano*[1]

[1]Food Quality & Design Group, Wageningen University & Research, Bornse Weilanden 9, 6708 WG Wageningen, the Netherlands; [2]Current address: Meatable BV, Alexander Fleminglaan 1, 2613 AX Delft, the Netherlands; [3]Current address: Danone Nutricia Research, Uppsalalaan 12, 3584 CT Utrecht, the Netherlands; [4]Protifarm, Harderwijkerweg 141B, 3852 AB Ermelo, the Netherlands; [5]Current address: Maple Leaf Foods, 6897 Financial Drive, Mississauga, ON L5N 0A8, Canada; *maryia.mishyna@wur.nl

[#]These authors contributed equally to this work.

Received 18 October 2023 | Accepted 12 August 2024 | Published online 28 August 2024

Abstract

The impact of blanching and storage temperature (–20 °C and –80 °C) on the formation of volatile organic compounds and changes in quality characteristics of lesser mealworm (*Alphitobius diaperinus*) larvae was examined over seven weeks. Acid and peroxide values, pH, and colour of lesser mealworm larvae were significantly affected by the studied variables. The changes in these characteristics as well as in the volatile profile indicated occurring lipid oxidation and lipid hydrolysis processes during the storage. The intensity of these reactions was mostly influenced by blanching treatment and storage time, and a lesser extent by storage temperature. It was proposed that changes in composition and intensity of volatiles can be used for rapid quality assessment of lesser mealworm larvae during frozen storage. Thus, the selection of optimum storage conditions for lesser mealworm larvae is important for the prevention of undesirable chemical reactions and preservation of the quality of insect biomass.

Keywords

edible insects – food quality – lesser mealworm – lipid oxidation – volatile compounds

1 Introduction

Interest in edible insects as a sustainable food alternative has emerged in recent years. One of the reasons is that insect production has a significantly lower environmental footprint with less land and water use and lower greenhouse gases emission in comparison to traditional livestock (van Huis and Oonincx, 2017). From a nutritional point of view, insects are a good source of protein, essential amino acids and unsaturated fatty acids. Additionally, insects contain considerable amounts of vitamins such as vitamin B_{12} and minerals such as iron (Rumpold and Schlüter, 2013).

Production of insects for human consumption is linked to their processing (i.e. blanching, grinding) and storage. It was shown that the storage behaviour of whole unprocessed insects by freezing is affected by the presence of antifreeze proteins, which can inhibit

Published with license by Koninklijke Brill BV | DOI: 10.1163/23524588-00001051
 | ISSN: 2352-4588 (online)

ice crystals growth and thus maintain water unfrozen (Graham *et al.*, 1997). Unfrozen water could favour biochemical reactions such as lipid oxidation and lipid hydrolysis resulting in undesirable colour and flavour formation (Leygonie *et al.*, 2012). In particular, lipid oxidation products can react with amino acids producing Strecker aldehydes and ketoacids (Hidalgo and Zamora, 2016), which are important for flavour of foods. Dynamic of lipid oxidation can be assessed by analysis of volatile compounds and detection markers of lipid oxidation such as hexanal and propanal (Grebenteuch *et al.*, 2021). Moreover, unfrozen water in insects at –20 °C favours protein hydrolysis resulting in changes in chemical properties and protein functionalities already during the first week of storage (Wessels *et al.*, 2020). It indicates that storage temperature and time can be important factors for the preservation of insect quality during storage.

The microbial activity of edible insects, largely due to insect gut microbiota, should be also taken into consideration to ensure the safety of insects for human consumption. Moreover, microbial activity could have an adverse effect on the quality profile of stored insects (Klunder *et al.*, 2012; Wynants *et al.*, 2018). In this regard, blanching is widely used as a microbial decontamination technique to reduce microbial loads and prevent browning of edible insects (Mancini *et al.*, 2019; Wynants *et al.*, 2018). It was shown that blanching of yellow mealworm larvae in boiling water for 10-40 s leads to considerable log reductions of total viable count, Enterobacteriaceae, lactic acid bacteria, yeasts and molds, and can be used as a pretreatment for refrigerated storage (Vandeweyer *et al.*, 2017). Additionally, blanching before storage can inactivate the enzymes that cause lipid oxidation and browning (Cacchiarelli *et al.*, 2022; Larouche *et al.*, 2019; Reyes De Corcuera *et al.*, 2004). Another study demonstrated that beside assuring microbial safety, blanching of yellow mealworm larvae can also improve colour characteristics and the composition of macro-nutrients (Ribeiro *et al.*, 2024). While heat treatment is an important step from a safety point of view, it leads to the denaturation of insect proteins resulting in the alteration of techno-functional properties of proteins and their potential application as food ingredients (Mishyna, Keppler and Chen, 2021).

Previous studies demonstrated that both processing and storage conditions affect quality of insect oils on an example of *Rhynchophorus phoenicis*. In particular, prolonged freezing and electrical drying at 50 °C affects oil stability, while refrigeration for less than 3 days or freezing for less than a month could preserve lipid quality (Tiencheu *et al.*, 2013). Therefore, the selection of optimum storage conditions is important for preservation of nutritional, functional, and sensory characteristics of edible insects which can be affected by numerous undesirable reactions during storage. The quality of insect biomass also affects the quality of further produced insect-derived food ingredients and foods with edible insects. So far, to the best of our knowledge, the influence of low-temperature storage on volatile profiles and quality-associated indicators of edible insects was not previously studied. Therefore, this study aimed to estimate the effect of blanching, storage temperature (–20 °C and –80 °C), and storage time (7 weeks) on the volatile profiles and quality indicators of lesser mealworm larvae.

2 Materials and methods

Materials

Larvae of *Alphitobius diaperinus* (lesser mealworm) at 28 days old were provided by Protifarm Holding NV (Ermelo, the Netherlands). Not blanched larvae were prepared by snap freezing of alive larvae with liquid nitrogen. The blanching step was performed at 85 °C for 1 min, and insects were provided by Protifarm Holding NV. Afterwards, not blanched and blanched larvae were stored in sealed plastic buckets at –20 °C and –80 °C (EvoSafe™-series, Snijders Labs, Tilburg, the Netherlands) for 7 weeks.

pH and colour analysis

Larvae slurries were prepared by blending frozen lesser mealworm larvae with demineralised water in 1:2 (w/w) larvae/water ratio for 1 min to obtain a homogenous mixture. Then, larvae slurries were used for pH and colour measurements. The values of pH were measured using a pH meter. Colour evaluation was performed using a colour meter (Elscolab BV, Ede, the Netherlands) to monitor the browning intensity over time. Colour was analysed for L, a, and b parameters, and the browning index was calculated based on these parameters according to Pathare *et al.* (2013).

Lipid analyses

Lipid extraction

The lipid extraction method was based on Bligh and Dyer method (Bligh and Dyer, 1959). The volumes of chloroform (RS, CARLO ERBA Reagents GmbH, Emmendingen, Germany), methanol (HPLC gradient, Actu-All Chemicals BV, Oss, the Netherlands) and water

were kept in proportions of 2:2:1.8 (v/v/v) by taking into account the water content of the not blanched (48%) and blanched (42%) larvae samples. After mixing the larvae samples with the abovementioned solvents, the mixture was vortexed for 1 min and centrifuged for 10 min at 2,000 rpm at 20 °C (Heraeus Multifuge X3R, Thermo Scientific, Waltham, MA, USA). After centrifugation, the upper layer was taken. Then 1:1 (v/v) chloroform:methanol mixture was added to this upper layer and after centrifugation (10 min, 2,000 rpm, 20 °C) the lower layer was collected. The solvent from this layer was evaporated at 40 °C using a vacuum rotary evaporator (R-215, Buchi, Flawil, Switzerland) to obtain the lipid fraction.

Peroxide value

Peroxide value was determined by using a modified method according to Jeon *et al.* (2016). One gram of lipid was dissolved in a 25 mL solution of glacial acetic acid (ACS, ISO, Reag. Ph. Eur., VWR Chemicals)-isooctane (ACS, Reag. Ph. Eur., VWR Chemicals, Amsterdam, the Netherlands) in 3:2 (v/v) ratio, then 1 mL of saturated potassium iodide (ISO, Reag. Ph Eur, Merck KGaA, Darmstadt, Germany) solution was added to the mixture and mixed in the dark for 1 min. Further, 30 mL of distilled water (Veolia Water Technologies Netherlands B.V., Ede, the Netherlands) and 1 mL of 1% starch (ISO, Merck KGaA, Darmstadt, Germany) solution (w/v) were added, and the mixture was titrated with 0.002 N sodium thiosulfate (Merck KGaA, Darmstadt, Germany) until the blue colour disappeared. Peroxide value was expressed as meq O_2/kg insect oil.

Acid value

The acid value was determined by using the modified method based on Jeon *et al.* (2016). The neutralised solvent was 96% ethanol (GPR Rectapur, VWR Chemicals, Amsterdam, the Netherlands) and phenolphthalein-methanol solution (ACS, Reag. Ph. Eur, Merck KGaA, Darmstadt, Germany) was used as a colour indicator. Firstly, the colour indicator solution was added to the required amount of solvent in a ratio of 2:125 (v/v) and was neutralised with alkali to a faint, but permanent pink colour. Subsequently, the insect oil sample (0.5 g) was added to the neutralised solution and the mixture was shaken until the sample was completely dissolved in the solution. Then the mixture was titrated with 0.1 N standard potassium hydroxide (Merck KGaA, Darmstadt, Germany) to the first permanent pink colour (30 s) of the same intensity as that of the neutralised solvent. The acid value was expressed as mg KOH/g insect oil.

Volatile compounds analysis with GC-MS

Volatile compounds of lesser mealworm larvae were analyzed with headspace (HS) solid-phase microextraction (SPME) and GC-MS. Extraction of volatile compounds was done by using a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (2 cm) fiber (Sigma-Aldrich, St. Louis, MO, USA). First, 2 g of frozen larvae were weighed into 10 mL headspace vials sealed with 20 mm PTFE/silicone magnetic caps (10 ml × 23 × 46 mm; Phenomenex, Torrance, CA, USA) and incubated at 40 °C for 10 minutes. Subsequently, the extraction of volatile compounds to the SPME fiber was done for 10 minutes at 40 °C followed by 10 minutes of desorption at the same temperature. The volatile compounds were injected into a GC-MS (Thermo TraceGc, Breda, the Netherlands) system with a Stabilwax-DA column (30 m × 0.25 mm, 1 μm) (Restek, Bellefonte, PA, USA) via an autosampler (TriPlus, Thermo Scientific, TX, USA). The following temperature program was used: initial temperature 40 °C for 3 minutes, then the temperature was raised to 220 °C with a rate of 10 °C/min. The final temperature of 220 °C was held for 5 minutes. Carrier gas helium was fed with a constant flow rate at 1 mL/min and the MS ion source was maintained at 225 °C in full scan. Electron impact mode (EI/MS) was set at 70 eV with the mass range of 33-250 *m/z*. All samples were measured in triplicate. Identification of the volatile compounds was done by comparing fragmentation mass spectra of the samples to data available from the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) library.

Statistical analysis

All experiments were performed in triplicate. The results were reported as mean values ± standard deviation. IBM SPSS Statistics 23 was used to perform one-way ANOVA on all data. A post-hoc Tukey HSD was used to identify the significant differences. Differences were considered significant at $P < 0.05$. Peak areas of volatile compounds obtained with GC-MS were used for Principal Component Analysis (PCA) with scaling and mean centring by using R programming language (R Core Team, 2013).

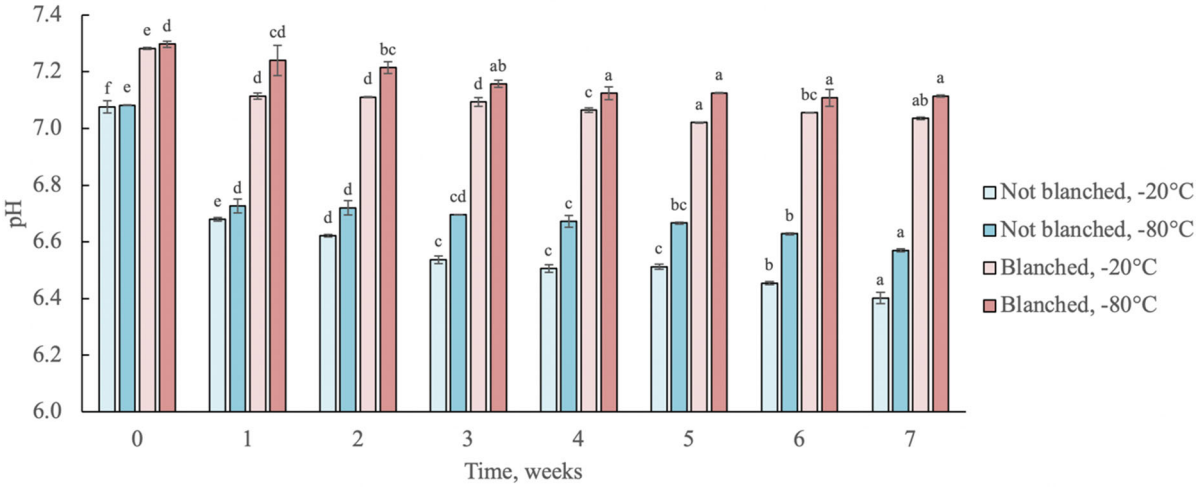

FIGURE 1 Changes in pH values of not blanched and blanched mealworm larvae during 7 weeks of storage at –20 °C and –80 °C. Different letters indicate difference ($P < 0.05$) of means among storage times within the same sample.

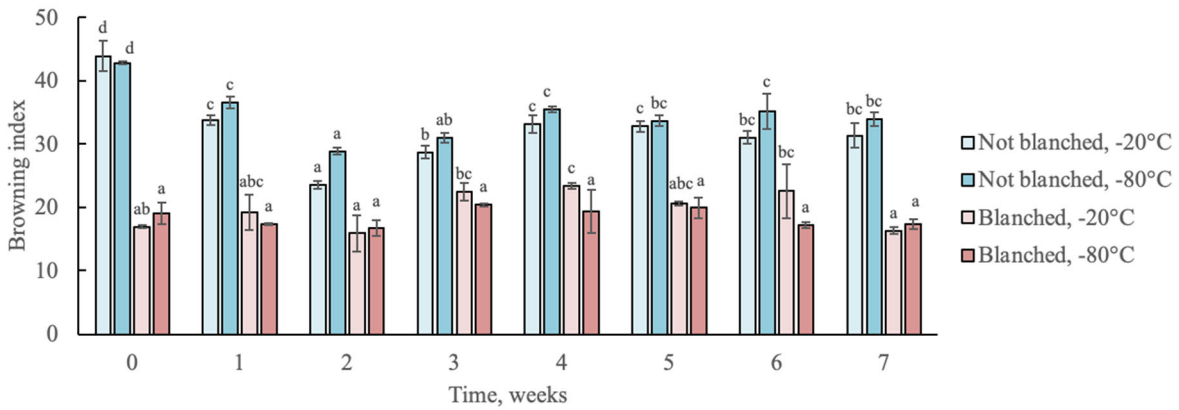

FIGURE 2 Browning index of not blanched and blanched lesser mealworm during 7 weeks of storage at –20 °C and –80 °C. Different letters indicate difference ($P < 0.05$) of means among storage times within the same sample.

3 Results and discussion

Changes in pH, colour, peroxide and acid values

pH

Blanching treatment led to an increase in pH of larvae from 7.1 to 7.3 (Figure 1), that is similar to the previously reported pH increase of lesser mealworms after blanching (Wynants *et al.*, 2018). A subsequent gradual decrease in pH values of both blanched and not blanched larvae was observed at both temperatures during 7 weeks of storage. In particular, a significant decrease from the initial pH of 7.1 to 6.4 (–20 °C) and 6.6 (–80 °C) of not blanched larvae can be caused by the release of hydrogen ions as a result of protein hydrolysis during storage. It can be due to the presence of unfrozen water as it has been previously demonstrated for lesser mealworms stored at –20 °C (Wessels *et al.*, 2020). Moreover, native lipases of insects can cause lipid hydrolysis leading to the release of free fatty acids as has been shown for larvae of flesh fly stored at –20 °C (Cabot and Lumb, 1981). Free fatty acids in turn cause a decrease in pH values. In fact, the pH decrease was less pronounced in blanched larvae with pH changes from around 7.3 to 7.0 (–20 °C) and 7.1 (–80 °C) that could be related to the inactivation of native enzymes during the blanching process and thus preventing protein and lipid hydrolysis and change in pH (Wessels *et al.*, 2020).

Colour

At day 0, browning index of blanched larvae (16.9-19.0) was significantly lower than that of not blanched larvae (42.8-43.9) that can be attributed to the inactivation of enzymes causing browning. This trend continued during 7 weeks of storage at both temperatures, i.e. browning index of blanched larvae (15.9-23.4) was significantly lower ($P < 0.05$) compared to not blanched larvae (23.5-43.9) indicating the occurrence of browning reactions in not blanched larvae (Figure 2). Fluctua-

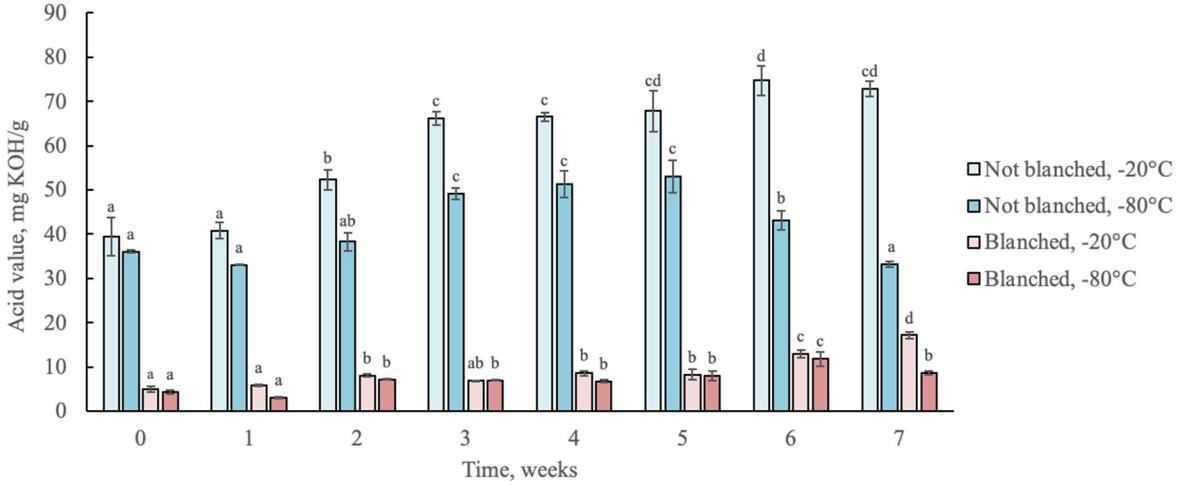

FIGURE 3 Changes in acid values of lipids from not blanched and blanched lesser mealworm during 7 weeks of storage at –20 °C and –80 °C. Different letters indicate difference ($P < 0.05$) of means among storage times within the same sample.

tions in colour were in general more pronounced in not blanched larvae. So, the browning index of not blanched larvae significantly decreased during the first 2 weeks to 23.5 and 28.9 at –20 °C and –80 °C, respectively; this observation requires further research for explanation. Further storage of not blanched larvae led to an increase in browning index and variations between 28.7 and 33.2 at –20 °C and 31.0-35.4 – at –80 °C. These changes as a function of storage temperature can be attributed to the presence of phenoloxidase previously reported as an enzyme that can cause browning during grinding of lesser mealworm (Janssen *et al.*, 2017). Colour formation in ground not blanched larvae can be also related to iron-phenolic complexation. The contribution of this phenomenon into browning might be less pronounced in comparison, for instance, to black soldier fly due to low iron content in lesser mealworm (Janssen *et al.*, 2019). In contrast to not blanched larvae, browning index of blanched larvae stored at –20 °C varied between 15.9 and 23.4. In this case, lower browning index of blanched larvae could be explained by the inactivation of enzymes during the blanching process at higher temperatures (>70 °C) (Mancini *et al.*, 2019). There were no significant changes in browning index of blanched larvae stored at –80 °C for 7 weeks, that can be related to the combined effect of enzyme inactivation and low temperature.

Peroxide value

Peroxide value indicates primary lipid oxidation processes and is one of the oil quality indicators. Peroxide values of blanched and not blanched larvae stored for 7 weeks at –20 °C and –80 °C are shown in Supplementary Table S1. Peroxide values varied between 0.41 and 1.34 meq O_2/kg insect oil except for one value of 3.46 meq O_2/kg insect oil (blanched, –20 °C, 1 week of storage). These values are below the maximum recommended limit of peroxide value of ≤5 meq O_2/kg fat recommended for yellow mealworm oil (Turck *et al.*, 2021) indicating a good quality of larvae oil in terms of lipid oxidation. However, peroxide values of all samples fluctuated without a clear trend during storage. In this case, an increase in peroxide values may indicate accumulation of primary oxidation products (Jeon *et al.*, 2016), while a decrease in these values can be referred to hydroperoxides decomposition to various oxidation products (Guo *et al.*, 2019). It should be taken into account that the decomposition of peroxides to secondary products may lead to an underestimation of the degree of oxidation when measuring peroxide concentration (Ross and Smith, 2006). In terms of volatiles, it has been previously found that secondary degradation products from lipid oxidation appear after time lag to peroxide formation (Grebenteuch *et al.*, 2021).

Acid value

As a measure of the amount of free fatty acids present, the acid values of lipids from blanched and not blanched larvae stored at –20 °C and –80 °C for 7 weeks are shown in Figure 3. In general, acid values of blanched larvae were significantly lower (2.96-17.15) than those of not blanched (33.02-74.73) indicating prevention of any reactions that might cause acid production by heat treatment. Although both types of larvae showed a trend for the increase of acid value during the storage, the increase was more pronounced for not blanched larvae. In particular, acid values of not blanched larvae stored at –20 °C increased during the storage from

39.37 to 72.77 mg KOH/g, indicating a faster hydrolysis rate than blanched larvae and being in line with the trend of a decrease in pH value. Correspondingly, a less noticeable increase in acid values was observed for not blanched larvae stored at –80 °C with an increase from 36.07 mg KOH/g to 53.03 mg KOH/g in 5 weeks and then decrease to the initial level (33.13 mg KOH/g) by week 7. Lower acid values of the samples that were stored at –80 °C can indicate faster enzymatic reactions at higher storage temperatures, being in this study at –20 °C. Moreover, the acidity of blanched larvae was a few times lower than those of not blanched larvae that can be related to enzyme inactivation caused by heat treatment.

Changes in volatile profile

Study of volatile compounds is important for the assessment of the quality of foods (Costello *et al.*, 2023). In this study, a total of 39 volatile organic compounds (VOCs) were identified in the headspace of blanched and not blanched lesser mealworm larvae at day 0 (Table 1). Overall changes in the volatile profile of the larvae stored at –20 °C and –80 °C for 7 weeks are shown in Supplementary Figure S1. The identified VOCs were from different compound classes namely aldehydes, acids, ketones, alcohols, alkanes, aromatic compounds, esters, nitriles, pyrroles and sulphur compounds. Similarly, a broad spectrum of volatiles was previously identified in other edible insect species (Kröncke *et al.*, 2019; Mishyna *et al.*, 2020; Tzompa-Sosa *et al.*, 2019; Yeo *et al.*, 2013).

For an overall evaluation of VOCs emission during storage of lesser mealworm larvae, a PCA was performed and the resulting PCA biplot with the first two principal components (PCs) is shown in Figure 4. The first two PC accounted for 53.8% of the total variance. PC2 discriminated the samples based on the blanching process with high negative loadings of volatile acids (e.g. 2-methyl propanoic acid, octanoic acid, 3-methyl butanoic acid) and (Z)-2-penten-ol in not blanched larvae whereas high positive loadings for 2-butanone, 3-methyl-butanal, dimethyl disulfide and nonanal in blanched larvae. High negative loadings of volatile acids in blanched larvae indicates that these volatile compounds were more dominant in not blanched larvae. This observation in volatile acids also correlates well with the higher pH decrease and acid values observed in not blanched larvae stored for 7 weeks at –20 °C. PC1, on the other hand, discriminated the samples based on storage time. There was no clear separation based on storage temperature neither on PC1, nor PC 2. Thus, blanching and storage time were more influential on VOC profiles compared to the storage temperature (–20 °C and –80 °C).

The variations with storage time observed in VOC profile of not blanched larvae were much higher than in blanched larvae (Table 1, Supplementary Figure S1), as it can also be seen on the PCA biplot. This can be explained by the heat treatment applied during blanching process that leads to the denaturation of the native proteases and lipases of lesser mealworm larvae and lowering of microbial decontamination in the lesser mealworm larvae (Vandeweyer *et al.*, 2017). As a result, due to the lower enzymatic activity, the concentration of VOCs emitted from blanched and not blanched larvae differed already on day 0 (Table 1). Among these compounds, blanched and not blanched larvae differed in 24 volatile compounds ($P < 0.05$): blanched larvae emitted significantly higher levels of ketones and aldehydes, while not blanched larvae contained higher amounts of volatile acids. The effect of storage temperature and duration on volatiles from blanched and not blanched larvae is discussed below.

Acids

The content of volatile acids in all samples differed and fluctuated depending on storage temperature and storage time (Supplementary Figure S1). Overall, the content of major volatile acids such as acetic acid and 2-methyl propanoic acid remained significantly higher in not blanched larvae for most time points at both temperatures compared to blanched larvae. For acetic acid, it can be related to the reduction of total aerobic count and lactic acid bacteria by blanching treatment (Vandeweyer *et al.*, 2017). Insect gut microbiota in its turn can be involved in fermentation processes with the emission of acetic acid (Pinu and Villas-Boas, 2017). Similar to our study, acetic acid was previously detected in oil extracted from lesser mealworms, yellow mealworms and crickets (Tzompa-Sosa *et al.*, 2019) and as a volatile from unprocessed locusts and silkworms (Mishyna *et al.*, 2020). It has been also suggested that 2-methyl propanoic acid from Dubia cockroach oil is produced by gut microflora as well (Tzompa-Sosa *et al.*, 2019). Interestingly, a steady decline of 2-methyl propanoic acid was observed for not blanched larvae stored at both temperatures with the minimum values at weeks 6 and 7. It can be assumed that the decomposition of 2-methyl propanoic acid during the storage and the absence of processes that could lead to a detectible increase in the emission of this compound.

TABLE 1 Headspace volatile compounds (peak area ± standard deviation) of not blanched and blanched lesser mealworm larvae at day 0

Volatile compound	Class	Not blanched (n = 6)	Blanched (n = 6)	*P*-value
Acetic acid	Acid	246717 ± 79379 b,*	29190 ± 6065 a	<0.01
Propanoic acid, 2-methyl	Acid	31277 ± 8819 b	2586 ± 1267 a	<0.01
Butanoic acid, 3-methyl	Acid	48988 ± 15918 b	3293 ± 1020 a	<0.01
Hexanoic acid	Acid	40267 ± 9284 b	23959 ± 11830 a	0.02
Octanoic acid	Acid	88120 ± 18602 b	26936 ± 9695 a	<0.01
Nonanoic acid	Acid	8711 ± 602 b	7313 ± 477 a	<0.01
1-Decanol, 2-ethyl-	Alcohol	48034 ± 8470 b	26003 ± 1903 a	<0.01
1-Decanol, 2-methyl-	Alcohol	8973 ± 2812 a	8657 ± 1008 a	0.80
3-methyl-2-heptanol	Alcohol	191546 ± 35953 a	158418 ± 40775 a	0.17
1-Octanol, 2-butyl-	Alcohol	6588 ± 2906 a	10093 ± 3081 a	0.07
(Z)-2-Penten-1-ol	Alcohol	60364 ± 12112 b	2169 ± 1642 a	<0.01
1-Hexadecanol	Alcohol	7094 ± 986 a	9194 ± 1174 b	0.01
1-Hexadecanol, 2-methyl-	Alcohol	4463 ± 1130 a	5825 ± 338 b	0.02
Propanal, 2-methyl-	Aldehyde	746410 ± 580061 a	885047 ± 409354 a	0.64
Butanal, 3-methyl-	Aldehyde	45348 ± 17427 a	73235 ± 8315 b	0.01
Nonanal	Aldehyde	5757 ± 2540 a	15693 ± 3846 b	<0.01
Decane	Alkane	45068 ± 14770 a	35952 ± 3049 a	0.17
Tridecane	Alkane	332149 ± 64342 a	275031 ± 19248 a	0.06
Nonane, 3,7-dimethyl-	Alkane	139525 ± 24137 b	96388 ± 9471 a	0.00
Octane, 2,3-dimethyl	Alkane	49045 ± 13463 b	36030 ± 4155 a	0.05
Nonane, 4,5-dimethyl-	Alkane	346961 ± 91261 b	213394 ± 37445 a	0.01
Undecane, 2,6-dimethyl-	Alkane	76911 ± 12858 a	66443 ± 5307 a	0.10
Decane, 5-propyl-	Alkane	5158 ± 2763 a	3521 ± 2078 a	0.27
Undecane, 4-methyl	Alkane	365 ± 138 a	652 ± 626 a	0.30
9-methylheptadecane	Alkane	76837 ± 14537 a	64035 ± 4250 a	0.07
Dodecane	Alkane	77793 ± 18479 a	70706 ± 3226 a	0.38
Toluene	Aromatic	27589 ± 20304 a	14699 ± 13363 a	0.22
p-Xylene	Aromatic	121372 ± 16146 a	148931 ± 17407 b	0.02
Heptylcyclohexane	Aromatic	6551 ± 1829 a	8032 ± 736 a	0.10
4-Cyanocyclohexene	Aromatic	1486 ± 398 a	1696 ± 317 a	0.34
Acetic acid, 2-ethylhexyl ester	Ester	61307 ± 11829 b	25871 ± 5965 a	<0.01
Benzoic acid, 4-ethoxy-, ethyl ester	Ester	120059 ± 23500 a	113162 ± 26377 a	0.64
2-Butanone	Ketone	82215 ± 6361 a	353724 ± 130671 b	<0.01
2,3-Butanedione	Ketone	42159 ± 5380 a	54322 ± 4140 b	<0.01
3-hydroxy-2-Butanone	Ketone	144198 ± 75073 b	14425 ± 3618 a	<0.01
Cyclopentanone, 2-methyl	Ketone	205 ± 143 a	11704 ± 1002 b	<0.01
Acetonitrile	Nitrile	92445 ± 13415 a	88062 ± 4948 a	0.47
Pyrrole	Pyrrole	155386 ± 38016 b	4343 ± 321 a	<0.01
Dimethyl disulfide	Sulphur compound	450 ± 374 a	82649 ± 4507 b	<0.01

*Different letters indicate significant differences of each compound between the samples.

Hexanoic acid was previously reported in vacuum-dried yellow mealworm (*Tenebrio molitor*) (Kröncke *et al.*, 2019), freeze-dried house crickets (*Acheta domesticus*), Jamaican field crickets (*Gryllus assimilis*) (Khatun *et al.*, 2021), and frozen fresh and salted boiled male giant water bugs (Kiatbenjakul *et al.*, 2015). In this study, hexanoic acid content fluctuated without a clear trend as a function of blanching and storage conditions. Its presence can be related to numerous occurring reactions, but the exact mechanism still should be studied. For instance, hexanoic acid is known as a product of oleic acid oxidation. Oleic acid is prone to oxidation

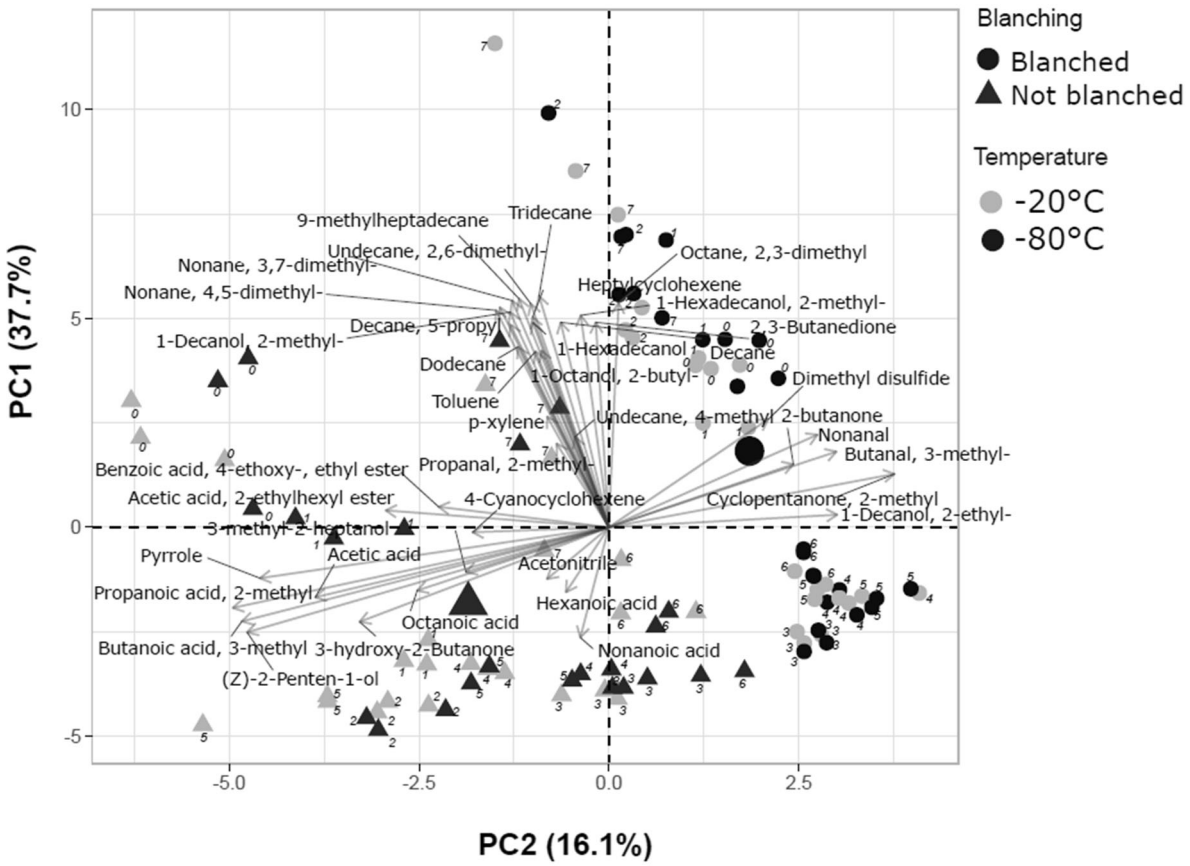

FIGURE 4 Principal Component Analysis (PCA) biplot of volatile profile of not blanched (NB, ▲) and blanched (B, ε) lesser mealworm during 7 weeks of storage at –20 °C (grey colour) and –80 °C (black colour). The biggest triangle (▲) and dot (ε) indicate the centre of the not blanched and blanched larvae clusters, respectively. Numbers on the dots and triangles indicate storage weeks.

due to its unsaturation and is one of the dominant fatty acids in lesser mealworm, followed by palmitic acid and linoleic acid (Adámková *et al.*, 2016). Oxidation of oleic acid might also be a cause for an increase in the emission of octanoic acid from blanched larvae after 5 weeks of storage at both temperatures in comparison to day 0. However, the octanoic acid content of not blanched larvae lowered after 2-3 and 6-7 weeks of storage at both temperatures.

Similar to our study, nonanoic acid was previously reported in lesser mealworm oil (Tzompa-Sosa *et al.*, 2019). The amount of produced nonanoic acid did not change significantly in all samples during the first week of storage, but a significant increase was observed between second and fifth weeks. Further, between weeks 4 and 6, not blanched larvae emitted more nonanoic acid at both temperatures than blanched larvae, which might indicate more intensive linolenic acid oxidation.

Aldehydes

Aldehydes are important breakdown products of secondary lipid oxidation that contribute to rancid off-flavours and possess low threshold values (Ross and Smith, 2006). Volatiles 2-methyl-propanal, nonanal, and 3-methyl-butanal were identified in the headspace of blanched and not blanched lesser mealworms. In particular, nonanal represents a special interest to assess the quality of lesser mealworm during the storage as it was proposed as a marker for lipid oxidation, for instance, for silver carp (*Hypophthalmichthys molitrix*) (Kunyaboon *et al.*, 2021). In this study, nonanal was determined in blanched larvae on day 0 with a three times higher amount for not blanched larvae. This can be due to oleic acid oxidation (Khatun *et al.*, 2021) caused by the thermal degradation of oleic acid during blanching of mealworms as has been shown for olive oil (Nunes *et al.*, 2013). Storage did not have a strong effect on the formation of nonanal; and nonanal content remained higher for blanched larvae over 7 weeks in comparison to not blanched larvae (Supplementary Figure S1). As a naturally occurring volatile, nonanal was previously identified as volatile secretions from the European stink bug *Graphosoma lineatum* (Šanda *et al.*, 2012), volatile defensive secretions of three species of Pyrrhocoridae (Krajicek *et al.*, 2016).

Other aldehydes such as 2-methyl-propanal and 3-methyl-butanal were identified in lesser mealworms on day 0. While 2-methyl-propanal content did not differ between blanched and not blanched larvae, 3-methyl-butanal was emitted at a significantly higher amount from blanched mealworms than from not blanched. It can be caused by the blanching-induced formation of the Strecker aldehyde such as 3-methyl-butanal (Rainer Cremer and Eichner, 2000).

Alcohols

Volatile alcohols from lesser mealworm larvae are represented by 2-ethyl-1-decanol, 2-methyl-1-decanol, 3-methyl-2-heptanol, 2-butyl-1-octanol, 2-ethyl-1-decanol, (Z)-2-penten-1-ol, 1-hexadecanol, and 2-methyl-1-hexadecanol. On day 0, blanched larvae emitted a significantly lower amount of 2-penten-1-ol and 2-ethyl-1-decanol than not blanched larvae that is in accordance with the study that blanching with water resulted in a decrease of volatile alcohols and ketones of *Cordyceps militaris* (Wu *et al.*, 2019). No significant effect of storage was observed on 2-penten-1-ol of blanched larvae at both temperatures for 7 weeks. In not blanched larvae, 2-penten-1-ol content fluctuated during the storage with a gradual decrease to the lowest values after 6 weeks of storage. However, these values were not higher than on day 0 that might indicate the decomposition of 2-penten-1-ol during the storage.

Ketones

Ketones 2-butanone, 2,3-butanedione, 3-hydroxy-2-butanone, and 2-methyl cyclopentanone were identified as volatiles from blanched and not blanched lesser mealworm larvae. 2-butanone is known as a pheromone of *Strategus aloeus* (Coleoptera) along with 3-pentanone and *sec*.-butyl acetate (Rochat *et al.*, 2000), and a study on semiochemicals of confused flour beetle *Tribolium confusum* identified 2-butanone in adults and larvae (Abuelnnor *et al.*, 2010). Volatile 2-butanone was determined in the headspace from rack oven-dried and vacuum-dried *Tenebrio molitor* larvae (Kröncke *et al.*, 2019) and in freeze-dried locusts and microwave-dried silkworms (Mishyna *et al.*, 2020). On day 0, blanched larvae emitted a significantly higher amount of 2-butanone than not blanched that can be related to the exposure to heat, and this trend was observed during the storage in weeks 3-5 and 7 (Supplementary Figure S1). The lowest emission was observed for not blanched larvae stored at –80 °C.

In this study, blanched larvae had higher 2,3-butanedione content than not blanched larvae on day 0. It was previously reported, that 2,3-butanedione can be generated as a result of Maillard reaction via a single pathway involving only glucose carbon atoms as has been demonstrated in D-glucose/L-alanine Maillard model systems (Yaylayan and Keyhani, 1999). 2,3-Butanedione was also identified in mussel extract and suggested to contribute to the desired flavor of cooked mussels (Le Guen *et al.*, 2000) and as a predominant odorant in the headspace of fish sauce (Lapsongphon *et al.*, 2015). Storage of not blanched larvae led to lowering of 2,3-butanedione emission despite the temperature and a gradual increase from the 5th week. A similar increase in 2,3-butanedione content at the end of the studied period was determined for blanched larvae, that can indicate its formation during prolonged larvae storage.

Thus, this study revealed the variations in volatile profiles of lesser mealworms as an effect of storage conditions and blanching. However, it should be taken into account, that further processing insects into food ingredients will also have an effect on flavour formation and sensory characteristics of insect food ingredients (Perez-Santaescolastica *et al.*, 2022). In addition, further development of food products comprises a combination of various food ingredients and processing steps that influence the resulting flavour of a product (Paravisini and Guichard, 2016).

4 Conclusions

Quality characteristics (pH, acid and peroxide values, color) of lesser mealworm larvae were found to be affected by pre-storage heat treatment (i.e. blanching) and storage conditions. The associated changes in volatile profile indicated the course of such reactions as lipid oxidation and lipid hydrolysis throughout seven weeks of storage. The extent of these events was primarily affected by blanching treatment and storage time, and least by storage temperature. A combination of blanching treatment and storage at –80 °C generally retards and inhibits the formation of oxidation-related volatiles during the storage and prevents lowering pH, increasing acid and peroxide values, as well as preserves the colour. Thus, blanching treatment and low-temperature storage can be recommended for the preservation of lesser mealworm larvae quality, while the storage time is important to take into consideration especially when storing lesser mealworm larvae at –20 °C. Moreover, analysis of insect volatiles can provide an indication of undergoing chemical reactions in

lesser mealworms larvae and can be an instrument for fast screening of the quality of insect biomass.

Supplementary material

Supplementary material is available online at: https://doi.org/10.6084/m9.figshare.26554471

Conflict of interest

The authors have no conflict of interest to declare.

References

Abuelnnor, N., Jones, P., Ratcliffe, N.M., de Lacy Costello, B. and Spencer-Phillips, P.T., 2010. Investigation of the semiochemicals of confused flour beetle *Tribolium confusum* Jaquelin du Val and grain weevil *Sitophilus granarius* (L.) in stored wheat grain and flour. In: Proceedings of the 10th International Working Conference on Stored Product Protection, 27 June to 2 July 2010 Estoril, Portugal. https://doi.org/10.5073/jka.2010.425.089

Adámková, A., Kourimská, L., Borkovcová, M., Kulma, M. and Mlček, J., 2016. Nutritional values of edible Coleoptera (*Tenebrio molitor*, *Zophobas morio* and *Alphitobius diaperinus*) reared in the Czech Republic. Potravinarstvo 10: 663-671. https://doi.org/10.5219/609

Bligh, E.G. and Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37: 911-917.

Cabot, M.C. and Lumb, R.H., 1981. The activity of a low temperature lipase in the larvae of *Sarcophaga bullata* (Diptera: Sarcophagidae). Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 68: 325-328. https://doi.org/10.1016/0305-0491(81)90106-1

Cacchiarelli, C., Fratini, F., Puccini, M., Vitolo, S., Paci, G. and Mancini, S., 2022. Effects of different blanching treatments on colour and microbiological profile of *Tenebrio molitor* and *Zophobas morio* larvae. LWT 157: 113112. https://doi.org/10.1016/j.lwt.2022.113112

Costello, B. de L., Wieczorek, M.N. and Drabinska, N., 2023. Editorial: The use of volatile compounds analysis for the assessment of food and beverage quality. Frontiers in Nutrition 10. https://doi.org/10.3389/fnut.2023.1250634

Graham, L.A., Liou, Y.C., Walker, V.K. and Davies, P.L., 1997. Hyperactive antifreeze protein from beetles. Nature 388: 727-728. https://doi.org/10.1038/41908

Grebenteuch, S., Kroh, L.W., Drusch, S. and Rohn, S., 2021. Formation of secondary and tertiary volatile compounds resulting from the lipid oxidation of rapeseed oil. Foods 10: 2417. https://doi.org/10.3390/foods10102417

Guo, X., Lu, S., Wang, Y., Dong, J., Ji, H. and Wang, Q., 2019. Correlations among flavor compounds, lipid oxidation indices, and endogenous enzyme activity during the processing of Xinjiang dry-cured mutton ham. Journal of Food Processing and Preservation 43: 1-15. https://doi.org/10.1111/jfpp.14199

Hidalgo, F.J. and Zamora, R., 2016. Amino acid degradations produced by lipid oxidation products. Critical Reviews in Food Science and Nutrition 56: 1242-1252. https://doi.org/10.1080/10408398.2012.761173

Janssen, R.H., Canelli, G., Sanders, M.G., Bakx, E.J., Lakemond, C.M.M., Fogliano, V. and Vincken, J.-P., 2019. Iron-polyphenol complexes cause blackening upon grinding *Hermetia illucens* (black soldier fly) larvae. Scientific Reports 9: 2967. https://doi.org/10.1038/s41598-019-38923-x

Janssen, R.H., Lakemond, C.M.M., Fogliano, V., Renzone, G., Scaloni, A. and Vincken, J.P., 2017. Involvement of phenoloxidase in browning during grinding of *Tenebrio molitor* larvae. PLOS ONE 12: 1-14. https://doi.org/10.1371/journal.pone.0189685

Jeon, Y.-H., Son, Y.-J., Kim, S.-H., Yun, E.-Y., Kang, H.-J. and Hwang, I.-K., 2016. Physicochemical properties and oxidative stabilities of mealworm (*Tenebrio molitor*) oils under different roasting conditions. Food Science and Biotechnology 25: 105-110. https://doi.org/10.1007/s10068-016-0015-9

Khatun, H., Claes, J., Smets, R., De Winne, A., Akhtaruzzaman, M. and Van Der Borght, M., 2021. Characterization of freeze-dried, oven-dried and blanched house crickets (*Acheta domesticus*) and Jamaican field crickets (*Gryllus assimilis*) by means of their physicochemical properties and volatile compounds. European Food Research and Technology 247: 1291-1305. https://doi.org/10.1007/s00217-021-03709-x

Kiatbenjakul, P., Intarapichet, K. and Cadwallader, K.R., 2015. Characterization of potent odorants in male giant water bug (*Lethocerus indicus* Lep. and Serv.), an important edible insect of Southeast Asia. Food Chemistry 168: 639-647. https://doi.org/10.1016/j.foodchem.2014.07.108

Klunder, H.C., Wolkers-Rooijackers, J., Korpela, J.M. and Nout, M.J.R., 2012. Microbiological aspects of processing and storage of edible insects. Food Control 26: 628-631. https://doi.org/10.1016/J.FOODCONT.2012.02.013

Krajicek, J., Havlikova, M., Bursova, M., Ston, M., Cabala, R., Exnerova, A., Stys, P. and Bosakova, Z., 2016. Comparative analysis of volatile defensive secretions of three species of Pyrrhocoridae (Insecta: Heteroptera) by gas

chromatography-mass spectrometric method. PLOS ONE 11: e0168827. https://doi.org/10.1371/journal.pone.0168827

Kröncke, N., Grebenteuch, S., Keil, C., Demtröder, S., Kroh, L., Thünemann, A.F., Benning, R. and Haase, H., 2019. Effect of different drying methods on nutrient quality of the yellow mealworm (*Tenebrio molitor* L.). Insects 10: 1-13. https://doi.org/10.3390/insects10040084

Kunyaboon, S., Thumanu, K., Park, J.W., Khongla, C. and Yongsawatdigul, J., 2021. Evaluation of lipid oxidation, volatile compounds and vibrational spectroscopy of silver carp (*Hypophthalmichthys molitrix*) during ice storage as related to the quality of its washed mince. Foods 10. https://doi.org/10.3390/foods10030495

Lapsongphon, N., Yongsawatdigul, J. and Cadwallader, K.R., 2015. Identification and characterization of the aroma-impact components of Thai fish sauce. Journal of Agricultural and Food Chemistry 63: 2628-2638. https://doi.org/10.1021/jf5061248

Larouche, J., Deschamps, M.H., Saucier, L., Lebeuf, Y., Doyen, A. and Vandenberg, G.W., 2019. Effects of killing methods on lipid oxidation, colour and microbial load of black soldier fly (*Hermetia illucens*) larvae. Animals 9. https://doi.org/10.3390/ani9040182

Le Guen, S., Prost, C. and Demaimay, M., 2000. Characterization of odorant compounds of mussels (*Mytilus edulis*) according to their origin using gas chromatography-olfactometry and gas chromatography-mass spectrometry. Journal of Chromatography A 896: 361-371. https://doi.org/10.1016/S0021-9673(00)00729-9

Leygonie, C., Britz, T.J. and Hoffman, L.C., 2012. Impact of freezing and thawing on the quality of meat: review. Meat Science 91: 93-98. https://doi.org/10.1016/j.meatsci.2012.01.013

Mancini, S., Fratini, F., Tuccinardi, T., Turchi, B., Nuvoloni, R. and Paci, G., 2019. Effects of different blanching treatments on microbiological profile and quality of the mealworm (*Tenebrio molitor*). Journal of Insects as Food and Feed 5: 225-234. https://doi.org/10.3920/JIFF2018.0034

Mishyna, M., Haber, M., Benjamin, O., Martinez, J.J.I. and Chen, J., 2020. Drying methods differentially alter volatile profiles of edible locusts and silkworms. Journal of Insects as Food and Feed 6: 405-415. https://doi.org/10.3920/jiff2019.0046

Mishyna, M., Keppler, J.K. and Chen, J., 2021. Techno-functional properties of edible insect proteins and effects of processing. Current Opinion in Colloid & Interface Science 56: 101508. https://doi.org/10.1016/j.cocis.2021.101508

Nunes, C.A., Souza, V.R. de, Corrêa, S.C., Silva, M. de C. da C. e, Bastos, S.C. and Pinheiro, A.C.M., 2013. Heating on the volatile composition and sensory aspects of extra-virgin olive oil. Ciência e Agrotecnologia 37: 566-572. https://doi.org/10.1590/s1413-70542013000600010

Paravisini, L. and Guichard, E., 2016. Interactions between aroma compounds and food matrix. In: Guichard, E., Salles, C., Morzel, M. and Le Bon, A.-M. (eds.) Flavour: from food to perception. John Wiley & Sons, Inc., Hoboken, NJ, USA, pp. 208-234. https://doi.org/10.1002/9781118929384.ch9

Pathare, P.B., Opara, U.L. and Al-Said, F.A.J., 2013. Colour measurement and analysis in fresh and processed foods: a review. Food and Bioprocess Technology 6: 36-60. https://doi.org/10.1007/s11947-012-0867-9

Perez-Santaescolastica, C., De Winne, A., Devaere, J. and Fraeye, I., 2022. The flavour of edible insects: a comprehensive review on volatile compounds and their analytical assessment. Trends in Food Science & Technology 127: 352-367. https://doi.org/10.1016/j.tifs.2022.07.011

Pinu, F.R. and Villas-Boas, S.G., 2017. Rapid quantification of major volatile metabolites in fermented food and beverages using gas chromatography-mass spectrometry. Metabolites 7: 1-13. https://doi.org/10.3390/metabo7030037

Rainer Cremer, D. and Eichner, K., 2000. The reaction kinetics for the formation of Strecker aldehydes in low moisture model systems and in plant powders. Food Chemistry 71: 37-43. https://doi.org/10.1016/S0308-8146(00)00122-9

Reyes De Corcuera, J., Cavalieri, R. and Powers, J., 2004. Blanching of foods. In: Heldman, D.R. (ed.) Encyclopedia of agricultural, food and biological engineering. Marcel Dekker Inc., New York, USA. https://doi.org/10.1081/E-EAFE-120030417

Ribeiro, J.C., Marques, J.P., Fernandes, T.R., Pintado, M.E., Carvalho, S.M.P. and Cunha, L.M., 2024. Effect of blanching, storage and drying conditions on the macro-composition, color and safety of mealworm *Tenebrio molitor* larvae. LWT 191: 115646. https://doi.org/10.1016/j.lwt.2023.115646

Rochat, D., Ramirez-Lucas, P., Malosse, C., Aldana, R., Kakul, T. and Morin, J.P., 2000. Role of solid-phase microextraction in the identification of highly volatile pheromones of two Rhinoceros beetles *Scapanes australis* and *Strategus aloeus* (Coleoptera, Scarabaeidae, Dynastinae). Journal of Chromatography A 885: 433-444. https://doi.org/10.1016/S0021-9673(99)01049-3

Ross, C.F. and Smith, D.M., 2006. Use of volatiles as indicators of lipid oxidation in muscle foods. Comprehensive Reviews in Food Science and Food Safety 5: 18-25. https://doi.org/10.1111/j.1541-4337.2006.tb00077.x

Rumpold, B.A. and Schlüter, O.K., 2013. Nutritional composition and safety aspects of edible insects. Molecular Nutrition & Food Research 57: 802-823. https://doi.org/10.1002/mnfr.201200735

Šanda, M., Žáček, P., Streinz, L., Dračínský, M. and Koutek, B., 2012. Profiling and characterization of volatile secretions from the European stink bug *Graphosoma lineatum* (Heteroptera: Pentatomidae) by two-dimensional gas chromatography/time-of-flight mass spectrometry. Journal of Chromatography B 881-882: 69-75. https://doi.org/10.1016/j.jchromb.2011.11.043

Tiencheu, B., Womeni, H.M., Linder, M., Mbiapo, F.T., Villeneuve, P., Fanni, J. and Parmentier, M., 2013. Changes of lipids in insect (*Rhynchophorus phoenicis*) during cooking and storage. European Journal of Lipid Science and Technology 115: 186-195. https://doi.org/10.1002/ejlt.201200284

Turck, D., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K.I., Kearney, J., Maciuk, A., Mangelsdorf, I., McArdle, H.J., Naska, A., Pelaez, C., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vinceti, M., Cubadda, F., Frenzel, T., Heinonen, M., Marchelli, R., Neuhäuser-Berthold, M., Poulsen, M., Prieto Maradona, M., Schlatter, J.R., van Loveren, H., Ververis, E. and Knutsen, H.K., 2021. Safety of dried yellow mealworm (*Tenebrio molitor* larva) as a novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 19: 1-29. https://doi.org/10.2903/j.efsa.2021.6343

Tzompa-Sosa, D.A., Yi, L., van Valenberg, H.J.F. and Lakemond, C.M.M., 2019. Four insect oils as food ingredient: physical and chemical characterisation of insect oils obtained by an aqueous oil extraction. Journal of Insects as Food and Feed 1: 1-14. https://doi.org/10.3920/jiff2018.0020

van Huis, A. and Oonincx, D.G.A.B., 2017. The environmental sustainability of insects as food and feed. A review. Agronomy for Sustainable Development 37: 43. https://doi.org/10.1007/s13593-017-0452-8

Vandeweyer, D., Lenaerts, S., Callens, A. and 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 Control 71: 311-314. https://doi.org/10.1016/j.foodcont.2016.07.011

Wessels, M.L.J., Azzollini, D. and Fogliano, V., 2020. Frozen storage of lesser mealworm larvae (*Alphitobius diaperinus*) changes chemical properties and functionalities of the derived ingredients. Food Chemistry 320: 126649. https://doi.org/10.1016/j.foodchem.2020.126649

Wu, X., Zhang, M., Bhandari, B. and Li, Z., 2019. Effect of blanching on volatile compounds and structural aspects of *Cordyceps militaris* dried by microwave-assisted pulse-spouted bed freeze-drying (MPSFD). Drying Technology 37: 13-25. https://doi.org/10.1080/07373937.2018.1433685

Wynants, E., Crauwels, S., Verreth, C., Gianotten, N., Lievens, B., Claes, J. and Van Campenhout, L., 2018. Microbial dynamics during production of lesser mealworms (*Alphitobius diaperinus*) for human consumption at industrial scale. Food Microbiology 70: 181-191. https://doi.org/10.1016/J.FM.2017.09.012

Yaylayan, V. and Keyhani, A., 1999. Origin of 2,3-pentanedione and 2,3-butanedione in D-glucose/L-alanine Maillard model systems. Journal of Agricultural and Food Chemistry 47: 3280-3284. https://doi.org/10.1021/jf9902292

Yeo, H., Youn, K., Kim, M., Yun, E., Hwang, J., Jeong, W. and Jun, M., 2013. Fatty acid composition and volatile constituents of *Protaetia brevitarsis* larvae. Preventive Nutrition and Food Science 18: 150-156. https://doi.org/10.3746/pnf.2013.18.2.150