Vitamin D fortification of selected edible insect species through UVB-treatment

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Abstract

Edible insects are receiving increased interest worldwide, due to being more sustainable than more established animal foods. It has also been demonstrated, that they form vitamin D_3 after treatment with UVB-light. However, only limited research about this potential fortification process has been conducted, especially regarding ready to use powdered insects.

Thus, the vitamin D_3 content of freeze-dried, powdered and UVB-treated yellow mealworm (*Tenbrio molitor*), migratory locust (*Locusta migratoria*) and two-spotted cricket (*Gryllus bimaculatus*) was investigated. Samples were analyzed via HPLC and identity of vitamin D_3 was verified via standard addition and spectrum analysis.

UVB-treated migratory locusts and two-spotted crickets did not contain quantifiable amounts of vitamin D_3. UVB-treated mealworms however showed substantial amounts of vitamin D_3 (8.95-18.24 µg/g dry matter). Thus, UVB-treatment of powdered mealworm is an effective approach to enhance its vitamin D_3 content and even modest serving sizes can supply the recommended daily intake of vitamin D.

Keywords: Edible insect, mealworm, vitamin D, UVB-treatment, HPLC analysis

1 Introduction

Edible insects are consumed by at least 2 billion people worldwide and form an integral part of their diet. While the countries where insect consumption is common are mainly found in the (sub)tropical countries of the Global South, there is a steadily increasing interest in the countries of the northern Hemisphere regarding insect consumption ((van Huis et al., 2013)van Huis et al. 2013, Tang et al. 2019, Reverberi 2020). One of the chief reasons of this increased interest in edible insects is, that their production is more sustainable compared to more established animal food sources, such as beef, pork, and even chickens, by needing considerably less feed, water and space and causing fewer CO_2 emissions (Oonincx et al. 2012; van Huis et al. 2021). Additionally, edible insects are generally a good source of important nutrients, containing high amounts of protein, iron, zinc and several B-vitamins, such as riboflavin (Rumpold & Schlüter 2013; Tang et al. 2019). In recent years, they have received some more attention, as a potential source of dietary vitamin D, due to their demonstrated ability to form said vitamin after treatment with UVB-light (Oonincx et al. 2018, van Huis et al. 2021). Edible insects contain 7-dehydrocholesterol, which they synthesize from plant sterols (camposterol, stigmasterol) obtained from their feed (Svoboda et al 1999, Cheseto et al. 2015). After contact with UVB-light (290-315 nm) 7-dehydrocholesterol undergoes a photoisomeriztation reaction, followed by a thermoisomerization reaction, resulting in the formation of vitamin D_3, one of the two main forms of vitamin D. The other main form is vitamin D_2, which is found in fungi and is formed from ergosterol, following the same reaction steps (Charoenngam et al. 2019; Jiang et al. 2020).

Vitamin D, regardless if vitamin D_2 or D_3, fulfills important functions in the calcium-phosphate-homeostasis and thus bone metabolism and has also been shown to influence the innate and adaptive immune system, as well as the pancreas, the heart and skeletal muscles. Therefore, vitamin D deficiency (below 50 nmol/L), which is increasingly seen as a worldwide problem, is a main cause for osteomalacia and rickets and has been associated with the development of infectious diseases, such as tuberculosis, and certain forms of cancer (Charoenngam et al. 2019; Amrein et al. 2020; Martens et al. 2020). Although humans, could potentially cover all

84 their needed vitamin D through natural synthesis in their skin, this option is often limited due to
85 factors like skin pigmentation, clothing habits, latitude of residence and age, necessitating a
86 certain uptake of vitamin D through dietary sources (Bonjour et al. 2018). As, only a few foods,
87 such as fatty fish and UVB-treated mushrooms are good dietary sources of vitamin D
88 (Lamberg-Allardt 2006, Jiang et al. 2020), establishing UVB-treated edible insects as a
89 potential new sustainable food source for this important vitamin seems sensible. However,
90 there is only limited research on this topic, mostly conducted on living insects reared under
91 UVB-lamps (Oonincx et al. 2018; Bah-Nelson et al. 2021; Michaels et al. 2022).
92 Studies on the vitamin D_2 enrichment of edible mushrooms have shown, that UVB-light is much
93 more effective, when treating already harvested mushrooms and to either slice or powder them
94 to increase the surface area. Additionally, the UVB-treatment of mushrooms, reported in the
95 literature can be completed within 20 minutes or less (Kristensen et al. 2012; Nölle et al. 2017;
96 Jiang et al. 2020). This is considerable shorter, than in the time frames described in the insect
97 rearing scenarios, where lamps had run for up to 24 hours (Oonincx et al. 2018, Bah-Nelson
98 et al. 2021; Michaels et al. 2022). Hence, it would probably be more practical for insect
99 producers to treat an already usable insect powder with UVB-light, than rearing insects under
100 UVB-lamps. However, there have been basically no studies in this area with only a very recent
101 position paper published by EFSA (Turck et al. 2023) on the safety of UVB-treated vitamin D
102 enriched mealworm powder.
103 Therefore, the aim of this work was to confirm the presence of vitamin D in the powder of UVB-
104 treated edible insects and subsequently to quantify the levels of vitamin D. While there are
105 reports of vitamin D_2 in edible insects (Oonincx et al. 2018) this study focused on vitamin D_3,
106 as this vitamin D form is natively synthesized by the insect itself, while the formation of vitamin
107 D_2 is more likely a sign of fungal cohabitants.

2 Materials & Methods

2.1 Sample material and UVB-light treatment

Three insect species, yellow mealworm (*Tenebrio molitor*), migratory locust (*Locusta migratoria*) and two-spotted cricket (*Gryllus bimaculatus*) were selected for the experiments. Selection of yellow mealworm and migratory locust was based on them being recently allowed for food production in the European Union (European Commission 2021, 2022), while the two-spotted cricket is a major species in the insect rearing industries of Thailand and Eastern Africa (Reverbi 2020, Tanga et al. 2021). All samples were bought from an online zoo shop based in Germany. Samples were frozen at -80°C and subsequently freeze-dried for approximately 22 h (Model: Lyoquest, Telstar, Terassa, Spain). As early grinding tests showed that the samples could contain a fair amount of fat, they were cooled again at -80°C for 3-4 h beforehand, to promote a better grinding behavior. Grinding was conducted by using a high-powered blender (Waring Commercial Inc., USA). After completing the preparation steps samples were stored at -80°C. Samples were tested for residual water by drying in an oven (Memmert GmbH & Co. KG, Schwabach, Germany) for 22 h at 105 ± 2° C, using duplicate analytical portions, each weighing about 2 ± 0.1 g. All samples were mixed with 10 g of dried sea sand, to ensure an even distribution and to avoid clumping of the sample material. For the UVB-light treatments 2.5 ± 0.1 g of ground sample material was put into individual 100x20 mm plastic petri dishes (Greiner Bio-One GmbH, Frickenhausen, Germany) and smoothed out into the thinnest possible layer. These petri dishes were then put into a UV-chamber (Opsytec Dr. Groebel GmbH, Ettlingen, Germany) and treated with UVB-light at room temperature for about 4 min until a dose of 1.5 J/cm^2 (5.75 mW/cm^2) was reached. Untreated insect powders were used as control for all experiments. Sample material was stored at -80° C prior to analyses.

2.2 Vitamin D_3-Extraction

The extraction method for vitamin D_3 was based on the extraction method by Nölle et al. (2017), with some modifications. Samples were extracted in quadruplicate analytical portions. For the first extraction step 2.0 g ± 0.005 g of both UVB-treated and untreated insect samples were transferred into 50 mL Falcon tubes and mixed with 20 mL HPLC-grade Ethanol (Carl Roth

GmbH, Karlsruhe, Germany), 5 mL 50% potassium hydroxide (Carl Roth GmbH, Karlsruhe, Germany) and 1,333 mL sodium ascorbate [1.75 g of ascorbic acid (Carl Roth GmbH, Karlsruhe, Germany) in 10 mL sodium hydroxide with a concentration of 1mol/L (Merck, Darmstadt, Germany)]. Samples were saponified overnight for 18h, while being agitated with a rolling mixer RM-810 (Sysmex Deutschland GmbH, Norderstedt, Germany). On the next day samples were mixed with 10 mL of saturated sodium chloride solution (Carl Roth GmbH, Karlsruhe, Germany) to improve phase separation. Vitamin D_3 was then extracted by adding 10 mL of n-Hexane (Carl Roth GmbH, Karlsruhe, Germany) to each sample, followed by centrifugation at 4°C for 10 min at 4020 RCF (Thermo Fisher Scientific, Waltham, USA). The n-hexane phase was transferred into a new 50 mL falcon tube and the extraction step was repeated twice. The pooled n-hexane phases were washed three times with deionized water (Merck Millipore, Darmstadt, Germany) until pH-neutrality was reached. The washed n-hexane phases were subsequently evaporated to dryness with a vacuum concentrator (Thermo Fisher Scientific, Waltham, USA) and immediately redissolved in tetrahydrofuran (Carl Roth GmbH, Karlsruhe, Germany). To remove potential solid impurities samples were filtered through a syringe filter (Membrane Solutions, Auburn, USA) prior to analysis.

2.3 HPLC-analysis

Vitamin D_3 analysis was conducted using a HPLC-system consisting of a DGU-20A3R degassing Unit, two LC-20AT pumps, a SIL- 20ACHT auto sampler and a CBM-20A communication module (Shimadzu GmbH, Duisburg, Germany). The column was a Reprosil 80 ODS-2, analytical column, 250 x 4.6 mm, 3 µm particle size (Dr. A. Maisch HPLC GmbH, Ammerbuch-Entringen, Germany) One pump carried an eluent composed of 770 mL acetonitrile (Carl Roth GmbH, Karlsruhe, Germany), 140 mL deionized water (Merck Millipore, Darmstadt, Germany) and 90 mL tetrahydrofuran (Carl Roth GmbH, Karlsruhe, Germany) per L (A). The other pump carried pure tetrahydrofuran (B). During the first 10 min the ratio between (A) and (B) was 80:20, followed by a rapid increase of (B) during minutes 10 and 11 min until 100% (B) was reached. This ratio was held for 3 minutes to clean the column from potential interfering substances. During minute 14 to 15 the ratio between (A) and (B) decreased to the

former 80:20 and was held there for 5 min. to equilibrate the HPLC system for the following analysis. The flow rate during this total analysis time of 20 minutes was 2 mL/minute. The injection volume was 10 µL and the UV-signal was registered at 265 nm, with a frequency of 12.5 Hz. Vitamin D_3 detection limit was 0.5 µg/mL. To verify the identity of vitamin D_3 in the samples the UV/VIS absorption spectrum of the vitamin D_3 standard solution was compared with the spectra of the substances found within the extracted samples. To ensure unambiguous identification, it was closely observed that the maximum of the substance's absorption spectrum did not deviate by more than ± 2 nm from the maximum of the absorption spectrum of the standard (Yılmaz et al. 2003). Additionally, 100 µL of a 200 µg/mL standard solution of vitamin D_3 (Enzo Life Sciences GmbH, Lörrach, Germany) were added to 100 µL aliquots of extracted sample. This mixture was measured via HPLC, under the aforementioned conditions. After the measurement, the sample with added standard addition was compared to a sample without added standard. If the peak height or peak area was increased by the standard addition, it can be assumed that this peak is the sought substance. For quantification of vitamin D_3 an external standard curve consisting of six calibration standards with concentrations of 5, 20, 40, 60, 80 and 100 µg/mL was used. The calibration standards were prepared from the dilution of a vitamin D_3 stock solution with a concentration of 5 mg/mL (Enzo Life Sciences GmbH, Lörrach, Germany). Vitamin D_3 contents were calculated to µg/g dry matter (d.m.). The LabSolutions software (Shimadzu GmbH, Duisburg, Germany) was used for instrument control, data acquisition and analysis.

3 Results & discussion

3.1 Verification of vitamin D_3 generation through UVB-treatment

Chromatograms of untreated mealworms samples showed no discernible or only miniscule peaks at the time point (6.5 ± 0.3 min) where the vitamin D_3 standard solution eluted, when measuring a vitamin D_3 standard solution, while chromatograms of both two-spotted crickets and migratory locusts already showed clear peaks (Figure 1 & 2). However, regardless of the peaks' respective sizes all were quickly determined not to be vitamin D_3 via spectrum analysis.

These findings are largely in accordance with published literature, where levels of vitamin D_3 are either below the level of detection or where samples were found to contain only small amounts (0.003-0.19 µg/g dry matter) of vitamin D_3, presumably due to some exposure of insect samples to sunlight before analysis (Finke 2002 & 2015, Oonincx et al. 2010, Mlček et al. 2019). In contrast, chromatograms of UVB-treated mealworm samples clearly showed the formation of large peaks at this time point, indicating that at least some kind of new substance formed after the UVB-treatment. Since the standard addition of vitamin D_3, resulted in a marked increase in the area under the curve of the potential vitamin D_3 peaks giving a first confirmation, that the newly formed substance was in fact vitamin D_3 (Figure 1). The spectrum analysis of the respective peaks further verified this substance as vitamin D_3, as the spectrum of the potential vitamin D_3 peaks corresponded with the spectrum of vitamin D_3 (Figure 3). These findings are in accordance with the position paper published by EFSA (Turck et al. 2023), that also reports vitamin D_3 formation in UVB-treated powdered mealworms.

On the other hand, while the UVB-treatment two-spotted crickets and migratory locusts also resulted in the formation of new peaks with similar retention times as vitamin D_3 these were considerably smaller and less separated, compared to UVB-treated mealworm samples (Figure 2). In addition, the absorption spectra of these newly formed peaks deviated strongly from the spectrum of vitamin D_3 (Figure 3). Therefore, confirming tests via standard addition of vitamin D_3 were suspended and both species were excluded from further quantification analyses. The findings of this study concerning two-spotted crickets are supported by recent studies (Bah-Nelson et al. 2021, Michaels et al. 2022), who also did not find that UVB-treatment of this insect leads to any detectable formation of vitamin D_3. A potential reason for this finding is the melanin-rich pigmentation of these insects, which might shield them from UV-light (Bah-Nelson et al. 2021). Although the samples in this study were ground prior to UVB-treatment, the resulting powder was still relatively dark, thus a shielding effect by melanin rich pigments might have still occurred. It is also possible that in all three studies vitamin D_3 was produced, but in such miniscule amounts, that it could not be detected by the respective analysis methods. As the photobiology of vitamin D_3 in insects is still an understudied field it could also

be possible that two-spotted crickets are simply not able to produce vitamin D_3 after UVB-treatment. Whatever the underlying cause, two-spotted crickets do not seem to be promising candidates for vitamin D enrichment trials. In contrast to our findings on migratory locusts, Ooninxc et al. (2018) were able to detect levels of approximately 0.019 µg D_3/g dry matter in UVB-treated migratory locusts in their experiments (data was converted from fresh weight by following the authors' assumption of 30% dry matter in migratory locusts). There are several explanations for this discrepancy. Since the vitamin D_3 contents found by Ooninxc et al. (2018) are relatively low, similar to what can be found in untreated insects, it is again possible that the migratory locusts in this study formed similar amounts of vitamin D_3, which were below the limit of detection of the used analysis method. It is also possible that these small amounts were obfuscated by the substances already present in the migratory locusts, which showed similar retention times but widely different UV/Vis spectra compared to vitamin D_3. Another reason might be potential differences in the sterol composition of the untreated migratory locusts used in the respective studies. Since insects can produce 7-dehydrocholesterol but are not able to synthesize the precursor sterols (Svoboda 1999), the migratory locusts used by Oonincx et al. (2018) might have had a higher uptake of these sterols (camposterol, stigmasterol) due to their feed, compared to the migratory locusts used in this study and were hence able to produce vitamin D_3. Also, Oonincx et al. (2018) raised their migratory locusts under UV-lamps from nymph stage to adulthood, while they migratory locusts used in this study were already purchased as adult animals. As 7-dehydrocholesterol is used primarily for the synthesis of ecdysteroids, which are needed for proper insect development (Svoboda 1999), there is a possibility that in certain species, subadult insects can synthesize 7-dehydrocholesterol and are losing this ability as adults, thus hindering vitamin D_3 production. However, pretests conducted at our department, comparing UVB-treated nymph migratory locusts and adult specimen, showed no differences between the two developmental stages but confirmed our finding of no detectable vitamin D_3 in this insect species (data not shown). Apart from vitamin D_3 UVB-treatment of 7-dehydrocholesterol also produces several other photo-products such as lumisterol, suprasterol I and II, and 5,6-transvitamin D_3 (Volmer et al. 2015). Oonincx et al.

(2018) used UPLC/MS measurements for the quantification of vitamin D_3. While liquid chromatography coupled with mass spectrometry is widely considered the gold standard of vitamin D quantification, there is a certain risk of isobaric interference of these isomers with both vitamin D_3 and vitamin D_2, due to having comparable mass spectra, leading to a distortion of the peak and to either misidentification of these substances as vitamin D or an overestimation of the quantified vitamin D content (Volmer et al. 2015, Sommer et al. 2022). Thus, further studies, ideally ones comparing mass spectrometric methods with methods producing UV/Vis spectra, need to be conducted in the future.

3.2. Quantification of vitamin D_3 in mealworms

In accordance with the findings presented and discussed in the previous chapter none of the untreated mealworm samples showed quantifiable levels of vitamin D_3, while the UVB-treated mealworm powder showed varying vitamin D_3 contents ranging from 8.95 to 18.94 µg/g dry matter with a mean value of 14.27± 2.88 µg/g dry matter. These differences in vitamin D_3 content can be explained by differing levels of 7-dehydrocholesterol in the mealworm powder, as mealworms from different harvesting cycles were extracted during the course of the study. Another reason could be a certain inhomogeneity of the ground mealworm powder, which still contained small pieces of less ground mealworm and might have caused a non-uniform extraction process. In future studies samples will be sieved prior to analysis to ensure homogenous analytical portions. Regardless of these observed differences the amounts of vitamin D_3 found in this study are on average 55 times higher than the vitamin D_3 contents of UVB-treated mealworms analyzed Ooninxc et al. (2018). There are several factors, that might explain this difference. Firstly, the mealworms used in this study were already powdered which leads to a considerable increase in surface area, which facilitates the sample's penetration with UVB-light (Nölle et al. 2017). Secondly UV-lamps used by Ooninxc et al. (2018) mimic natural sun-light, which consists mainly of UVA-light and is therefore less effective in generating vitamin D, than the pure UVB-light used in this study (Jasinghe & Perera 2006). Furthermore, the UVB-irradiance in our study was much higher (5.75 mW/cm^2) than the highest UVB-irradiance (90 µW/cm2) reported by Ooninxc et al. (2018). Interestingly the mean

amounts of vitamin D_3 found in this study are also about 25 times higher than the vitamin D_3 contents that are reported by EFSA (Turck et al. 2023) although their data is also based on UVB-treated mealworm powder. This could again be due to differences in the UV-treatment method, yet the position paper does only state that the mealworm powder is treated with UV-light without any information on the used UV-source, the duration of the treatment or the target doses, allowing only to speculate that this is indeed the responsible factor. Further explanations for the differences between the findings of this study and both literature sources are again potential variations in the sterol composition of the analyzed mealworms due to different feeding, or differences in the analytical methods used to quantify vitamin D_3. With a recommended daily vitamin D intake of 20 µg or 800 IU, amounts 1.1-2.5 g of UVB-treated mealworm powder would be sufficient to cover the recommended daily intake of vitamin D, making UVB-treated vitamin D_3 enriched mealworms into a highly effective vehicle to reduce the prevalence of vitamin D deficiency (Charoenngam et al. 2019; Amrein et al. 2020). Yet, acceptance to consume mealworms as a food is rather limited in Western countries (Caparros Megido et al. 2016) and substantially limits their great potential in delivering vitamin D to consumers. Given the low amounts of mealworm powder necessary to provide the daily needed doses of vitamin D UVB-treated mealworm powder could be incorporated as a fortifying ingredient in familiar foods, such as bread or noodles or burger patties, potentially increasing consumer acceptance by masking the unfamiliar insect (van Huis et al. 2013; Caparros Megido et al. 2016). While no animal or human studies on the bioavailability of vitamin D_3 from edible insects could be found in the literature, a recent study by Morand-Laffargue et al. (2023) demonstrated that the also fat-soluble provitamin β-carotene from black soldier fly larvae, is bioavailable to gerbils. Furthermore, studies on other animal-based foods, such as vitamin D_3 fortified cow's milk and yogurt and a study on UVB-treated Vitamin D_2 enriched button mushrooms, all demonstrated a good bioavailability of the respective vitamin D form in humans (Urbain et al. 2011; Bonjour et al. 2018; Kruger et al. 2019). As consuming >8 g of UVB-treated mealworm powder would already exceed the upper limit for exogenous vitamin D intake of 100 µg/day it is commendable that EFSA sets the allowed amount of UVB-

treated Vitamin D enriched mealworms at amounts between 1-4 g/100 g of food (Turck et al. 2023). Still, a hypervitaminosis with toxic side effects is rare in healthy individuals and is almost exclusively caused by overdosing vitamin D supplements for several months (Amrein et al. 2020; Marcinowska-Suchowierska et al. 2020).

4 Conclusion

To our knowledge this is first study ever to investigate the effects of a UVB-treatment on the Vitamin D_3 content of ready to use edible insect powders. While, no quantifiable amounts of vitamin D_3 could be detected in both migratory locusts and two- spotted crickets, the results of this study prove that UVB-treatment of ready to use mealworm powder leads to the de novo formation of substantial, but variable amounts vitamin D_3 (8.95-18.94 µg/g dry matter) in the sample material, which could nonetheless greatly contribute to the recommended daily intake of vitamin D_3. Since the number of foods, that are rich in any form of vitamin D is comparatively small (Lamberg-Allardt 2006) this is a rather valuable finding. Future studies should be conducted in this area to investigate the potential vitamin D_3 enrichment of more edible insect species. Potential candidates could be house crickets (*Acheta domesticus*) or lesser mealworms (*Alphitobius diaperinus*). Factors, such as the currently limited acceptance of insect-based foods or a low but certain risk of hypervitaminosis, might be a hurdle in the dissemination of UVB-treated insect powders. Provided that the vitamin D_3 content of enriched insects is closely monitored and the use of these powders, is mainly focused on fortification, treating edible insect powders with UVB-light shows high potential for the future development of safe vitamin D rich food products.

Authors' contribution

Nils Nölle: Conceptualization, Methodology, Writing - Original draft **Aranya Hörnstein:** Investigation, Methodology, Writing- Review & Editing **Christine Lambert:** Supervision, Writing – Review & Editing

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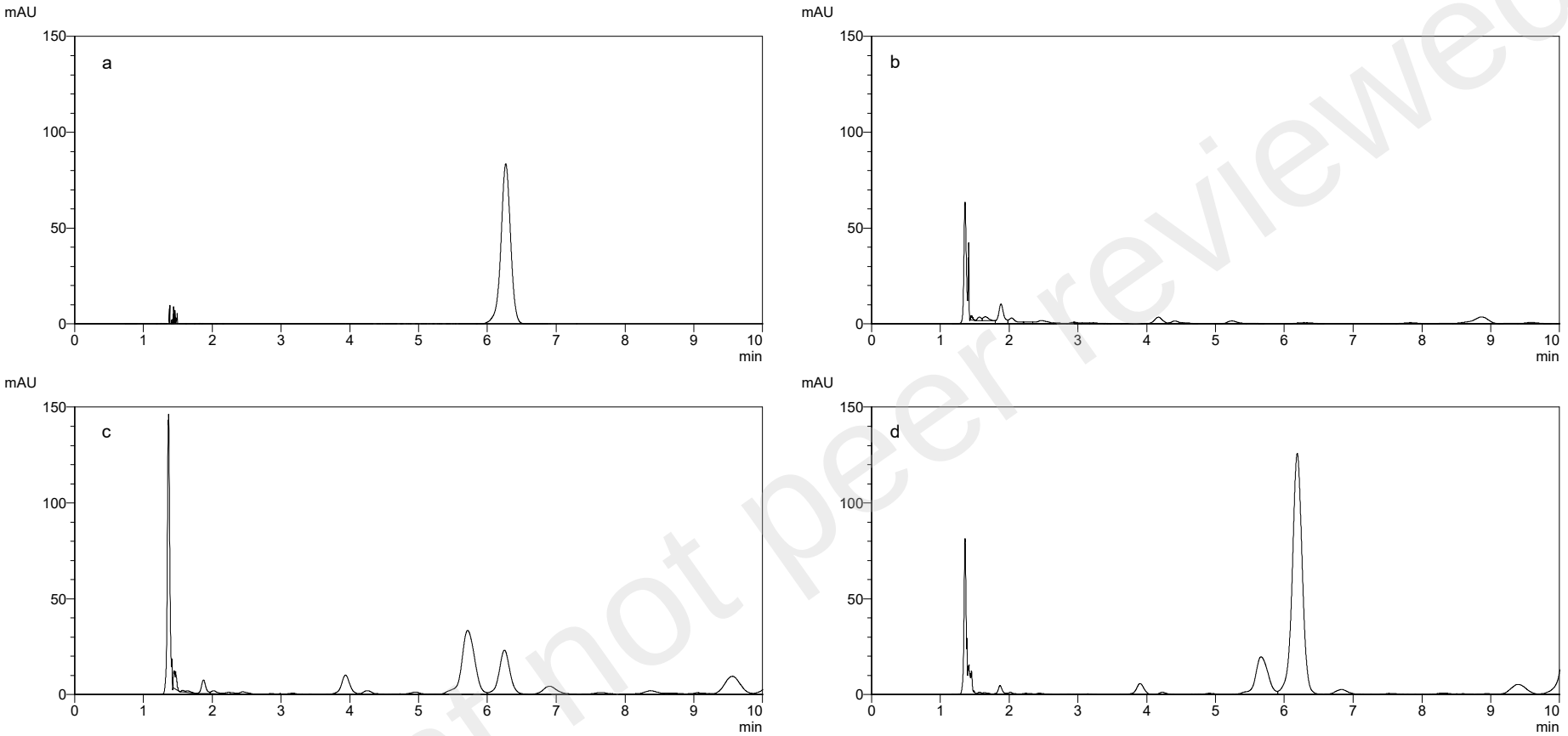

Figure 1: HPLC chromatograms of a 80 µg/mL vitamin D3 standard solution (a) untreated mealworm powder (b), UVB-treated mealworm powder (c) and UVB-treated mealworm powder with added vitamin D_3 standard solution (200 µg/mL) (d). All samples were freeze-dried prior to analysis. UVB-treated powders were obtained by subjecting sample material to artificial UVB-light at room temperature for about 4 min. until a dose of 1.5 J/cm^2 (5.75 mW/cm^2) was reached. Untreated mealworm powder shows no discernible peaks at the time point (6.5 ± 0.3 min) where the vitamin D_3 standard solution elutes. UVB-treated mealworm powder shows the formation of a large peak at this time point. The standard addition of vitamin D_3, results in a marked increase in the area under the curve of the potential vitamin D_3 peak strongly indicating, that this newly formed substance is in fact vitamin D_3.

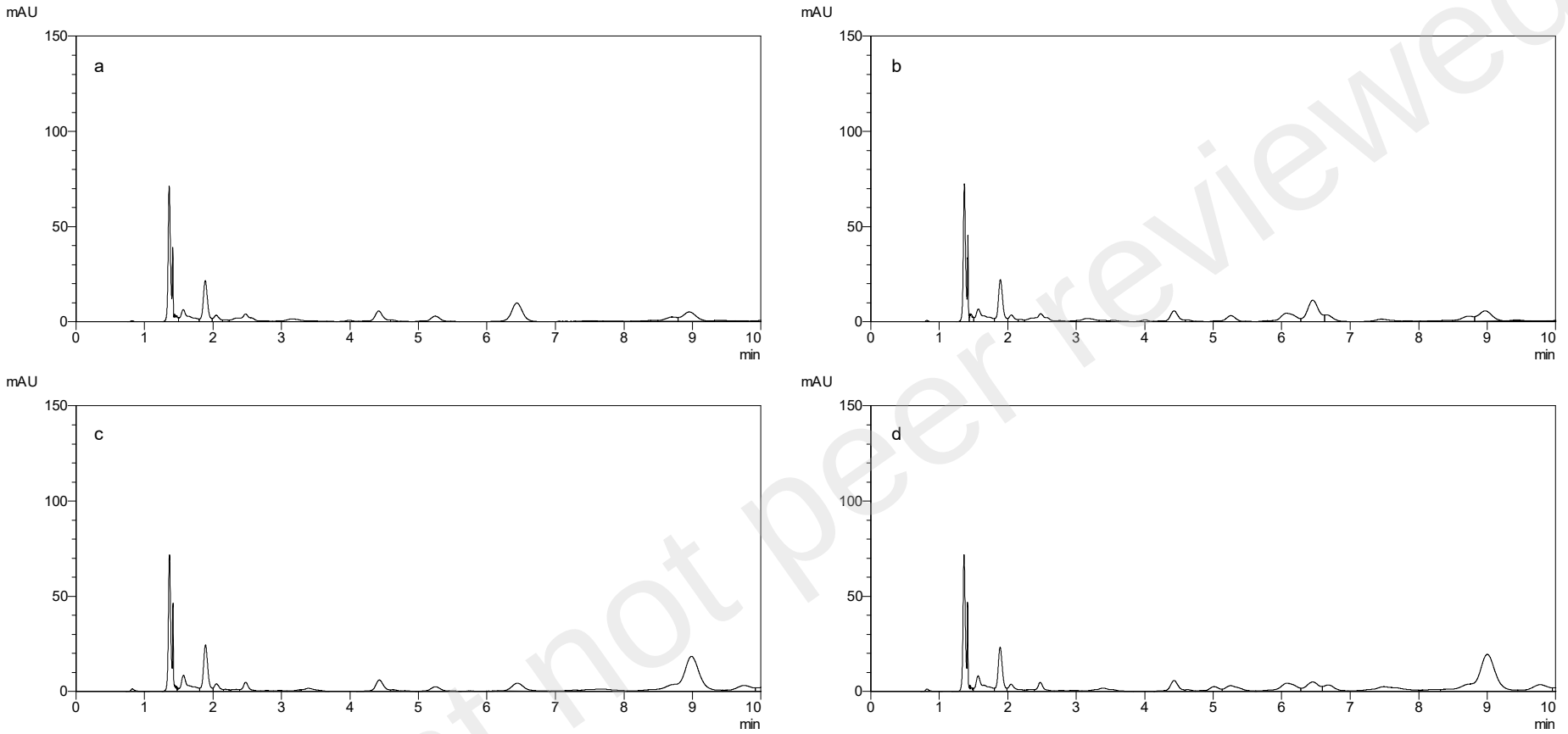

Figure 2: HPLC chromatograms of untreated migratory locust powder (a), UVB-treated migratory locust powder (b) untreated two-spotted cricket powder (c) and UVB-treated two-spotted cricket powder (d). All samples were freeze-dried prior to analysis. UVB-treated powders were obtained by subjecting sample material to artificial UVB-light at room temperature for 260.84 ± 4.63 s until a dose of 1.5 J/cm^2 (5.75 mW/cm^2) was reached. Untreated powders of both two-spotted crickets and migratory locusts showed discernible peaks at the time point (6.5 ± 0.3 min) where the vitamin D_3 standard solution elutes. UVB-treatment of two-spotted crickets and migratory locust powder results in the formation of new peaks close to the retention times of vitamin D_3.

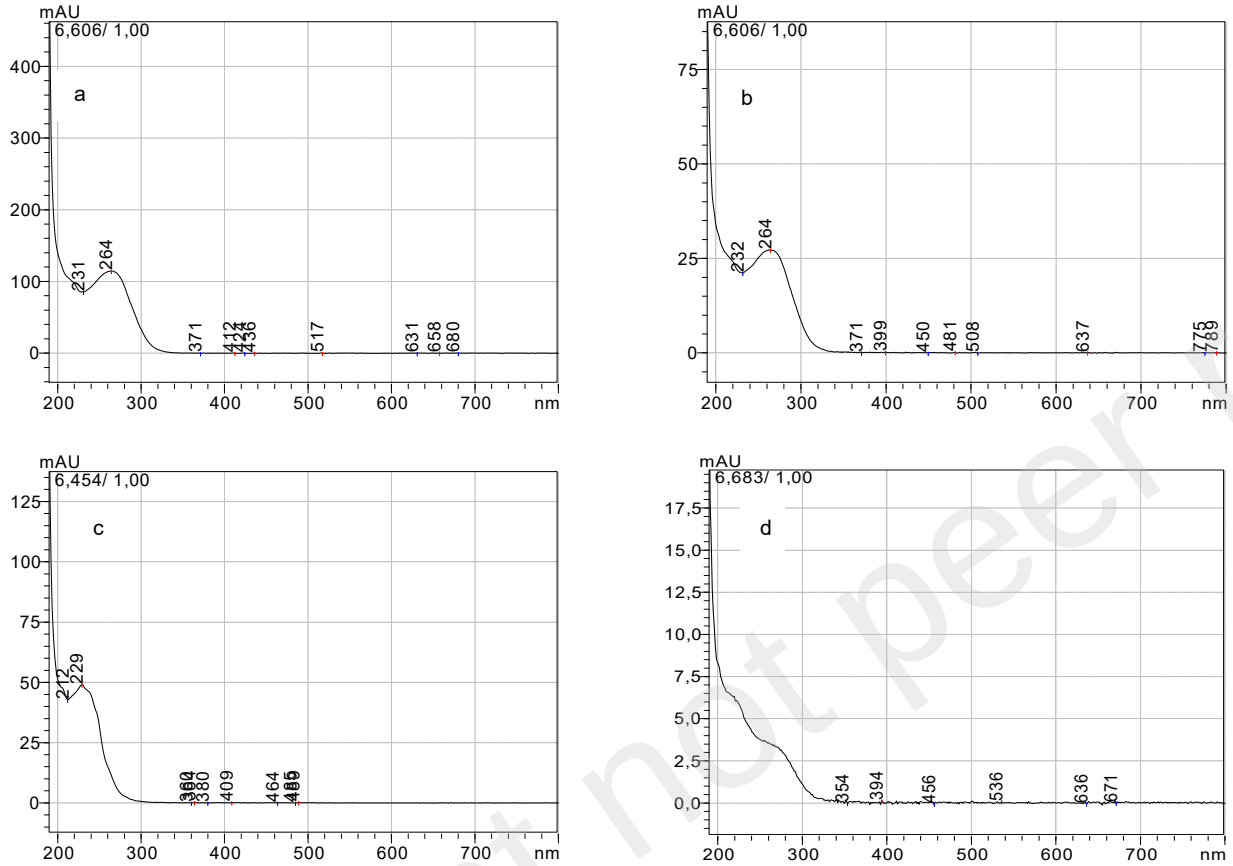

Figure 3: Exemplary UV-Vis spectra of a vitamin D_3 standard solution (80 µg/mL) (a), and UV-treated mealworm (b), migratory locust (c), and two-spotted cricket (d) samples. Spectra were taken from peaks eluting at 6.5 ± 0.3 min., the time frame were vitamin D_3 eluted under the used chromatographic conditions. As the spectrum obtained from UVB-treated mealworm powder corresponds with to the spectrum of the vitamin D_3 standard solution, the formation of vitamin D_3 in UVB-treated mealworm powder is confirmed. However, the spectra of both two-spotted crickets and migratory locusts, deviate strongly from the spectrum of the vitamin D_3 standard, indicating that no discernible amount of vitamin D_3 was formed after UVB-treatment.