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Short communication

Direct identification of edible insects by MALDI-TOF mass spectrometry



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ABSTRACT

The consumption of edible insects (entomophagy) will gain greater significance facing the increasing global population, which is suggested to reach 9 billion people in 2050 (FAO., 2009). Due to their high amount of proteins, fatty acids, vitamins, and minerals insects represent a valuable source of essential nutrients.

While the consumption of insects is very common in many countries of Africa and Asia, there is a far smaller acceptance for entomophagy in Western cultures. Though, products such as noodles or burger paddies made from insect meal have a better compliance and can already be purchased in some countries of the European Union. This processing step however involves the risk of adulteration, because there is no more possibility to authenticate the insects once they are ground.

The aim of this study was to investigate whether edible insects could be measured and distinguished by MALDI-TOF MS (matrix-assisted laser desorption ionization-time of flight mass spectrometry). Therefore, different kinds of edible insects (buffalo worms, mealworms, crickets and grasshoppers) were purchased via online shops and ground subsequently. The insect powder was extracted by vigorously shaking in diluted formic acid and measured by MALDI-TOF MS. The measurement provided reproducible as well as specific mass spectra and enabled a precise differentiation of the different species.

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1. Introduction

By 2050, nine billion people are estimated to live on our planet. Subsequently, food security and the supply with high-quality protein sources and essential nutrients is a global challenge (FAO., 2009; United Nations, 2015). Insects contain significant amounts of essential fatty acids, minerals, vitamins and a high percentage of protein (Finke, 2002). According to the FAO they will play an important role as a future protein source in human nutrition (FAO., 2009). While people in Africa and Asia are accustomed to eat insects, they are rarely used in the diet of Western countries. Despite that, the interest in insects as a potential protein source is growing. Production is easy and fast, only little of space is needed and a great variety of raw materials could be used as feed. Moreover, the conversion rate of feed into insect biomass is very efficient

(Premalatha, Abbasi, Abbasi, & Abbasi, 2011).

Nevertheless, not all insects are edible; some can contain venoms (Raimon, 1963). Very little research has been done until now to assess the potential carry-over of pesticides, mycotoxins and plant toxins by insects into the food chain. Insects can also carry potentially pathogenic bacteria in or upon themselves and therefore may represent a serious health hazard for humans (Klunder, Wolkers-Rooijackers, Korpela, & Nout, 2012; Schlüter et al., 2016; Van Huis et al., 2013). In addition, it is discussed whether insects can trigger allergenic reactions in humans, similar to reactions caused by crustaceans (Belluco et al., 2013; FAO., 2010).

Eating entire insects would hardly be accepted as potential human food in Europe. Alternatively, whole insect meal, defined fractions or protein extracts might be used as or could be added to food. Methods are needed for identifying these components in order to verify correct declarations and to prevent a possible misuse. Currently available publications dedicated to the identification of insects as well as relevant insect species are summarized in Table 1. To our knowledge, there is currently no protein extraction protocol available for identifying whole edible insects.

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Table 1 Publications on the analysis of insects by MALDI-TOF MS.

Insects	Mass range	Reference
Drosophila melanogaster	2–40 kDa	Campbell (2005)
Mantophasmatodea	0.8–3 kDa	Predel, Roth, Neupert, and Picker (2005)
Aphid spp.	3–25 kDa	Perera, Vargas, and Jones (2005)
Drosophila spp.	1.8–15 kDa	Feltens, Görner, Kalkhof, Gröger-Arndt, and von Bergen (2010)
Culicoides spp.	2–30 kDa	Kaufmann et al. (2011)
Culicoides spp.	2–20 kDa	Steinmann, Pfluger, Schaffner, Mathis, and Kaufmann (2013)
Glossina spp.	2–20 kDa	Hoppenheit et al. (2013)
Phlebotomus spp.	2–25 kDa	Dvorak et al. (2014)
Flea species	2–20 kDa	Yssouf et al. (2014b)
Mosquito species	2–20 kDa	Yssouf et al. (2014a)
Culicoides spp.	2—20 kDa	Samboua et al. (2015)

For these reasons, we conducted a study to develop a MALDI-TOF mass spectrometry-based method for the detection of insect-specific proteins. The protein extraction was applied to whole ground insects that are available at the European market for human consumption, i.e. crickets, grasshoppers, mealworms, and buffalo worms. This method can be easily applied in routine laboratories using automatic measurement and identification tools.

2. Materials and methods

2.1. Chemicals and laboratory equipment

Glass beads (SiLiBeads, 1.0-1.2 mm) were obtained from VWR

(Darmstadt, Germany). CHCA (α-cyano-4-hydroxycinnamic acid), aqua dest. and acetone were purchased from Fluka (Dagebüll, Germany), trifluoracetic acid (TFA) and formic acid were obtained from Merck (Hamburg, Germany), and ethanol p.a. was bought from VWR (Darmstadt, Germany).

The matrix for MALDI-TOF MS was prepared with the following ingredients: 14 mg CHCA solved in 1 ml organic solvent (500 μ l acetone, 475 μ l aqua dest., 25 μ l TFA).

2.2. Insect samples

All analyzed insects were labeled as suited for human consumption. The following freeze-dried and edible insects were

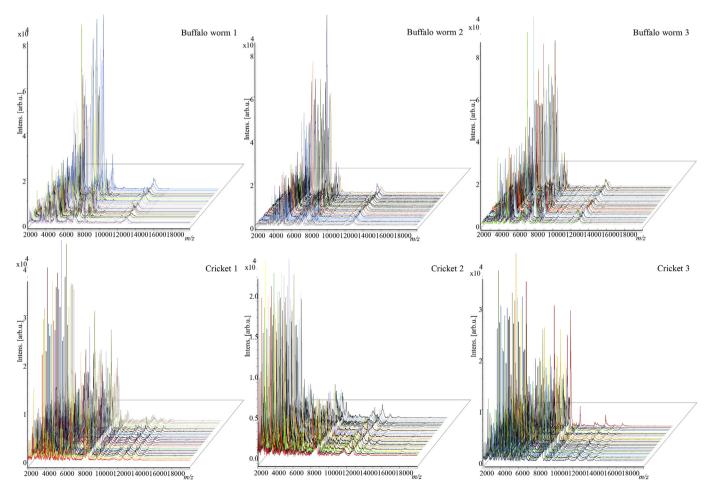


Fig. 1. Spectra of edible insects used for reference spectra (MSP) creation, buffalo worms (*Alphitobius diapernius*, n=3), crickets (*Acheta domesticus*, n=3), grasshoppers (*Locusta migratoria*, n=2) and mealworm (*Tenebrio molitor*, n=3).

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