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Effect of freezing and drying processes on the molecular traits of edible yellow mealworm

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ABSTRACT

Yellow mealworm (*Tenebrio molitor* L.) represents a highly sustainable source of proteins for food and feed. Industrial production of mealworm meals for food and feed must count on optimized processing methods based on a deep knowledge of nutritional and quality aspects. Viable mealworm larvae (VL) were frozen at −20 °C and dried under two different thermal conditions, namely high-temperature-short-time (HTST, 90 °C for 1.5 h) and low-temperature-long-time (LTLT, 50 °C for 62 h). Proximate composition, fatty acid analyses by gas-chromatography and metabolic profiling by means of proton nuclear magnetic resonance (^{1}H NMR) spectroscopy were carried out and ^{1}H NMR data investigated with multivariate data analysis (MVDA). While fatty acid profiles did not indicate significant differences among treatments, ^{1}H NMR highlighted relevant molecular alterations associated to LTLT drying (95% of detected metabolites are altered by LTLT). In particular, detrimental hydrolysis of triacylglycerols (TAG) was favored during LTLT drying (approximately 25% reduction of TAG in LTLT compared to VL), accompanied by the enrichment of the free amino acid pool. Larvae composition was only minimally affected by the freezing process, with only 15% of the metabolite pool affected.

Industrial relevance: Sustainable industrial production of insect-derived products (insect meal, oil, and other extracts) must count on efficient processes and high standard quality. Freezing and drying are the most critical processing operations in insect industry, since they can strongly affect the quality of final products. It is demonstrated here that low-temperature-long time drying processes negatively affect insect products quality, while freezing and high-temperature-short time drying do not have a significant impact. Molecular details of nutrient degradation processes are provided.

1. Introduction

New sustainable protein sources for direct or indirect human food consumption are being explored in an attempt to solve the coming food insecurity problem due to the growing world population. Starting from January 2018, the European Union has redefined the procedure of commercialization of novel food categories including insects and derived products according to Regulation (EU) No 2015/2283 and 2017/893. In this context, massive production of yellow mealworm *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae represents a promising alternative to livestock products and vegetables. Yellow mealworm is already produced and sold in various forms in North America and European countries such as the Netherlands, Belgium, Great Britain, France, Germany and Italy (van Huis et al., 2013). Various edible insect fractions could potentially be obtained from the insect, e.g. proteins (including enzymes), oils and polysaccharides (chitin) (Ravzanaadii, Kim, Choi, Hong, & Kim, 2012). Mealworm larvae are commercialized for both food and feed purposes. Animal feed production, especially the fastly growing sector of aquafeeds, is becoming a very interesting opportunity for the insect-related economy (Tomberlin et al., 2015). Mainly due to cultural reasons, entomophagy is less practiced in developed countries than in developing ones, being in the former sometimes hindered by the unaccepted appearance of insects and insect larvae. Therefore, processing insect larvae into powders could likely

Abbreviations: LTLT, low-temperature-long-time drying; HTST, high-temperature-short-time drying; MVDA, multivariate data analysis; ^{1}H NMR, proton Nuclear Magnetic Resonance; GC, Gas Chromatography; VL, viable larvae; FL, frozen larvae; LD50, larvae dried at 50 °C for 62 h (LTLT drying); LD90, larvae dried at 90 °C for 1.5 h (HTST drying); FAME, fatty acid methyl esters; PCA, Principal Component Analysis; OPLS-DA, orthogonal partial least square discriminat analysis; ASCLAN, Automatic Spectroscopic data Categorization by CLustering ANalysis

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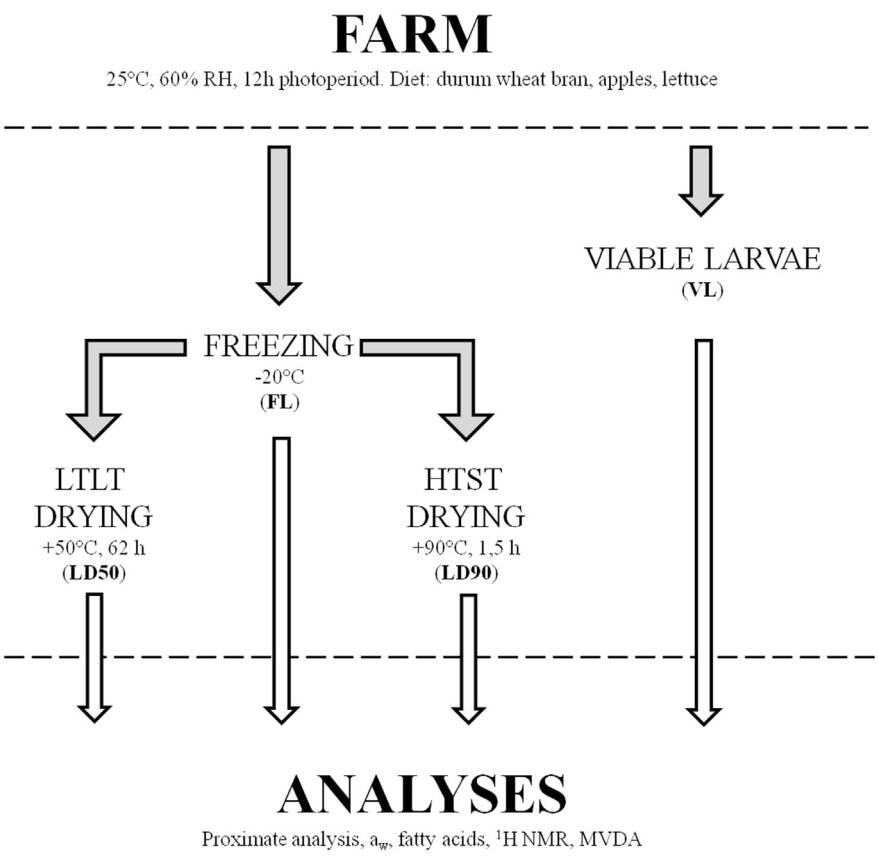

Fig. 1. Diagram of the processing steps adopted on yellow mealworm larvae from viable to dried processed larvae. VL = viable larvae; FL = frozen larvae; LD50 = larvae dried at 50 °C for 62 h (LTLT = low temperature long time drying treatment); LD90 = larvae dried at 90 °C for 1.5 h (HTST = high temperature short time drying treatment).

increase consumers' acceptance (Megido et al., 2014). Of course, different technological processing methods are supposed to affect the nutritional and functional properties of the primary material as well as those of the desired final product. In this sense, a sustainable industrial production requires a proper balance between technological efficiency of the process, manufacturing cost and product quality. Usually, a freezing process is applied in order to kill, preserve and store live larvae (Dossey, Tatum, & McGill, 2016) before drying process. Dried larvae and insect meals of *T. molitor* are commercially available from Asiatic, North American and European producers. In experimental trials, likely paralleled by industrial and household productions, larvae have been dried at different combinations of temperatures and time. For example, low temperature-long time treatments (50 °C for 24 h) (Klasing, Thacker, Lopez, & Calvert, 2000) or 3 days (Ramos-Elorduy, Avila Gonzalez, Rocha Hernandez, & Pino, 2002) were performed in previous studies, but also high temperatures for shorter time (100 °C for 200 min) have been used (Yingchang et al., 1996). Since traditionally the drying process is mainly done by leaving the larvae in the sun for extended periods of time (Azzollini, Derossi, & Severini, 2016) some researchers studied the nutritional quality of yellow mealworm dried in the sun for 2 days (Ng, Liew, Ang, & Wong, 2001).

Protein, fat and chitin contents of insect larvae may vary significantly and in turn may greatly influence processing results. The choice and optimization of technological processes depends not only on the technological, functional and nutritional properties of the starting raw materials, but also on the quality of the fractions that can be achieved. The available methods for processing insects to produce food and feed need to be evaluated regarding their capacity to both guarantee the highest nutritional and quality standards in the final products and to remove undesired, insect-specific components and contaminants (e.g. toxins and antinutrients) (Payne, Scarborough, Rayner, & Nonaka, 2016). In order to deeply investigate the overall molecular reorganization of mealworm larvae throughout the different processing steps, and to monitor possible degradation processes, metabolomics can be seen as a very suitable approach. ^{1}H NMR metabolomics allows exhaustive assessment of small molecular metabolites both qualitatively and quantitatively and can provide a reasonable molecular fingerprinting of a living or processed organism under specific conditions (Kodani, Miyakawa, Komatsu, & Tanokura, 2017; Viant, 2007). The aim of the present study was to distinguish the metabolic variation between fresh, frozen and dried yellow mealworm larvae. In particular, the molecular impact of a preliminary freezing and two different thermal conditions for drying were tested by means of quantitative ^{1}H NMR-based metabolomics approach in combination with multivariate data analysis (MVDA), proximate composition and fatty acid profile. The information collected will be useful for the optimization of suitable processing protocols able to preserve as much as possible the metabolic features, i.e. quality and nutritional aspects, of dried yellow mealworm larvae meals.

2. Materials and methods

2.1. Experimental insects

Yellow mealworms used in this study were obtained from a laboratory culture maintained at CNR, Institute of Ecosystem Study of Sassari. Larvae and adult beetles were fed exclusively feed-grade durum wheat bran and discarded or unmarketable vegetables and fruits (mainly lettuce and apples) as a water source. The colony was kept in a rearing room at 25 °C, 60% relative humidity under a 12 h:12 h light - dark photo regime. Adults and larvae were reared in 400 × 600 × 100 mm plastic boxes. Adults were maintained in groups of 1000. Eggs laid from adults on the bottom of the box were separated weekly and larvae were reared for a period of 16–18 weeks until their harvest as mature larvae. A small portion of larvae was separately reared to obtain new adults earmarked to replace died ones. New bran was provided weekly if necessary and vegetable were provided bi-weekly. An average of 1 kg of mature larvae was harvested from each