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# Cold atmospheric pressure plasma processing of insect flour from *Tenebrio molitor*: Impact on microbial load and quality attributes in comparison to dry heat treatment

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ABSTRACT

In this study, the applicability of semi-direct cold atmospheric pressure plasma (CAPP) during postharvest processing of *Tenebrio molitor* flour is investigated. Besides analyzing the decontamination efficacy, plasma-induced impact on techno-functionality, protein solubility, composition and structure was determined and compared to heat induced effects.

Following CAPP treatment, the total microbial load of the *Tenebrio* flour of 7.72 $\log_{10}$ cfu/g was reduced to 7.10 (1 min), 6.72 (2.5 min), 5.79 (5 min), 5.19 (7.5 min), 5.21 (10 min) and 4.73 (15 min) $\log_{10}$ cfu/g. With increasing exposure to CAPP, protein solubility at pH 4 almost linearly decreased to a minimum of 54%. Water binding capacity decreased from 0.79 to 0.64 $g_{water}/g$ whereas oil binding capacity increased from 0.59 to 0.66 $g_{oil}/g$. Gel electrophoresis revealed a decrease of all protein fractions at pH 4 whereas at pH 10 the band pattern significantly shifted to protein fractions with higher molecular weights.

*Industrial relevance:* Edible insects are rich in valuable protein, fat, fibre, minerals and micronutrients. Although a wide range of species represent a valuable alternative protein source that could contribute to food and feed security, they are industrially hardly exploited. The tailored application of proper processing technologies could lead to novel insect-based high-protein food and feed products with unique functional properties supporting the increase in acceptability among potential consumers. Current research concentrates on developing processing chains including innovative nonthermal approaches. Cold atmospheric pressure plasma (CAPP) has gained attention as an effective technology for the decontamination and modification of fresh and dry agricultural products. In the postharvest chain of edible insects, the application of CAPP could contribute to the development of safe and high-quality insect-based products in the food and feed sector.



## 1. Introduction

The expanding population is expected to grow to >9.7 billion by 2050 according to the United Nations (United Nations, 2015). As the demand for food will increase dramatically within the forthcoming decades, alternative and sustainable sources of highly nutritious and sustainable food in combination with innovative processing techniques are required.

Edible insects are highly nutritious and can contain high amounts of fat, protein, vitamin, fibre and minerals, thus representing an interesting to date underexploited food source. Existing and new processing pathways need to be adapted or developed in order to produce insect-based safe food ingredients of a high quality, which could be incorporated into numerous consumer items, such as meat substitutes and protein-fortified dry products, including cereals, bars, and snack foods. Whole edible insects represent a traditional food in many parts of the world and are eaten by approx. 2 billion people worldwide (van Huis, 2013). Besides the foreseen development of an effective insect fractionation into a protein, a fat and a chitin rich fraction (Bußler, Rumpold, Jander, Rawel, & Schlüter, 2016), the production of safe and readily processable insect-based intermediates and products represents a wise strategical step towards the industrial use of insects in the food and feed sector. Therefore, it is important to use sustainable processing technologies and handling along the food chain. For an economic and safe industrial mass production of edible insects excessive research is required regarding cost-effective rearing methods and post-harvest processing technologies including the development of effective decontamination, modification and storage procedures (Rumpold & Schlüter, 2013).

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In recent years, significant research effort has been focused on developing and evaluating a multitude of novel nonthermal food technologies with the goal to avoid undesirable effects generated when conventional thermal processes are applied to food matrices such as loss in vitamins and "freshness", undesirable changes in color, texture and taste and protein denaturation. Thereby the research work is primarily motivated by consumer demands for high quality and minimally processed food, whilst ensuring microbiological and chemical safety. In the field of insect processing, too, the application of nonthermal technologies could offer enormous benefits compared to using conventional traditional procedures.

Cold atmospheric pressure plasma (CAPP) also qualifies as a new discipline in food processing. As the CAPP technology was found to be capable of effectively inactivating a wide range of microorganisms including spores and viruses (Baier et al., 2014; Birmingham, 2004; Surowsky, Fröhling, Gottschalk, Schlüter, & Knorr, 2014) it has been considered as an emerging nonthermal technology for the improvement of food safety. Although CAPP offers a promising technology in the different fields of food and feed processing, presently, the only commercial application of CAPP technology in food industries is limited to polymer processing used for food packaging applications (Pankaj, Bueno-Ferrer, Misra, Milosavljević, O'Donnell, Bourke, et al., 2014). It is well-known that CAPP also modifies the structure of materials in the micro- to nanometer range (Attri & Choi, 2013; Pankaj, Bueno-Ferrer, Misra, Milosavljević, O'Donnell, Bourke, et al., 2014; Pankaj et al., 2014b) and researchers found that, similar to the plasma application in material science, CAPP is capable of modifying wet and dry surfaces of agricultural and food products (Grzegorzewski, Rohn, Kroh, Geyer, & Schlüter, 2010; Khanal, Knoche, Bußler, & Schlüter, 2014). Up to now, the unique feature is only used in the non-food sector. Using and transferring knowledge from those research fields and industrial branches to food science and technology may offer an innovative approach for the targeted surface modification und functionalization of powdery and bulky food and feed materials.

Main objective of this study was to investigate the possible use of the CAPP technology for the decontamination and modification of flour produced from mealworms (*Tenebrio molitor*) and to compare the effects obtained with those induced by a traditional thermal treatment. For this purpose a dielectric barrier discharge (DBD) setup with air as the working gas was used as it is applicable for the treatment of larger goods, especially in solid dry powder or granular form. Operation in air reduces the costs when compared to the use of noble gases and the DBD system is a promising choice in order to adopt the CAPP technology for food industry. Besides investigating the plasma and heat induced inactivation of the native microorganism flora, special focus was set on monitoring the process-specific impact on quality, techno-functional and protein properties which will very likely provide specific application possibilities of insect-based intermediates and products. Furthermore, interest was also directed towards analyzing the contained *Tenebrio* proteins in order to gain deeper knowledge of plasma-induced changes in protein solubility, structure and composition which may provide a base for the targeted use of the CAPP technology as a tool for functionalization and modification of insect-based products.

## 2. Material and methods

### 2.1. Sample preparation

In this study, high-protein insect flour produced from *T. molitor* served as test material. Mealworm larvae were purchased from a local breeder (Futtertier-Shop.de, Eisenhüttenstadt, Germany), separated from frass by sieving, then packaged in freezer bags, subsequently inactivated by freezing and stored at −20 °C. *Tenebrio* flour was produced by pureeing frozen larvae with distilled water (1:1 *w*/w) at 4 °C, subsequent freezing at −20 °C, freeze drying (Christ Alpha 1-4, Christ Gefriertrocknungsanlagen, Osterode, Germany) and fine grinding in a coffee mill (Clatronic KSW 3307, Clatronic International GmbH, Kempen, Germany).

### 2.2. Cold atmospheric pressure plasma treatment

For semi-direct CAPP treatment of the insect flour, a surface dielectric-barrier air-discharge (SDBD) system was used. The setup is described in detail elsewhere (Bußler et al., 2015; Bußler, Steins, Ehlbeck & Schlüter, 2015). CAPP was generated by applying a sinusoidal voltage of 8.8 $kV_{PP}$ at a frequency of 3.0 kHz using air as working gas. CAPP treatment of 4.75 g of *Tenebrio* flour was conducted in a Petri dish, which was fixed on a shaker at a distance of 12 mm below the plasma source. Thin layers of *Tenebrio* flour were evenly spread over the base area (50.3 $cm^2$) of the Petri dish in order to ensure homogeneity of treatments. Samples were agitated continuously (350 rpm) on the rotary shaker during exposure to CAPP for up to 15 min. The sample temperature during CAAP treatment was measured according to Bußler et al. (2015). Thermal load of the flour did not exceed 67 °C for the selected plasma application.

### 2.3. Thermal treatment

Thermal treatment of the *Tenebrio* flour was carried out in a drying cabinet by applying temperatures of 20, 40, 60, 80, 100, 120 and 140 °C. Glass Petri dishes (base area 50.3 $cm^2$) were preheated to the respective temperature subsequent to addition of the flour samples followed by thermal treatment of 15 min, which was terminated by removing the *Tenebrio* flour from the drying cabinet and transferring it into a cooled Petri dish.

### 2.4. Microbial analysis

Total viable count of the *Tenebrio* flour was analyzed by mixing and homogenizing 3 g of flour and 27 g of 0.1% casein–peptone-solution (CPS) in a sterile filter stomacher bag (Bag Mixer Interscience, St. Nome, France) at a speed level of 8 for 2 min. The homogenate was then serially diluted with CPS in Rotilabo®-microtest plates (96er U-profile, Roth, Germany), and 50 μL of each dilution was spread on plate count agar and incubated at 30 °C for 72 h to determine the number of colony forming units per g on a dry matter basis ($cfu/g_{DM}$). The detection limit of plate count analyses was 200 $cfu/g_{DM}$.

### 2.5. Mass loss and pH

Thermal and plasma-induced mass loss of the samples was determined by differential weighing. Shifts in pH were determined in the suspension of flour and 0.1% CPS (Inolab Terminal 740 pH measurement device, WTW, Weilheim, Germany). During further analysis, the pH values of the protein extracts in buffered systems (pH 4 and 10) were measured.

### 2.6. Characterization of techno-functional properties

#### 2.6.1. Crude protein, crude fat and dry matter content

Crude protein content ($N_{Kjel}$, conversion factor 6.25) was analyzed using the method by Kjeldahl (Kjeldatherm Turbosog, Titrino plus 848, Gerhardt Analytical Systems, Königswinter, Germany), according to DIN EN 25663: Digestion and distillation (Kjeldahl Sampler System K-370/371) were conducted as described by the Association of German Agricultural Investigation and Research Institutions (VDLUFA, 1976). Crude fat content of the *Tenebrio* flour was determined according to the filter bag (Filterbags XT4, ANKOM Technology, New York, USA) method Am 5-04 (AOCS 2005). Dry matter (DM) content was determined via oven drying method (105 °C, 48 h).

#### 2.6.2. Water (WBC) and oil binding capacity (OBC)

WBC of the *Tenebrio* flour was measured using the method by Smith and Circle (1978), modified by Quinn and Paton (1979). Therefore 0.5 g (±0.009 g) of *Tenebrio* flour was weighted into a centrifuge beaker and