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

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Abstract

Edible insects are repeatedly being considered as underutilized food candidate due to their high nutritional value. Although frequent acceptance of whole insects to consumer is still challenging, incorporation of insects in food as powder or paste has been suggested to increase their acceptability. Hence *Acheta domesticus* and *Gryllus assimilis* were subjected to freeze drying, oven drying, and blanching to acquire a clear insight on the impact of the processing methods on the nutritional and physical properties of both species. Result showed that these three methods had limited effects on the proximate composition, fatty acid and amino acid profile but had significant impact on other parameters for both species. Vitamin B12 in *A. domesticus* did not change but *G. asiimilis* changed due to the treatment. Freeze drying showed better lightness (L* value) and lower browning index for both cricket species. Oven drying and blanching resulted in lower lipid oxidation values than freeze drying for both cricket species; though

lipid oxidation was in all cases well below the rejection limit. Esters were the predominant group of volatile compound in freeze and oven dried cricket while least amount of volatiles were found in blanched cricket. The volatiles resulted from fatty acid oxidation were higher in freeze dried crickets, while Maillard reaction products were found in oven dried samples. Although freeze drying showed higher colour quality, oven drying exerted superior effect in terms of nutritional and flavour characteristics followed by blanching treatment.

Keywords: *Acheta domesticus*, *Gryllus assimilis*, Freeze drying, Oven drying, Nutritional characteristics, Volatile compounds

Introduction

Edible insects are gaining more and more attention to researchers and policymakers all around the world as they contain valuable proteins and fats with a high amounts of essential amino acids and fatty acids [1,2]. Most of these insects are reported to contain high amounts of phosphorus, potassium, calcium, iron, and vitamin B12 which could reduce the micronutrient deficiencies in developing countries [3]. Further, food development and regulatory bodies are attracted to edible insects due to their ecological and economic advantages. Farming of edible insects can diminish the burden from aquaculture, agriculture, and animal husbandry as it requires significantly less land, feed and water [4].

Traditionally, edible insects have played an essential role as part of human nutrition in many countries in Asia, Africa and Latin America [1]. Despite being consumed by at least 2 billion people worldwide [5], entomophagy is not a common practice in many countries all over the world. Knowledge gaps regarding the nutrition and safety of insects and products thereof, along with negative attitude towards insects, might be the possible reasons, since edible insects have never played a substantial role in their food cultures. As a result, introducing insects as food in these countries is a significant challenge. Incorporation of insects into everyday food items is one of the documented strategies to overcome the challenges

53 associated with poorly accepting insects as food [6]. This was also agreed by a study conducted on a meat
54 replacer in Denmark which reported the increased consumer acceptance when insects were incorporated
55 invisibly in food products identical to familiar ones [7]. This suggests that introducing insect as powder or
56 paste in a food product could be a viable option to increase human consumption.
57 Different drying techniques, including freeze drying and oven drying, are used worldwide to process and
58 preserve food industrially. However, processing methods are reported to have particular effects on
59 nutritional quality due to the change of physical or chemical composition of nutrients upon exposure to
60 high or low temperatures, which is true for insects as well. For instance, freeze dried *Tenebrio molitor*
61 larvae (mealworm) was reported to have a higher vitamin B12 content and lower fat oxidation compared
62 to microwave dried mealworms [8], while higher protein solubility and fat oxidation were found in freeze
63 dried mealworm than that of rack oven dried ones [9]. In another research oven-dried (66 °C for 24 h)
64 giant jewel beetles (*Sternocera Orissa*) were reported to contain higher essential and non-essential amino
65 acids concentrations compared to freeze dried beetles [10]. Furthermore, solar-drying was reported to
66 reduce folic acid (47 – 66 %), ascorbic acid (25 – 55 %), niacin (6 – 26 %), retinol (30 – 56 %), riboflavin (29
67 – 46 %), pyridoxine (9 – 13 %), and α-tocopherol (9 – 30 %) present in grasshoppers (*Ruspolia differens*)
68 and termites (*Macrotermes subhylanus*) [11]. Jeon et al., reported that roasting of mealworms resulted in
69 an increase of their δ-tocopherol contents, oleic acid contents, and colour intensity while their α- and γ-
70 tocopherol contents and linoleic acid decreased [12].
71 Along with the nutritive value, the sensory attributes of foodstuffs, including insects, are greatly
72 influenced by the processing techniques. A range of volatiles like aldehydes, ketones and alcohols are
73 formed during drying of mealworms, which are responsible for flavouring characteristics of the dried
74 product [13]. For example, Ssepuuya et al. reported that roasted *Ruspolia differens* contained higher
75 quantities of hexanal, heptanal, and 2-heptanone compared to boiled ones [14]. Kroncke et al. showed
76 that vacuum drying produced more aldehydes and acids by lipid oxidation than that of freeze drying and

77 oven drying of mealworms [13]. Furthermore, microwave drying of locusts (*Locusta migratoria manilensis*)
78 and silkworms (*Bombyx mori*) resulted in more typical Maillard reaction products as well as lipid oxidation
79 aldehydes compared to freeze and oven drying [15].
80 Thus, it can be summarized that several efforts have been taken during the last few years towards
81 investigating processing effects on the nutritional quality of edible insects. Nevertheless, considering the
82 diversity of the processing effects and insect species, the findings are low. House cricket (*Acheta*
83 *domesticus*) and Jamaican field cricket (*Gryllus assimilis*) are considered as potential source of nutrients
84 but processing effects on their nutrients and flavour compounds has not been studied elaborately. Hence,
85 this study aims to gain a clear insight on how the nutrients and flavour compounds of both cricket species
86 are affected by freeze drying, oven drying and blanching conditions.

87 ## Materials and methods

88 **Processing of the insects**

89 In this study, three processing treatments (i.e. blanching, freeze drying, and oven drying) were applied to
90 *Acheta domesticus* and *Gryllus assimilis*. Both insects were purchased from Nusect (Ledegem, Belgium) in
91 sub imago stage. After starving for 24 hours, insects were killed by freezing at – 20 °C. Drying parameters
92 were optimized previously (data not shown) to achieve an a_w value below 0.60. For oven drying, after
93 thawing at 4 °C temperature in a fridge (Miele K 14820 SD ED/CS), the crickets were placed with a 3 cm
94 thick layer in a glass plate and dried in a forced-air oven (UFB500, Memmert) at 65 °C for 20 hours. Dried
95 samples were ground to a powder by a grinder (DPA141, Moulinex). For freeze drying, frozen samples
96 were kept at - 80 °C (UXF40086V, Thermo Fisher Scientific LLC) for 15 minutes after which they were
97 ground immediately to a powder to avoid it turning into paste. After grinding, the samples were placed in
98 freeze dryer (Lyovapor L200, Büchi) for 40 hours. Cricket powders were stored at 4°C until further analysis.
99 To obtain a blanched paste, samples were thawed at 4 °C and transferred into boiling water, kept there
100 for 40 seconds, cooled immediately afterwards with cold water of 10 °C and turned into paste by a kitchen

grinder (AR110830, Moulinex). Freshly prepared paste was used for every analysis, except for the fat content, fatty acid and amino acid analysis, where the paste samples were freeze dried prior to analysis.

Proximate composition and water activity

The moisture content of prepared powders and paste was determined by forced air oven (UFB500, Memmert) at 105 °C for 17 hours [16]. Water activity (a_w) of all the samples was measured by using LabMaster a_w, Novasina (Lachen, Switzerland) as described by Vandeweyer et al. [16]. Crude fat was determined gravimetrically using the Soxhlet extraction process. In brief, crude fat from the sample was extracted using petroleum ether (technical grade, VWR Chemicals) for 17 hours. After extraction, the solvent was removed using a rotary evaporator (Rotavapor R-200, Büchi) [17]. Crude protein was determined by the Kjeldahl method with pure acetanilide (UCB) used as reference standard and 6.25 as the protein conversion factor [8]. To determine the ash content, the samples were heated in porcelain crucibles (VWR International) in a muffle furnace (LT 5/12, Nabertherm) at 550 °C until constant weight was obtained [17]. Chitin content was determined according to the method described by Ssepuuya et al. [18] where the samples were first demineralized using 1.0 M HCl (technical grade, VWR Chemicals) followed by deproteinization with 1.0 M NaOH (technical grade, VWR Chemicals) . All results are expressed as g/100 g of dry matter (DM).

Amino acid profile

The amino acid composition of the defatted samples was measured according to the procedure described by Ssepuuya et al.[18]. Fat free samples were subjected to acid and alkaline hydrolysis with 6 M HCl and 4 M LiOH, respectively. After the neutralization and centrifugation, derivatisation of the amino acids was accomplished with 6-aminoquinoyl-N-hydroxysuccimidly carbamate (AQC) reagent (Waters, USA). Separation and quantification of the amino acids was performed by a Waters Acquity UPLC H-class equipped with a Waters Cortecs® UPLC C18 column (2.1 mm x 150 mm, 1.6 μm) coupled to a Waters QDa mass detector in Selected Ion Recording (SIR) mode. Analytical standards (Sigma Aldrich) were used for

calibration. The Empower™ 3 software (Waters) was used for processing the data. The chemical score (*CS*) of each essential amino acid was calculated by dividing the amount of the essential amino acid present in cricket protein by the same amino acid in the reference pattern suggested by WHO/FAO, 2007 for adults multiplied by 100 [19]. The chemical score of the cricket proteins corresponds with the *CS* of the first limiting amino acid.

Fatty acid profile

To determine the fatty acid composition, fatty acid methyl esters (FAMEs) were produced by esterification of extracted fats with methanolic KOH solution by adding 20 % of BF_3-methanol solution (Sigma-Aldrich) as described by Ssepuuya et al. [14]. Fatty acid composition was measured by a GC–MS (GC 7820A/5977E MSD, Agilent Technologies, USA) fitted with a SLB-IL60 column (30 m x 0.25 mm x 0.2 µm, Sigma-Aldrich, St. Louis, USA) with helium as carrier gas. The initial oven temperature was 40 °C for 4 minutes, which subsequently increased at 5 °C/min up to 280 °C and the run time was 55 minutes. Analytical standards (Sigma Aldrich) for each fatty acid were used for calibration. The MS setting was in SIM mode with electron impact ionization of 70 eV. Fatty acids were quantified by MassHunter software (Agilent Technologies, Santa Clara, USA) while methyl tricosanoate (Sigma Aldrich) was used as the internal standard.

Fat oxidation

Fat extraction: To measure the fat oxidation in terms of the peroxide value and the *p*-anisidine value, fat was extracted by a modified Folch method (cold extraction) following the procedure described by Lenaerts et al., [8].In brief, a solvent mixture of chloroform-methanol in a 2:1 ratio was added to the sample and shaken vigorously for 5 minutes. Both solvents were of analytical grade (AnalaR NORMAPUR, VWR Chemicals). The suspension was filtered with a quantitative filter paper (454, VWR Chemicals) using a Buchner funnel and a vacuum filtration flask. The filter cake was extracted two more times with the chloroform-methanol mixture. After combining the extracts, the resulting two layers were separated by a separatory funnel. The organic phase containing fat was collected and solvent was evaporated at 45°C

for 30 minutes under vacuum (Büchi, V-300) using a rotary evaporator (Rotavapor R-200, Büchi, Switzerland) to obtain the fat.

Peroxide value: Peroxide values (*PV*) of the fats were measured according to the method described by Wo and Mao [20] with a minor modification. In brief, an acetic acid (ACS, Reag. Ph. Eur., VWR Chemicals) and *iso*-octane (technical grade, VWR Chemicals) solution in a 3:2 ratio was added to 2.5000 ± 0.01 g fat. After dissolving the fat, 0.500 ml potassium iodide (99 % ACS, VWR Chemicals) saturated in distilled water was added to the solution and mixed well for 30 seconds and then placed in dark. After one minute, 30 ml demineralized water was added to the solution and the mixture was immediately titrated with 0.0100 N sodium thiosulfate (ConvoL NORMADOSE, VWR Chemicals) using 1 % starch as indicator (potato starch, Analytical reagent, VWR Chemicals). A method blank was included as well. The following equation was used to calculate the peroxide value, expressed as milli-equivalents peroxide per kilogram of fat:

$$PV\ \left(\frac{\text{meq O}_2}{\text{kg fat}}\right) = \frac{\left(V_{\text{Na}_2\text{S}_2\text{O}_3,\text{sample}}\ (\text{ml}) - V_{\text{Na}_2\text{S}_2\text{O}_3,\text{blank}}\ (\text{ml})\right) \times N_{\text{Na}_2\text{S}_2\text{O}_3}\left(\frac{\text{meq}}{\text{ml}}\right)}{W_{\text{sample}}\ (\text{g})} \times 1000 \qquad \text{Eq. 1}$$

In this equation, $V_{\text{Na}_2\text{S}_2\text{O}_3,\text{sample}}$ is the added volume of sodium thiosulphate, $V_{\text{Na}_2\text{S}_2\text{O}_3,\text{blank}}$ is the volume of sodium thiosulphate required for the blank, $N_{\text{Na}_2\text{S}_2\text{O}_3}$ is the normality of the standard thiosulphate solution, and W_{sample} is the sample weight of the fat.

***p*-Anisidine value**: *p*-Anisidine value was measured by the method described by Tenyang et al., [21] with minor modification. Briefly, 1.00 ± 0.01 g of fat was weighed in a 25 ml volumetric flask and dissolved and diluted up to 25.00 ml with *iso*-octane (VWR, Chemicals). From this, 5.00 ml of the solution was taken in one test tube, and 5.00 ml of iso octane was taken in another test tube and kept in dark for eight minutes. The absorbance of the solution (A_S) was measured at 350 nm using a spectrophotometer (Genesys 10S UV–VIS, Thermo Scientific) while *iso*-octane was used as blank. After preparing, 1.00 ml of 0.25 % *p*-anisidine was added to 5.00 ml of fat solution and 5.00 ml of *iso*-octane and again kept in dark for eight

minutes. After eight minutes, the absorbance (A_S) of the solution was measured at 350 nm using the second solution as blank. *p*-Anisidine value was calculated using following equation:

$$pAV = \frac{100 \times Q\left(\frac{\text{g}}{\text{ml}}\right) \times V_{\text{sample}}\,(\text{ml})}{W_{\text{sample}}\,(\text{g})} \times 1.2 \times (B - A) \qquad \text{Eq. 2}$$

where V_{sample} is the volume in which the fat sample is dissolved (V_{sample} = 25.0 ml), W_{sample} is the weight of the fat portion tested, Q is the sample content of the measured solution based on which the *p*-anisidine value is expressed (Q = 0.01 g/ml), *B* = difference in absorbance between the reacted sample and reacted blank, *A* = difference in absorbance between the sample and pure *iso*-octane.

Vitamin B12

In order to determine the vitamin B12 content, samples of approximately 1.0000 g were weighed in a centrifuge tube for the extraction and analysis as described by Ssepuuya et al. [18]. In brief, 250 µL of a 1 % sodium cyanide (ACS reagent, Sigma Aldrich) and 20 ml of a 50 mM sodium acetate buffer (technical grade, VWR Chemicals) were added to both the samples and a blank. Subsequently, 300 mg of α-amylase (*Aspergillus oryzae,* ~1.5U/mg, Sigma Aldrich) was added to both the samples and the blank followed by incubation at 37 °C for 1 hour. Afterwards, the volumes were adjusted to 40.00 ml with demineralized water. Samples were heated for 30 minutes in a shaking (99 rpm) water bath at 95 °C (Julabo, SW22), cooled in an ice bath until the temperature was lower than 30 °C, and subsequently centrifuged at 1560 g (Heraeus Multifuge 3S) for 20 minutes. 2.00 ml of the solution was filtered using a sterile polyethersulfone syringe filter (pore size 0.2 µm, diameter 25 mm) (VWR international) in a sterile centrifuge tube (VWR International). 150 µL vitamin B12 assay medium, 150 µL standard, blank or diluted samples were added to their assigned wells of the microplate. After covering with adhesive foil, the microplate was incubated at 37 °C for 46 hours. After incubation, a microplate reader (VERSAmax EXTR, Molecular Devices, USA) was used to measure turbidity at 630 nm. The vitamin B12 content of the samples was measured against a standard curve using Softmax® 5 pro software and expressed as µg/100 g DM.

Colour evaluation

The colour measurements of the prepared powder and paste were performed with a colorimeter (CR-5, Konica Minolta, Tokyo, Japan) using the CIELAB colour space, where the L^* value represents the lightness, the a^* value the red-green direction of the colour and the b^* the yellow-blue direction. The colour was measured on five points of the petri dish containing cricket powder and paste with a measuring aperture of 30 mm and the specular component excluded. In addition, the browning index (*BI*) was determined for each measuring point and calculated by means of the following expression [14].

$$BI = 100 \times \frac{X - 0.31}{0.17} \quad \text{where} \quad X = \frac{a^* + 1.75 \times L^*}{5.645 \times L^* + a^* - 3.012 \times b^*} \qquad \text{Eq. 3}$$

Volatile compounds

Predominant volatile compounds in the headspace of samples were identified, separated and semi-quantified by means of an Agilent 7890 gas chromatograph coupled with a 5975C Agilent mass spectrometry system [14]. For semi-quantification of the volatile compounds, to 0.50 g of oven and freeze dried samples and to 2.00 g of blanched samples (due to high moisture content) 3.00 µL of the standard tridecanone (50, 3 µg/µl) (Sigma-Aldrich) diluted in methanol (Acros organics, HPLC-grade 99,8 %,) was added and the 20 ml headspace vial was hermetically sealed. Then each sample was incubated at 70 °C for 10 min in order to release the volatile compounds and extracted onto a 75 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (Sigma-Aldrich) for 20 min at 70 °C . After completing extraction, the fibre was transferred into the injector (250 °C) of the GC system using splitless injection mode and helium as a carrier gas with flow rate of 1 ml/min. Volatile compounds were separated on an apolar capillary column [ZB-5ms, *L* = 30 m, *ID* = 0.25 mm, and d_f = 0.25 µm (Zebron, Phenomenex)]. The GC oven temperature was initially set at 35 °C, held for 5 min and increased at 5 °C/min up to 200 °C, and finally from 200 °C to 245 °C at 15 °C/min, to ensure that all the volatiles were eluted and separated. The temperature of transfer line was maintained at 250 °C. The mass spectrometer

maintained at electron impact mode (ionization voltage of 70 eV) over a mass range from *m*/*z* 30 to 350 (scan mode) using no solvent delay and a threshold of 150. Volatile compounds were identified by comparing the mass spectrum of each peak to that from the NIST08 library. The semi-quantitative headspace concentrations of the identified volatile components were expressed as µg tridecanone equivalents/g of DM of freeze dried, oven dried, and blanched crickets. They were calculated as the area of the target ion of the volatile indicator component divided by the response factor of tridecanone.

Statistical analysis

All the analyses were conducted in triplicate unless stated otherwise. Statistical analysis was performed by using SPSS (Version 23, IBM Corporation). After testing the normality by Shapriro-Wilk test and homogeneity by Levene's test ($p > 0.05$), a one way ANOVA was used to determine the effect of processing (freeze drying, oven drying and blanching) on nutritional composition, the colour and the volatile compounds present in *A. domesticus* and *G. assimilis.* Means were separated using the Tukey's post hoc test. Kruskal Wallis test with Bonferroni post-hoc test was applied when variables were not homogenous. For all tests, a significance level of 0.05 was considered. Principal component analysis (PCA) was applied to the volatiles data using Unscrambler X version 10.5.1. (Camo Analytics, Oslo, Norway). A PCA-biplot is created as an overlay of a score plot and a loading plot.

Results and discussion

Effect of processing on the water activity, proximate composition and vitamin B12

The water activity (a_w), proximate composition and vitamin B12 content of all the processed samples from *Acheta domesticus* and *Gryllus assimilis* are presented in Table 1. Freeze dried and oven dried powder had lower water activities for both the cricket species, and ranged from 0.26 ± 0.02 to 0.32 ± 0.01. This a_w level is sufficient to retard microbial growth and activity. Generally the rate of quality deterioration in dry food systems by chemical reactions, including lipid oxidation and non-enzymatic reaction, are reduced at lower

240 a_w but lipid oxidation can increases at very low water activity (a_w <0.2) [22]. Therefore, our result suggest
241 that, the freeze dried and oven dried cricket powders only undergo reduced degradation reactions.
242 Regarding the proximate composition, *A. domesticus* was reported to contain higher protein (~75 g/100 g
243 DM), lower fat (~12 g/100 g DM) and lower fibre (~6 g/100 g DM) contents [23] compared to present
244 study, while other authors [24,25] reported lower protein (47-59 g/100 g DM) and higher fat (20- 26 g/100
245 g DM) contents. For *G. assimilis,* Jozefiak et al. and Adamkova et al. [26,27] found lower protein (~56 g/100
246 g DM) and higher fat(23- 32 g/100 g DM) than that of our results. The variation between the findings can
247 be due to different feed substrates and life stage of the insects [28].
248 **Table 1** Water activity (a_w), proximate composition (in g/100g DM) and vitamin B12 (in μg/100 g DM) of
249 freeze-dried, oven-dried and blanched *A. domesticus* and *G. assimilis*

Treatment	a_w	Moisture	Fat	Protein	Ash	Chitin	Vitamin B12
A. domesticus							
Freeze dried	0.26 ± 0.02^c	1.27 ± 0.10^c	15.31 ± 0.10^a	67.33 ± 0.47^b	5.41 ± 0.01^b	8.45 ± 0.33^a	9.08 ± 0.50^a
Oven dried	0.31 ± 0.01^b	3.96 ± 0.21^b	15.06 ± 0.13^a	68.55 ± 0.71^a	5.81 ±0.02^a	7.89 ± 0.48^a	9.35 ± 0.87^a
Blanched	0.97 ± 0.01^a	77.79 ± 0.31^a	15.28 ± 0.11^a	69.41 ± 0.50^a	5.03 ± 0.02^c	8.26 ± 0.53^a	9.60 ± 0.92^a
G. assimilis							
Freeze dried	0.32 ± 0.01^b	2.77 ± 0.25^b	21.19 ± 0.20^a	66.63 ± 0.08^a	4.19 ± 0.02^a	8.31 ± 0.48^a	11.37 ± 0.26^c
Oven dried	0.27 ± 0.03^b	2.83 ± 0.21^b	21.62 ± 0.20^a	65.08 ± 0.18^b	4.16 ± 0.01^a	7.99 ± 0.51^a	14.08 ± 0.54^b
Blanched	0.96 ± 0.03^a	70.54 ± 0.29^a	21.27 ± 0.49^a	67.25 ± 0.62^a	4.26 ± 0.14^a	8.33 ± 0.51^a	19.53 ± 0.03^a

250 Values are expressed as mean ± standard deviation (n = 3). Values in the same column with different
251 superscripts represent significant (p < 0.05) differences among the treatments of the same species
252 The processing methods applied did not affect significantly the crude fat and chitin content for both insect
253 species. Similar findings were reported by Kröncke et al. for *Tenebrio molitor* larvae (mealworm) [9],
254 where there was no significant effect on the proximate composition, except for the protein content of
255 freeze dried and oven dried samples. These findings comply with the results found for freeze dried *A.*
256 *domesticus* samples since they significantly (p < 0.05) exhibited a lower protein content in comparison
257 with oven dried and blanched samples. On the other hand, oven dried *G. assimilis* showed a lower protein
258 content than blanched and freeze dried samples. This reduction might be due to the utilization of amino

acids in non-enzymatic browning reactions during the oven drying process [29]. Another possible reason could be protein co-oxidation during freeze drying and/or oven drying since secondary oxidation products of lipid oxidation can induce protein oxidation by reacting with nucleophilic groups in proteins [30,31]. This fact is supported by the result of lipid oxidation discussed later (see Effect of processing on lipid oxidation) showing higher lipid oxidation during freeze drying. Further investigation is required to confirm the extent of protein co-oxidation occurred during freeze drying and oven drying of *A. domesticus* and *G. assimilis.* Regarding the ash content, *A. domesticus* showed significantly different values due to the processing, though *G. assimilis* had no significant difference in the ash content. In general, the effect of processing on the protein and ash content, regardless of the species, was numerically small and unlikely to influence their nutritional value.

Vitamin B12 present in *A. domesticus* and *G. assimilis* was also found to be affected differently by freeze drying, oven drying, and blanching. There was no significant effect of the processing methods on the vitamin B12 content of *A. domesticus.* These results are in accordance with Lenaerts et al., who also did not find any difference in the vitamin B12 content of blanched and freeze dried mealworm larvae [8]. In contrast, vitamin B12 of *G. assimilis* changed the greatly with the processing techniques applied. Blanched *G. assimilis* contained the highest amount of vitamin B12 followed by oven dried and freeze dried samples. The possible explanation might be that vitamin B12, is sensitive to air oxidation and light [32]. The higher amount of vitamin E (331 µg/g DM) present in *A. domesticus* [24] could prevent the oxidation of vitamin B12 during freeze drying and oven drying thus resulted no difference among the treatments. On contrary, presence of vitamin E is not confirmed in *G. assimilis,* it is possible that long time exposure during freeze drying and oven drying could lead to the degradation/oxidation of vitamin B12.

Effect of processing on the amino acid profile

The amino acid composition of freeze dried, oven dried, and blanched *A. domesticus* was found to be affected slightly (Supplementary Table 1) while it did not change for *G .assimilis*. For *A. domesticus,* among

the essential amino acids, histidine, isoleucine, and leucine were significantly higher in freeze dried samples compared to the oven dried and blanched samples.

Table 2 Chemical scores (*CS*) of essential amino acids present in freeze-dried, oven-dried and blanched of *A. domesticus* and *G. assimilis* proteins

CS	*A. domesticus*			*G. assimilis*			Scoring pattern* (mg/g of protein)
	Freeze dried	Oven dried	Blanched	Freeze dried	Oven dried	Blanched	
Met + cys	**98.71 ± 7.81**	**90.76 ± 11.19**	**98.09 ± 4.44**	**95.75 ± 6.14**	**95.22 ± 13.89**	**91.77 ± 10.72**	22
His	134.03 ± 1.51[a]	133.04 ± 3.54[a]	109.46 ± 3.81[b]	123.28 ± 15.24	114.86 ± 5.85	132.65 ± 6.52	15
Leu	136.19 ± 0.48[a]	136.89 ± 0.86[a]	128.13 ± 0.86[b]	134.95 ± 1.67	132.13 ± 3.68	136.17 ± 2.84	59
Lys	149.92 ± 4.46	148.28 ± 6.13	150.65 ± 2.03	148.28 ± 1.76	146.90 ± 2.54	150.68 ± 5.35	45
Trp	155.81 ± 7.32	155.83 ± 8.97	160.21 ± 8.91	144.55 ± 5.54	137.53 ± 8.60	149.05 ± 10.45	6
Ile	158.38 ± 0.73[a]	157.83 ± 1.79[a]	146.88 ± 0.69[b]	152.10 ± 4.26	151.08 ± 1.43	154.70 ± 3.40	30
Val	164.90 ± 3.07	167.70 ± 5.01	162.80 ± 1.85	163.92 ± 0.63	163.39 ± 2.32	166.65 ± 4.73	39
Thr	188.97 ± 2.85	186.01 ± 5.65	185.08 ± 2.53	182.01 ± 2.80	183.88 ± 4.76	185.16 ± 3.85	23
Phe + tyr	248.64 ± 4.35[b]	252.32 ± 7.83[b]	283.65 ± 2.55[a]	265.14 ± 3.94	272.99 ± 12.68	155.02 ± 4.20	38

Values are expressed as the mean ± standard deviation (n = 3). Values in the same row with different superscripts represent significant ($p < 0.05$) differences among the treatments of the same species.
*Scoring pattern according to WHO/FAO (2007) for adults. The values marked in bold indicate the first limiting amino acids

The lower amount of histidine in the oven dried and blanched samples confirmed that it is heat labile which was also reported by Wu & Mao in hot air dried grass carp [20]. On the other hand Fombong et al. did not find any significant differences in the amino acid content of freeze dried and oven dried *R. differens* [33]. This can be explained by the fact that amino acids could be affected during processing depending on the species, amino acid type and methods applied [34]. However, although statistically significant differences can be identified, they are of little relevance regarding the nutritional value, since the differences are small.

The chemical score (*CS*) represents the abundance of each essential amino acid (EAA) present in a food proteins in comparison with a reference proteins and is related to nutritional value of a proteins [20]. The *CS* of isoleucine, leucine and phenylalanine + tyrosine changed in the case of *A. domesticus* (Table 2). According to scoring reference from WHO/FAO, 2007 [19] the first limiting amino acids were methionine

302 + cysteine for both species in all processing conditions. However, it is was encouraging that a high *CS* (*CS*
303 > 90) was maintained in all the processed sample.

304 **Effect of processing on the fatty acid composition**

305 The predominant fatty acids of *A. domesticus* were linoleic acid, oleic acid, α-linolenic acid, palmitic acid,
306 and stearic acid, representing more than 90 % of the total fatty acid, while linoleic acid, oleic acid and
307 palmitic acid were the major fatty acids of *G. assimilis*, representing more than 80 % of the total fatty acids
308 (Table 3). The fatty acid composition of *A. domesticus* and *G. assimilis* in present study were comparable
309 with the result reported by Adámková et al., Barroso et al. and Finke [27,35,36].

310 **Table 3** Fatty acid composition of freeze-dried, oven-dried and blanched *A. domesticus* and *G. assimilis* (in
311 g /100 g of fat)

Fatty acids	*A. domesticus*			*G. assimilis*		
	Freeze dried	Oven dried	Blanched	Freeze dried	Oven dried	Blanched
Decanoic acid (C10:0)	0.07 ± 0.01^{a}	0.07 ± 0.00^{a}	0.08 ± 0.03^{a}	0.07 ± 0.01^{a}	0.07 ± 0.01^{a}	0.08 ± 0.01^{a}
Lauric acid (C12:0)	0.15 ± 0.02^{a}	0.14 ± 0.00^{a}	0.26 ± 0.09^{a}	0.19 ± 0.01^{a}	0.17 ± 0.02^{b}	0.27 ± 0.04^{a}
Myristoleic acid (C14:1)	0.52 ± 0.07^{a}	0.48 ± 0.04^{a}	0.49 ± 0.17^{a}	0.85 ± 0.05^{a}	0.77 ± 0.11^{a}	0.77 ± 0.11^{a}
Myristic acid(C14: 0)	0.24 ± 0.02^{a}	0.23 ± 0.00^{a}	0.27 ± 0.09^{a}	0.23 ± 0.03^{a}	0.22 ±0.03^{a}	0.25 ± 0.04^{a}
Pentadecanoic acid (C15: 0)	0.38 ± 0.04^{a}	0.36 ± 0.02^{a}	0.38 ± 0.12^{a}	0.29 ± 0.03^{a}	0.28 ±0.03^{a}	0.31 ± 0.05^{a}
Palmitic acid (C16:0)	19.34 ±0.06^{a}	18.85 ± 0.02^{a}	18.78 ± 1.57^{a}	29.57 ± 1.08^{a}	25.45 ± 0.36^{b}	25.94 ± 1.07^{b}
Palmitoleic acid (C 16:1)	0.54± 0.06^{a}	0.51 ± 0.03^{a}	0.53 ± 0.18^{a}	1.69 ± 0.22^{a}	1.62 ± 0.29^{a}	1.37 ± 0.21^{a}
Heptadecanoic acid (C17: 0)	0.48 ± 0.05^{a}	0.46 ± 0.03^{a}	0.47 ± 0.16^{a}	0.38 ± 0.03^{a}	0.35 ±0.04^{a}	0.39 ± 0.05^{a}
Cis-10-heptadecanoic acid (C17:1)	0.37 ± 0.05^{a}	0.35 ± 0.01^{a}	0.41 ± 0.14^{a}	0.35 ± 0.04^{a}	0.33 ± 0.04^{a}	0.39 ± 0.05^{a}
Stearic acid (C18:0)	12.16 ± 0.26^{a}	11.89 ± 0.06^{a}	12.28 ± 0.42^{a}	9.94 ± 0.40^{a}	8.82 ± 0.38^{a}	9.03 ± 1.17^{a}
Oleic acid (C18:1)	21.02 ± 0.20^{a}	20.95 ± 0.03^{a}	21.04 ± 0.09^{a}	28.21 ± 0.18^{a}	27.14 ± 0.27^{b}	26.07 ± 0.14^{c}
Linoleic acid (C18:2) (n6)	30.07 ± 0.57^{a}	30.76 ± 0.25^{a}	29.66 ± 1.87^{a}	26.06 ± 0.12^{b}	32.58 ±0.39^{a}	32.73 ± 0.88^{a}
α-Linolenic acid (C18:3) (n3)	13.59 ± 0.45^{a}	13.93 ± 0.04^{a}	14.30 ± 1.82^{a}	1.12 ± 0.11^{a}	1.26 ± 0.19^{a}	1.31 ± 0.20 ^{a}
Arachidonic acid (C20:4)	0.63 ± 0.14^{a}	0.59 ± 0.04^{a}	0.60 ± 0.20^{a}	0.66 ± 0.1^{a}	0.61 ± 0.11^{a}	0.66 ± 0.11^{a}
Eicosapentanoic acid (C20:5) (n3)	0.47 ± 0.15^{a}	0.42 ± 0.04^{a}	0.45 ± 0.15^{a}	0.38 ± 0.05^{a}	0.34 ± 0.01^{a}	0.43 ± 0.07^{a}
Total SFA	32.81 ± 0.07^{a}	32.01 ± 0.13^{a}	32.52 ± 0.67^{a}	40.69 ± 0.57^{a}	35.37 ± 0.13^{b}	36.27 ± 0.37^{b}
Total MUFA	22.44 ±0.11^{a}	22.29 ± 0.21^{a}	22.47 ± 0.40^{a}	31.10 ± 0.26^{a}	29.86 ± 0.17^{b}	28.61 ± 0.27^{c}
Total PUFA	44.75 ± 0.15^{b}	45.70 ± 0.23^{a}	45.01 ± 0.32^{b}	28.21 ± 0.32^{b}	34.86 ±0.10^{a}	35.12 ± 0.52^{a}

312 Values are expressed as mean ± standard deviation (n = 3). Values in the same row with different
313 superscripts represent significant ($p < 0.05$) differences among the treatments of the same species

314 The fatty acid profile of *A. domesticus* changed not significantly with the processing methods applied,
315 while several fatty acids (lauric acid, palmitic acid, oleic acid and linoleic acid) of *G. assimilis* did change
316 significantly. Fombong et al. did not find any changes in the fatty acid composition for freeze dried and
317 oven dried *Ruspolia differens* [33], while Lenaerts et al. and Kröncke et al. reported significant differences
318 in palmitoleic acid, palmitic acid and linoleic acid due to the freeze drying, oven drying and microwave
319 drying of mealworms [8,13]. The variation in fatty acid composition after the same processing methods
320 reported by different authors already explains that results could be different due to the insect species.
321 Another explanation for different results in our study might be the presence of higher vitamin E contents
322 (331 µg/g DM) in *A. domesticus* [24] which could preserve fatty acids by providing antioxidating effect
323 while presence of vitamin E in *G. assimilis* is not confirmed.

324 **Effect of processing on fat oxidation**

325 Fat oxidation during processing and storage of fat-rich foods is a major concern as it leads to the
326 development of off-flavours and off-odours. This aspect is crucial for foods rich in unsaturated fatty acids
327 due to their high susceptibility to fat oxidation. Lipid oxidation in processed foods is a complex process
328 and involves numerous mechanisms including auto-oxidation, photo-oxidation, and enzymatic
329 (lipoxygenase) oxidation [37]. Primary oxidation products, mainly hydroperoxides, can be measured by
330 the peroxide value (*PV*). The primary oxidation products are very unstable and rapidly decompose or
331 oxidize to secondary products, which can be quantified by the *p*-anisidine value (*pAV*).

332 **Table 4** Fat oxidation and colour values of freeze-dried, oven-dried and blanched *A. domesticus* and *G.*
333 *assimilis*

Processing methods	Lipid oxidation values		Colour values			
	Peroxide value (meq. O_2/kg fat)	*p*-Anisidine value	*L**	*a**	*b**	Browning index
A. domesticus						

Freeze dried	< LOD[†]	4.85 ± 0.11[a]	66.29 ± 0.55[a]	3.10 ± 0.08[c]	14.96 ± 0.33[a]	28.53 ± 0.91[c]
Oven dried	0.96 ± 0.02	3.64 ± 0.10[b]	36.02 ± 0.65[c]	5.54 ± 0.11[b]	14.24 ± 0.36[a]	60.68 ± 0.45[a]
Blanched	< LOD[†]	1.15 ± 0.14[c]	38.52 ± 0.55[b]	6.41 ± 0.20[a]	7.92 ± 0.31[b]	34.80 ± 1.50[b]
G. assimilis						
Freeze dried	< LOD[†]	5.60 ± 0.29[a]	64.15 ± 0.30[a]	3.12 ± 0.02[a]	15.31 ± 0.24[a]	30.33 ± 0.56[b]
Oven dried	0.67 ± 0.07	4.07 ± 0.15[b]	36.28 ± 0.57[c]	4.59 ± 0.57[a]	9.45 ± 0.29[b]	39.00 ± 0.73[a]
Blanched	< LOD[†]	1.83 ± 0.16[c]	50.51 ± 0.74[b]	4.27 ± 0.36[a]	6.91 ± 0.39[c]	20.59 ± 1.08[c]

Values are expressed as the mean ± standard deviation (n = 3). Values in the same column with different superscripts represent significant ($p < 0.05$) differences among the treatments of the same species. [†] Limit of detection (*LOD*) = 0.5 meq O_2/kg fat

The peroxide values of both *A. domesticus a*nd *G. assimilis* were less than the detection limit of 0.5 mEq O_2/kg fat for freeze dried and blanched sample (Table 4). These findings are in contrast with the result found by Lenaerts et al. who found much higher peroxide values (55.57 ± 8.13 meq O_2/kg fat to 125.13 ± 5.61 meq O_2/kg fat) for freeze dried and blanched plus freeze dried mealworms [8]. Although oven dried *A. domesticus* and *G. assimilis* showed peroxide values of 0.96 ± 0.02 meq O_2/kg fat and 0.67 ± 0.07 meq O_2/kg fat respectively, these values were well below the recommended level (*PV* < 2 meq O_2/kg) for edible oils by EFSA [38].

The *p*-anisidine values of processed *A. domesticus* and *G. assimilis* were significantly different and followed the same trend having the highest value for freeze dried samples and the lowest for blanched samples. In all cases the *p*-anisidine values were quite below the values recommended by EFSA (*pAV* < 20) [38]. Since *A. domesticus* contained much higher α-linolenic acid than *G. assimilis* (Table 2), it was expected that *A. domesticus* would result in a higher lipid oxidation than *G. assimilis*; which was, however, not the case as *G. assimilis* showed a slightly higher lipid oxidation value. Kroncke et al. also reported higher secondary oxidation values for freeze dried mealworms than for oven dried mealworms [13]. Possible reason for higher *p*-anisidine values in freeze dried crickets compared to oven dried and blanched crickets might be the porous structure of the freeze dried powder, which increases the susceptibility to fat oxidation [22]. Another explanation could be the activity of lipoxygenase enzyme in the freeze dried

sample, which was possibly inactivated during oven drying and blanching. However, further investigation is required to confirm the presence and activity of lipoxygenase enzyme in *A. domesticus* and *G. assimilis.* Further, the lower primary and secondary lipid oxidation found in our study than that of mealworms reported by Lenaerts et al. and Kroncke et al. [8, 9] might be due to the antioxidant activity exerted by higher amounts of vitamin E present in *A. domesticus* (331μg/g DM), as mentioned earlier [21,34]. On the contrary, mealworms were reported to contain lower vitamin E (20 μg/g DM) [39,40], mentioning that presence of vitamin E might depend upon diet fed rather than the life stage and species of the insect. To our knowledge there is no published result on the vitamin E content of *G. assimilis.*

Effect of processing on colour

The colour of food products is of major importance for consumer acceptability. It is clear from Table 4 that the colour parameters of *A. domesticus* and *G. assimilis* were significantly influenced by the processing methods employed and followed similar trends.

In general, freeze dried *A. domesticus* and *G. assimilis* exhibited significantly ($p < 0.05$) higher lightness (L^* value) compared to blanched and oven dried samples. This might be due to the inactivation of browning enzyme or absence of non-enzymatic browning reaction during freeze drying [41]. Redness (a^* value) of *G. assimilis* did not change while a^* value of *A. domesticus* changed significantly for all treatments. There was no difference in the yellowness (b^* value) between freeze dried and oven dried samples of *A. domesticus*, while the b^* value was significantly lower in blanched crickets for both species. Leaching of carotenoids pigment, responsible for yellowness, during blanching could be the possible explanation [42]. Similar findings of colour value (L^*, a^*, b^*) were also reported by Azzollini et al. for freeze dried, oven dried and blanched mealworm larvae [43].

Additionally, the browning index (*BI*) was significantly higher in oven dried samples compared to the freeze dried and blanched samples for both species. The presence of Maillard reaction products in the oven dried samples might be the possible reason of the higher *BI* values.

Effect of processing on formation of volatiles

The consumer acceptance and preference of food is largely determined by the flavour and aroma characteristics attributed by different volatile compounds including aldehydes, ketones, alcohols and organic acids. Generally most of the volatile compounds are produced by the oxidation (either chemical or enzymatic) of unsaturated fatty acids while Maillard reaction and Strecker degradation of free amino acids are also responsible for flavour development in food products[44].

Table 5 Semi quantitative concentration (in µg tridecanone equivalents/g DM) of volatiles identified in processed powder (freeze dried and oven dried) and paste (blanched) of *A. domesticus* and *G. assimilis*

		A. domesticus			*G. assimilis*		
	Volatiles	Freeze dried	Oven dried	Blanched	Freeze dried	Oven dried	Blanched
	Esters						
1	Methyl acetate	0.75 ± 0.22^{a}	1.68 ± 1.05^{a}	Nd	0.70 ± 0.01^{b}	1.86 ± 0.45^{a}	Nd
2	Methyl propanoate	0.04 ± 0.02^{a}	0.29 ± 0.30^{a}	Nd	0.09 ± 0.01^{b}	0.64 ± 0.05^{a}	Nd
3	Methyl 2-methylpropanoate	Nd	0.10 ± 0.11^{a}	Nd	Nd	0.15 ± 0.01^{a}	Nd
4	Methyl 3-methylbutanoate	Nd	0.59 ± 0.30^{a}	Nd	Nd	0.51 ± 0.05^{a}	Nd
5	Methyl butanoate	Nd	0.06 ± 0.03^{a}	Nd	Nd	0.22 ± .38^{a}	Nd
6	Methyl pentanoate	0.14 ± 0.04^{a}	0.02 ± 0.02^{b}	Nd	0.17 ± 0.05	0.03 ± 0.00	Nd
7	Methyl hexanoate	6.38 ± 1.74^{a}	0.40 ± 0.15^{b}	Nd	6.85 ± 2.20^{a}	0.35 ± 0.01^{b}	Nd
8	Methyl phenylacetate	0.01 ± 0. 01^{b}	0.80 ± 0.32^{a}	Nd	Nd	0.87 ± 0.1^{a}	Nd
9	Methyl octanoate	0.39 ± 0.11^{a}	0.41 ± 0.17^{a}	Nd	0.56 ± 0.24^{a}	0.15 ± 0.01^{b}	Nd
10	Methyl decanoate	1.13 ± 0.28^{a}	1.27 ± 0.57^{a}	Nd	0.58 ± 0.21^{a}	0.39 ± 0.04^{a}	0.01 ± 0.01^{b}
11	Methyl dodecanoate	1.81 ± 0.46^{a}	1.08 ± 0.50^{a}	0.01 ± 0.00^{b}	2.20 ± 0.79^{a}	0.87 ± 0.21^{b}	0.02 ± 0.01^{b}
12	Methyl tetradecanoate	1.39 ± 0.32^{a}	0.67 ± 0.36^{b}	Nd	1.65 ± 0.47	0.43 ± 0.16	0.01 ± 0.01
13	Methyl hexadec-9-enoate	0.21 ± 0.04^{a}	0.08 ± 0.05^{b}	Nd	0.29 ± 0.10	0.05 ± 0.02	Nd
14	Methyl hexadecanoate	8.32 ± 1.64^{a}	2.93 ± 1.61^{b}	0.06 ± 0.02^{b}	9.69 ± 1.93^{a}	1.50 ± 0.47^{b}	0.12 ± 0.07^{c}
15	Methyl octadeca-9,12-dienoate	0.62 ± 0.17^{a}	0.19 ± 0.09^{b}	Nd	0.76 ± 0.22	0.10 ± 0.02	0.01 ± 0.01
16	Methyl octadecanoate	0.48 ± 0.14^{a}	0.13 ± 0.07^{b}	0.01 ± 0.00^{b}	0.34 ± 0.16	0.05 ± 0.00	0.48 ± 0.14^{a}
	Total	*21.73 ± 5.11^{a}*	*10.69± 4.33^{b}*	*0.08 ± 0.03^{c}*	*23.94 ± 6.11^{a}*	*9.65 ± 1.48^{b}*	*0.20 ± 0.11^{c}*
	Aldehydes						
17	2-Methylbutanal	Nd	0.08 ± 0.14	Nd	Nd	0.15 ± 0.03	Nd
18	3-Methylbutanal	Nd	0.08 ± 0.14	Nd^{a}	Nd	0.24 ± 0.06	Nd
19	Pentanal	0.34 ± 0.12^{a}	Nd	Nd	0.33 ± 0.03^{a}	Nd	Nd
20	Hexanal	1.54 ± 0.61^{a}	0.02 ± 0.01^{b}	Nd	1.23 ± 0.09^{a}	0.03 ± 0.01^{b}	Nd
21	Nonanal	0.05 ± 0.02^{a}	0.03 ± 0.02^{ab}	0.01 ± 0.00^{b}	0.05 ± 0.00^{a}	0.04 ± 0.02^{a}	0.01 ± 0.01^{b}
22	2-Propyl-2-heptenal	0.11 ± 0.03^{a}	Nd	Nd	0.14 ± 0.01^{a}	Nd	Nd
23	2-Butyl-2-octenal	1.47 ± 0.41^{a}	Nd	Nd	1.50 ± 0.17^{a}	Nd	Nd
24	Decanal	Nd	0.03 ± 0.04^{a}	Nd	0.01 ± 0.01^{a}	0.06 ± 0.03^{a}	0.01 ± 0.02^{a}
25	Benzaldehyde	0.04 ± 0.02^{ab}	0.15 ± 0.08^{a}	0.02 ± 0.00^{b}	0.06 ± 0.01^{a}	0.08 ± 0.02^{a}	0.02 ± 0.01^{b}
26	Phenylacetaldehyde	Nd	0.90 ± 0.54^{a}	0.07 ± 0.01^{b}	Nd	0.50 ± 0.12^{a}	0.04 ± 0.01^{b}
	Total	*3.56 ±1.19^{a}*	*1.29 ± 0.62^{b}*	*0.10 ± 0.02^{b}*	*3.33 ± 0.29^{a}*	*1.31 ± 0.33^{b}*	*0.08 ± 0.05^{c}*

	Organic acids						
27	2-Methylbutanoic acid	Nd	0.26 ± 0.26	Nd	Nd	0.22 ± 0.38	Nd
28	3-Methylbutanoic acid	Nd	1.42 ± 0.49	Nd	Nd	1.79 ± 0.55	Nd
29	Hexanoic acid	1.79 ± 0.43	Nd	Nd	2.69 ± 0.65^{a}	Nd	Nd
30	Decanoic acid	Nd	0.16 ± 0.10^{a}	0.03 ± 0.01^{b}	0.03 ± 0.03^{a}	0.08 ± 0.02^{a}	0.02 ± 0.02^{a}
31	Dodecanoic acid	Nd	0.18 ± 0.03^{a}	0.04 ± 0.01^{b}	0.01 ± 0.02^{b}	0.10 ± 0.04^{a}	0.03 ± 0.02^{ab}
32	Tetradecanoic acid	Nd	0.12 ± 0.08^{a}	0.01 ± 0.00^{b}	0.01 ± 0.02	0.06 ± 0.04	0.01 ± 0.02
33	8,11-Octadecadienoic acid	0.76 ± 0.22^{a}	0.29 ± 0.13^{b}	Nd	0.83 ± 0.27	0.12 ± 0.04	Nd
	Total	*2.56 ± 0.57^{a}*	*2.43 ± 0.77^{a}*	*0.07 ± 0.02^{b}*	*3.56 ± 0.89^{a}*	*2.80 ± 0.81^{b}*	*0.07 ± 0.05^{c}*
	Ketones						
34	2-Heptanone	0.56 ± 0.26	Nd	Nd	0.59 ± 0.10^{a}	Nd	Nd
35	5-Methyl-3-hepten-2-one	0.23 ± 0.07	Nd	Nd	0.18 ± 0.15	Nd	Nd
36	(*E,E*)-3,5-Octadien-2-one	0.26 ± 0.09	Nd	Nd	0.30 ± 0.04^{a}	Nd	Nd
37	(*E,Z*)-3,5-Octadien-2-one	0.35 ± 0.11	Nd	Nd	0.45 ± 0.06^{a}	Nd	Nd
	Total	*1.40 ± 0.52*	*Nd*	*Nd*	*1.52 ± 0.15*	*Nd*	*Nd*
	Alcohols						
38	1-Octen-3-ol	0.40 ± 0.15^{a}	Nd	0.01 ± 0.00^{b}	0.30 ± 0.06^{a}	0.03 ± 0.01^{b}	0.01 ± 0.01^{b}
39	3,5-Octadien-2-ol	2.07 ± 0.76^{a}	Nd	Nd	2.11 ± 0.24^{a}	0.04 ± 0.01^{b}	Nd
	Total	*2.47 ± 0.90^{a}*	*Nd*	*0.01 ±0.00^{b}*	*2.40 ± 0.26^{a}*	*0.08 ± 0.02^{b}*	*0.01 ± 0.01^{c}*
	Sulphur containing compounds						
40	Benzothiazole	0.55 ± 0.18^{a}	Nd	Nd	0.56 ± 0.09^{a}	Nd	0.01 ± 0.00^{b}
41	**Methyl 3-(methylsulfanyl)propanoate**	Nd	0.19± 0.13^{a}	Nd	Nd	0.17 ± 0.02^{a}	Nd
42	1-(2-Thienyl)ethanone	0.13 ± 0.04^{a}	Nd	Nd	2.49 ± 0.44^{a}	Nd	Nd
	Total	*0.68 ± 0.21^{a}*	*0.19 ± 0.13^{b}*	-	*3.05 ± 0.52^{a}*	*0.20 ± 0.02^{b}*	*0.01 ± 0.00^{c}*
	Others						
43	Trimethylamine	2.70 ± 0.91^{a}	Nd	Nd	5.01 ± 0.75^{a}	Nd	Nd
44	2,5-Dimethylpyrazine	Nd	0.47 ± 0.26^{a}	Nd	Nd	0.30 ± 0.07^{a}	Nd
45	2-Pentylfuran	1.33 ± 0.58^{a}	0.26 ± 0.12^{b}	Nd	1.75 ± 0.27^{a}	0.29 ± 0.08^{b}	Nd
46	2-(Hexyloxy)heptane	3.39 ± 1.13^{a}	Nd	Nd	6.80 ± 0.92^{a}	Nd	Nd
47	3-Ethoxy-4-methoxyphenol	4.97 ± 1.36^{a}	Nd	Nd	5.58 ± 0.57^{a}	Nd	Nd
48	2-Butyl-5-methyl-3-(2-methylprop-2-enyl)-cyclohexanone	0.59 ± 0.13^{a}	Nd	Nd	0.55 ± 0.48	Nd	Nd
49	Unknown	5.25 ± 1.44^{a}	Nd	Nd	6.08 ± 0.69^{a}	Nd	Nd
	Total	*18.22 ± 5.48^{a}*	*0.72 ± 0.32^{b}*	-	*25.76 ± 2.59^{a}*	*0.71 ± 0.18^{b}*	-
	Grand total	***63.57 ± 10.02^{a}***	***15.33 ± 6.12^{b}***	***0.36 ± 0.21^{c}***	***50.61 ±12.88^{a}***	***10.12 ± 6.02^{b}***	***0.27 ± 0.07^{c}***

Values are expressed as the mean ± standard deviation (n = 3). Values in the same row with different superscripts represent significant (p < 0.05) differences among the treatments of the same species. *Nd = Not detected

A total of 48 volatile compounds and one unknown compound (Table 5) were tentatively identified in the headspace of freeze dried, oven dried, and blanched crickets which included esters, aldehydes, ketones, organic acids, alcohols, sulphur containing compounds and others.

In the present study, significantly higher amounts of volatiles were observed in the freeze dried crickets compared to the oven dried and blanched crickets. Esters were the most abundant volatiles representing 21.73 ± 5.11 µg/g DM in freeze dried *A. domesticus*, followed by the oven dried (10.69 ± 4.33 µg/g DM*)* and blanched samples (0.08 ± 0.03 µg/g DM). Similarly, freeze dried *G. assimillis* contained higher amounts (23.94 ± 6.11 µg/g DM) of esters than the oven dried (9.65 ± 1.48 µg/g DM) and blanched (0.20 ± 0.11 µg/g DM) samples. Esters are heat sensitive; thus, they are degraded during heat treatment. They can either be naturally present or can be formed by the esterification of alcohol and carboxylic acids. These esters are usually fragrant compounds having low odour threshold values and responsible for fruity notes found in different fruits and also in cured meat and fermented sausage [45]. Esters were reported to be present in abundance in freeze dried *Protaetia brevitarsis* larvae (the white-spotted flower chafer beetle) [46], while Domínguez et al. found higher amounts of esters in raw foul meat which was significantly reduced in microwave dried and roasted sample [45].

It was of interest to notice that the temperature (65 °C) applied during oven drying resulted in the development of some compounds which are known as Maillard reaction and Strecker degradation products. For example 2,5-dimethylpyrazine can be produced by Maillard reactions while Strecker degradation of phenylalanine, isoleucine, and leucine lead to the development of phenylacetaldehyde, 2-methylbutanal, and 3-methylbutanal respectively [47]. 2-methylbutanal, 3-methylbutanal and 2,5-dimethylpyrazine were also detected by Mishyna et al. in oven dried locust (*Locusta migratoria manilensis*) and silkworm (*Bombyx mori*) which were higher in microwave dried samples [15], while higher 2-methylbutanal concentrations were found in vacuum and freeze dried mealworms compared to crude mealworms [13]. Higher production of 2,5-dimethylpyrazine was also reported by Ma et al. for mushrooms dried at 60 °C for 9 hours [48]. This is in line with the fact the Maillard reaction does not necessarily require high temperatures, though the reaction rate increases greatly with increasing temperature [49].

Most of the aldehydes are secondary products of lipid oxidation and are associated with a fatty aroma. Aldehydes have a low odour detection threshold, hence even a small amount of them can contribute to food flavours. Among the aldehydes formed, hexanal and 2-butyl-2-octenal are predominant breakdown products in freeze dried sample, which indicate the oxidation of ω-6 fatty acids such as linoleic or arachidonic acid [50]. Similarly, Mishyna et al. reported that higher hexanal and 2-methylpropanal concentrations were found in freeze dried locusts (*Locusta migratoria manilensis*) compared to oven dried and microwave dried samples [15]. Furthermore, pentanal is an auto-oxidation product of linoleic acid having a malt and pungent odour note, while nonanal could be formed by the oxidation of oleic acid, imparting a fatty, citrus flavour. However, these aliphatic aldehydes were reduced dramatically or were absent in oven dried and blanched samples for both species. These findings are in agreement with those reported by de Torres et al. who found higher amounts of esters, aldehydes, and organic acids in freeze dried grape skin, compared to oven dried (at 60 °C) grape skin [51]. On the other hand, higher temperatures were reported to produce higher amounts of aldehydes in microwave dried locusts (*Locusta migratoria manilensis*) and silkworms (*Bombyx mori*) [15], while higher aldehydes concentrations were detected in vacuum dried mealworms [13]. Other oxidation products of linoleic acid such as, 1-octen-3-ol having an earthy, mushroom odour note and 2-pentylfuran, having an earthy, beany, green character were also found to be present at significantly higher levels in freeze dried crickets compared to the oven dried and blanched crickets for both the species. The volatiles produced by lipid oxidation formed during different processing methods, were in line with the secondary oxidation status of the fats expressed as the *p*-anisidine value (see section Effect of processing on fat oxidation) which was higher in the freeze dried samples compared to the oven dried and blanched samples. These findings could be explained by the fact that the Maillard reaction products, such as 2,5-dimethylpyrazine, were only found in oven dried samples, which were reported to inhibit or reduce fat oxidation [52]. Again, the porous nature of freeze dried powder could enhance its susceptibility to fat oxidation [22] and thus increased fat oxidation

volatiles. Another possible reason might be the action of the lipoxygenase (LOX) enzymes during freeze drying, which were most likely inactivated during oven drying and blanching as mentioned earlier. The latter is also supported by Kuribayashi et al. who stated that 1-octen-3-ol is formed from oxidation of PUFA by the action of lipoxygenase (LOX) enzyme [53].

Another predominant compound found is trimethylamine (TMA). TMA has a fishy odour and was found only in freeze dried samples of both insect species. TMA can be produced from trimethylamine oxide (TMAO) either by microbial reduction or by the action of the TMAOase enzyme, which is naturally present in fish [54,55]. TMA is also considered as an indicator of freshness of fish, with a rejection level of 12 mg/100 g. The presence of TMA only in freeze dried samples is consistent with the fact that the TMAOase enzyme continues its activity at freezing temperature and might be inactivated at 50 °C during oven drying and blanching [56]. Again, further investigation is required to confirm the presence and activity of TMAO and TMAOase enzymes in freeze dried *A. domesticus* and *G. assimilis*.

More organic acids were found in the oven dried samples than in freeze dried and blanched samples, except for hexanoic acid, which was higher in freeze dried samples. Organic acids found in this study are known to have much weaker aromas, except for hexanoic acid, which has a strong cheesy aroma [57]. Hexanoic acid along with 2-methylbutanoic acid and 3-methylbutanoic acid were also found in fresh frozen male giant water bugs (*Lethocerus indicus*) [58].

Cleavage of lipid hydroperoxides leads to the formation of alkanes, alkenes and ketones. Due to their relative high odour threshold, hydrocarbons are generally not considered to be essential contributors to the flavours of lipid-containing foods. On the other hand, 2-heptanone, which is produced by oxidation of saturated fatty acids, has a strong spicy, cheesy, and fruity odour note [59]. In addition, three sulphur containing compounds were detected in the processed samples which could be formed by the degradation of sulphur containing amino acids, more specifically methionine and cysteine, whereas 1-(2-thienyl)ethanone is known as a Maillard reaction product. As different compounds have different flavor

characteristics, it is expected that there will be a complex flavor mixture/combination in the processed sample, while imparting a specific flavour by a compound will depend on its odour threshold value (OTV) [60]. Further investigation could be conducted using GC-MS with olfactometric detection to find how each compound contributes to the overall flavor of the processed insects.

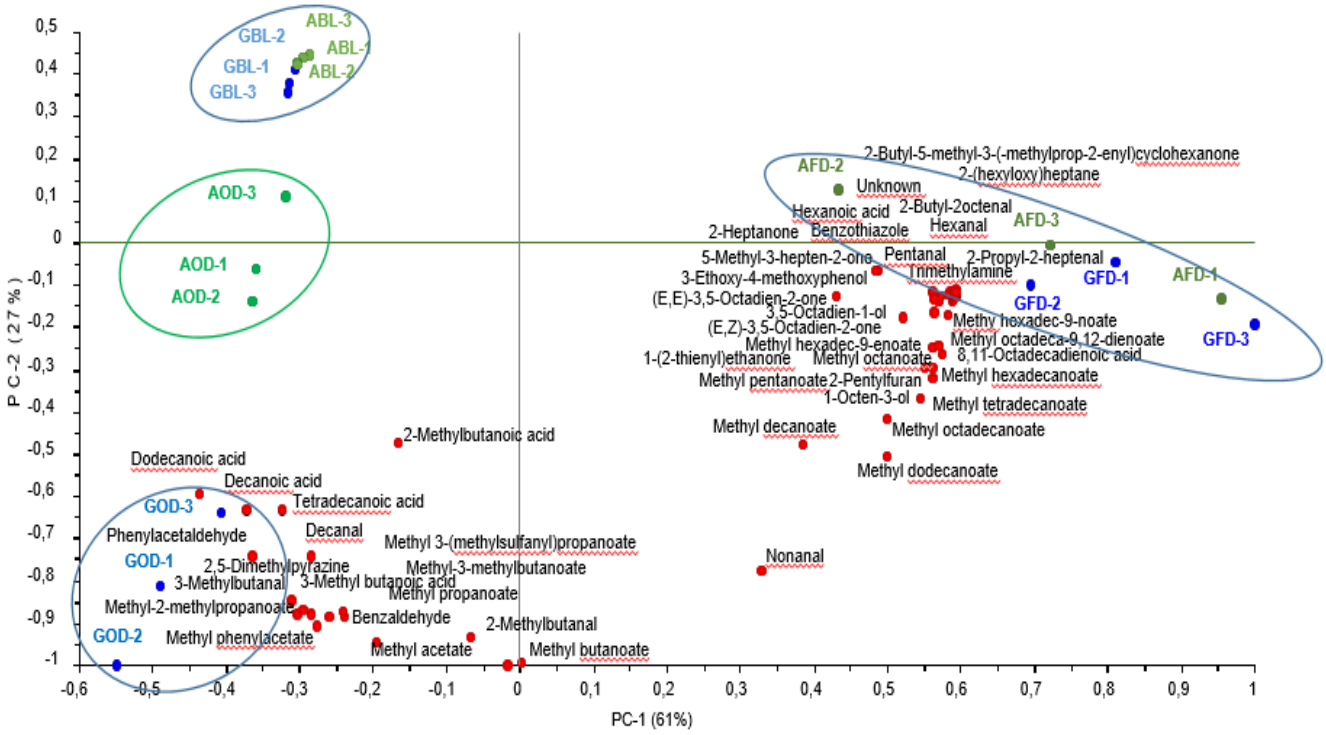


Figure 1 Bi-plot of the first two principal components. Black labels = loadings, blue labels = scores: GOD, GFD and GBL represent respectively oven dried, freeze dried and blanched *G. assimilis*, green labels = scores: AOD, AFD, and ABL represent respectively oven dried, freeze dried and blanched *A. domesticus*

To clearly visualize the relationship between *A. domesticus* and *G. assimilis* samples in terms of their processing methods and volatile components, PCA was performed (Figure 1). The bi-plot showed that the first two principal components (PC) explained 88 % of the total variability.

PC1, accounting for 61 % of the variance, discriminated between freeze dried and other processing methods. The freeze dried samples of both *A. domesticus* and *G. assimilis* were mainly characterized by high concentrations of methyl esters formed from long fatty acid chains, some aldehydes (pentanal and

hexanal), typical fatty acid oxidation components such as 2-pentylfuran, 1-octen-3-ol, 3,5-octadien-2-one and the off-flavour component trimethylamine. PC2, explaining 27 % of the variance, showed differences between blanched samples and oven dried samples.

No separation could be made between blanched samples, which were situated at the positive side of the PC2-axis. No characteristic volatiles were present in this part of the figure. At the bottom of the plot (negative axis), the oven dried samples of *G. assimilis* were located. The compounds of AOD are situated between blanched and GOD samples. This was because the volatiles present in GOD were slightly higher than that of AOD except for volatile acids (decanoic, dodecanoic and tetradecanoic acid), 2,5-dimethylpyrazine and methyl esters of short fatty acids chains. But in general the differences between both oven dried cricket species were low. Figure 1 also shows that the different flavour compounds were separated by the different processing methods rather than by cricket species.

Conclusions

Although both the *Acheta domesticus* and *Gryllus assimilis* are crickets, the impact of freeze drying, oven drying, and blanching on their physicochemical properties were not always the same. These processing methods had a limited effect on the fat, protein, ash, and chitin content of both species. Blanched and oven dried crickets had lower fat oxidation value compared to freeze dried ones for both species. Nevertheless, in all cases oxidation values were far below the acceptable limits. Vitamin B12 did not change in *A. domesticus*, while blanched *G. assimilis* contained a higher vitamin B 12 content followed by oven dried and freeze dried samples. Better colour values were observed in freeze dried samples in terms of lightness and browning index. Freeze dried samples contained a higher content of volatile compounds compared to oven dried and blanched samples for both species. Esters and aldehydes were the predominant group of compounds which could impart their characteristics flavour. Considering the aforementioned aspects, oven drying could be the advantageous processing method compared to freeze drying and blanching for both cricket species.

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Conflict of interest:

The authors declare that they don't have any conflict of interest.

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Supplementary Table 1 Amino acid of freeze-dried, oven-dried and blanched *A. domesticus* and *G. assimilis* (in g /100 g protein). Values are expressed as mean± standard deviation (n = 3). Values in the same row with different superscripts represent significant ($p < 0.05$) difference among the treatments of same species

	Acheta domesticus			*Gryllus assimilis*		
	Freeze dried	Oven dried	Blanched	Freeze dried	Oven dried	Blanched
Essential amino acids (EAA)						
Histidine	2.01± 0.03^{a}	1.63± 0.03^{b}	1.64 ± 0.06^{b}	1.85 ± 0.23^{a}	1.72 ± 0.09^{a}	1.64 ± 0.06^{a}
Lysine	6.75 ± 0.25^{a}	6.81 ± 0.26^{a}	6.78 ± 0.09^{a}	6.67 ± 0.08^{a}	6.61 ± 0.11^{a}	6.78± 0.09^{a}
Methionine	1.58 ± 0.15^{a}	1.30 ± 0.13^{a}	1.32 ± 0.08^{a}	1.39 ± 0.17^{a}	1.35 ± 0.21^{a}	1.32 ± 0.08^{a}
Valine	6.43 ± 0.15^{a}	6.54 ± 0.13^{a}	6.35 ± 0.07^{a}	6.39 ± 0.02^{a}	6.37 ± 0.09^{a}	6.35 ± 0.07^{a}

Isoleucine	4.75 ± 0.03[a]	4.49 ± 0.03[b]	4.41 ± 0.02[c]	4.56 ± 0.13[a]	4.53 ± 0.04[a]	4.41 ± 0.02[a]
Leucine	8.04 ± 0.03[a]	7.58 ± 0.11[b]	7.56 ± 0.05[b]	7.96 ± 0.10[a]	7.80 ± 0.22[a]	7.56 ± 0.05[a]
Phenylalanine	3.72 ± 0.03[a]	3.75 ± 0.04[a]	3.78 ± 0.09[a]	3.71 ± 0.09[a]	3.96 ± 0.34[a]	3.78 ± 0.09[a]
Tryptophan	0.93 ± 0.05[a]	0.94 ± 0.07[a]	0.96 ± 0.05[a]	0.87 ± 0.03[a]	0.83 ± 0.05[a]	0.96 ± 0.05[a]
Threonine	4.35 ± 0.08[a]	4.28 ± 0.08[a]	4.26 ± 0.06[a]	4.19 ± 0.06[a]	4.23 ± 0.11[a]	4.26 ± 0.06[a]
Total EAA	38.55 ± 0.13[a]	37.31 ± 0.26[b]	37.06 ±0.10[b]	37.60 ± 0.68[a]	37.40 ± 0.21[a]	38.20 ± 0.73[a]
Non-essential amino acids (NEAA)						
Arginine	8.15 ± 0.04[a]	7.36 ± 0.15[b]	7.61 ± 0.23[b]	7.62 ± 0.53[a]	7.57 ± 0.57[a]	7.61 ± 0.23[a]
Serine	5.29 ± 0.11[a]	4.56 ± 0.07[b]	4.67 ± 0.09[b]	5.33 ± 0.01[a]	5.16 ± 0.51[a]	4.67 ± 0.09[a]
Glycine	5.13 ± 0.20[b]	6.00 ± 0.22[a]	5.97 ± 0.01[a]	5.48 ± 0.12[a]	5.54 ± 0.07[a]	5.97 ± 0.01[b]
Aspartic acid	9.11 ± 0.33[a]	7.97 ± 0.22[b]	8.19 ± 0.18[b]	8.67 ± 0.28[a]	8.67 ± 0.44[a]	8.19 ± 0.18[a]
Glutamic acid	11.54 ± 0.07[b]	12.10 ± 0.30[a]	11 95 ± 0.05[ab]	11 50 ± 0.56[a]	11.97 ± 0.31[a]	11.95 ± 0.05[a]
Alanine	8.69 ± 0.09[b]	9.27 ± 0.19[a]	8.89 ± 0.22[ab]	8.92 ± 0.07[a]	8.84 ± 0.29[a]	8.89 ± 0.22[a]
Proline	7.21 ± 0.07[b]	7.90 ± 0.21[a]	7.85 ± 0.08[a]	7.80 ± 0.07[a]	7.70 ± 0.15[a]	7.85 ± 0.08[a]
Cystine	0.59 ± 0.07[b]	0.64 ± 0.05[b]	0.84 ± 0.03[a]	0.71 ± 0.04[a]	0.74 ± 0.10[a]	0.84 ± 0.03[b]
Tyrosine	5.73 ± 0.23[b]	6.89 ± 0.38[a]	7.00 ± 0.01[a]	6.36 ± 0.16[a]	6.41 ± 0.43[a]	7.00 ± 0.01[a]
Total NEAA	61.45 ± 0.13[b]	62.69 ± 0.26[a]	62.94 ± 0.10[a]	62.40 ± 0.68[a]	62.60 ± 0.21[a]	61.80 ± 0.73[a]

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694 **Supplementary Table 2** Retention time and Kovat indices of the volatile compounds present in freeze
695 dried, oven dried and blanched *Acheta domesticus* and *Gryllus assimilis*

Compounds	**Retention time (min)**	**Kovat Indices - Experiment**	**Kovat indices - Literature***
Methyl acetate	1.851	639	515[j]
Methyl propionate	2.547	659	621[f]
Methyl-2-methylpropanoate	3.323	683	684[x]
Methyl-3-methylbutanoate	5.95	762	778[l]
Methyl butanoate	4.129	707	717[f], 728[p]
Methyl pentanoate	7.744	815	821[f]
Methyl hexanoate	11.702	934	1040[a], 922[f], 924[h]
Methyl phenylacetate	20.131	1187	1178[i]
Methyl octanoate	18.534	1139	1127[h]
Methyl decanoate	24.363	1314	1325[h]
Methyl dodecanoate	29.514	1468	1525[d], 1479[u]

Methyl tetradecanoate	34.115	1606	1719[g], 1723[h], 1674[u]
Methyl hexadec-9-enoate	37.837	1718	1890[t], 1856[u]
Methyl hexadecanoate	38.343	1733	1927[d], 1924[h], 1875[u]
Methyl octadeca-9,12-dienoate	40.628	1802	2079[t]
Aldehydes			
2-Methylbutanal	3.004	673	593[a], 646[c], 662[k], 665[q]
3-Methylbutanal	2.874	669	654[a], 651[h], 649[k], 675[n]
Pentanal	3.551	690	693[k], 699[a]
Hexanal	6.809	787	799[a], 819[b], 799[h]
Nonanal	17.926	1121	1104[a], 1108[c]
2-propyl-2-heptenal	20.31	1192	
2-Butyl-2-octenal	25.697	1354	
Decanal	21.048	1214	1204[a], 1209[c], 1205[f]
Benzaldehyde	12.923	971	996[b], 962[f], 968[v], 971[w]
Phenylacetaldehyde	15.854	1059	1049[a], 1043[f], 1048[k], 1009[t], 1053[w]
Organic acids			
2-Methyl butanoic acid	10.543	899	956[b], 830[n]
3-Methyl butanoic acid	10.197	889	834[d], 810[n], 873[x]
Hexanoic acid	14.808	1027	996[h], 999[u]
Decanoic acid	25.515	1348	1375[h]
Dodecanoic acid	29.518	1469	1568[d]
Tetradecanoic acid	34.826	1628	1720[e]
8,11-Octadecadienoic acid	40.548	1799	
Ketones			
2-Heptanone	10.292	892	891[h], 888[a], 889[v], 865[a]
5-Methyl-3-hepten-2-one	14.561	1020	
(E,E)-3,5-Octadien-2-one	16.763	1086	1072[w]
(E,Z)-3,5-Octadien-2-one	17.542	1109	1096[w]
Alcohols			
1-Octen-3-ol	13.732	995	980[a], 942[b], 978[v]
3,5-Octadien-2-ol	15.755	1056	1040[q]
Sulpher compounds			
Benzothiazole	21.546	1229	1218[o], 1250[v]
Methyl 3-(methylthio)propanoate	15.216	1039	1023[f]

1-(2-Thienyl)ethanone	24.253	1311	
Others			
Trimethylamine	1.608	631	503[m]
2,5-Dimethylpyrazine	11.182	918	915[n], 911[s], 890[u]
2-Pentylfuran	14.048	1004	989[f], 969[u], 991[v], 994[w]
2-(Hexyloxy)heptane	17.291	1102	
3-Ethoxy-4-methoxyphenol	23.199	1279	
2-Butyl-5-methyl-3-(2-methylprop-2-enyl)cyclohexanone	30.495	1498	
Unknown	22.99	1273	

*= superscripts indicates the references of literature

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