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Pre-treated mealworm larvae and silkworm pupae as a novel protein ingredient in emulsion sausages



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ABSTRACT

The objective of this study was to determine the effects of adding pre-treated mealworm larvae ($Tenebrio\ molitor$) and silkworm pupae ($Bombyx\ mori$) flours on nutritional, physicochemical and textural properties of emulsion sausages. Whole freeze-dried insects were sequentially ground, defatted, and acid-hydrolyzed. Control sausage was formulated with 60% lean pork, 20% ice and 20% back fat, and insect treatments were prepared with replacement of 10% lean pork by each pre-treated insect flour. Defatting and/or acid hydrolysis significantly increased the protein content of two insect flours, but acid hydrolysis slightly decreased protein solubility (P=0.002). The addition of pre-treated insect flours had no impact on protein solubility of emulsion sausages, but increased cooking yield and hardness in a similar extent, regardless of pre-treating methods and insect types (P>0.05). Our results suggest that through separation processing, mealworm larvae and silkworm pupae can be further optimized as a novel protein ingredient for emulsified meat products.

Industrial Relevance: This study evaluated the nutritional and technological properties of pre-treated edible insects as a novel non-meat ingredient for the meat emulsion application. The results of the present study suggest that edible insect proteins can be practically utilized as a non-meat food ingredient in processed meat products or potentially other food applications without compromising the nutritional and technological properties of the products.

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1. Introduction

Food security is one of the most pressing challenges related to the world's growing population. In particular, there has been an increase in consumer demand for animal proteins, primarily in the form of meat products, which has been estimated to additional 200 million tonnes of meat production per year by 2050 (Bruinsma, 2009). This presents an urgent need to increase supply of protein from new and sustainable sources. Due to concern over food security caused by global population growth, edible insects have received growing interest as a sustainable protein source mainly because of their environmental and nutritional advantages (van Huis et al., 2013). Compared to conventional livestock, it has been reported that edible insects have a higher feed conversion efficiency (Nakagaki & DeFoliart, 1991; Smil, 2002), consume less natural resources such as water (Chapagain & Hoekstra, 2003; Ramos-Elorduy, González, Hernándezz, & Pino, 2002), and produce less greenhouse gases and ammonia (Oonincx et al., 2010).

While edible insects are undeniably rich sources of proteins and other nutrients (Rumpold & Schlüter, 2013a, 2013b; Yi et al., 2013), there still remain challenges and scientific knowledge gaps to fill. One of the challenges for promoting edible insects as a human food is unfavorable consumer perceptions, particularly in Western countries, where edible insects are not traditionally considered as a conventional food (Tan et al., 2015). In this regard, consumer acceptability would likely increase when insects are processed in less recognizable forms (e.g. extracted protein powders) and incorporated in food products as highquality protein/functional food ingredients (Verkerk, Tramper, van Trijp, & Martens, 2007). Edible insect proteins have been suggested as a functional food ingredient for providing technological benefits, such as emulsion capacity, gel-forming ability, and water/oil absorption ability (Omotoso, 2006; Osasona & Olaofe, 2010; Yi et al., 2013). Thus, it would be reasonable to postulate that insect proteins can be used as a novel protein source to partially substitute meat portion in processed meat products, while not compromising the nutritional and technological properties.

As available edible insects, mealworm larvae (*Tenebrio molitor*) and silkworm pupae (*Bombyx mori*) have been commercially farmed for human consumption in many regions of the world (Chen, Feng, & Chen, 2009; Hanboonsong, Jamjanya, & Durst, 2013; van Huis, 2013).

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Mealworm larvae and silkworm pupae contain high amounts of protein over 45 g/100 g dry matter (Rumpold & Schlüter, 2013a, 2013b; van Huis, 2013), while also including considerable amounts of other major components. To fully realize the potential of insect proteins in processed meat applications, separation of insect proteins from other major components, which may have adverse impacts on functional/technological properties, would be an essential step.

For example, mealworm larvae and silkworm pupae contain a large amount of fat (18.9-36%) and dry matter (30.1-35%) (Oonincx, van Broekhoven, van Huis, & van Loon, 2015; Ramos-Elorduy et al., 1997; Rao, 1994; Oonincx et al., 2015). In particular, mealworm larvae and silkworm pupae contain the high proportion of unsaturated fatty acids over 60% (Oonincx et al., 2015; Rao, 1994). This may result in an adverse impact on oxidation stability of edible insect-added products. Further, mealworm larvae and silkworm pupae include a hard exoskeleton that comprise over 1% of their mass, consisting of chitin-based structures (Finke, 2002; Ramos-Elorduy et al., 1997; Rumpold & Schlüter, 2013a). Although the chitin in edible insects is considered a source of dietary fiber in the human diet, it could reduce the digestibility when consumed (van Huis, 2013). Thus, an effective separation of fat and chitin at insect protein preparations would likely increase the protein concentration and improve its nutritional and/or technological values as a novel protein ingredient.

The overall objective of this study was to investigate the nutritional and technological value of pre-treated mealworm larvae and silkworm pupae as a novel protein ingredient for the meat emulsion application. We evaluated the chemical composition and protein functionality of pre-treated mealworm larvae and silkworm pupae flours (by defatting for fat removal and acid hydrolysis for chitin degradation) and determined the nutritional, physicochemical and textural characteristics of emulsion sausages after replacing 10% lean pork with the pre-treated mealworm larvae and silkworm pupae flours.

2. Materials and methods

2.1. Experimental design

An experimental design in this study was 2×3 factorials with two insect species (mealworm larvae, *Tenebrio molitor* and silkworm pupae, *Bombyx mori*) and three stepwise pre-treatment methods (grinding, defatting and acid hydrolysis). In application phase to an emulsion sausage, a regular formulation was prepared as a control sausage to compare the nutritional, physicochemical and textural characteristics of emulsion sausages.

2.2. Preparation of pre-treated mealworm larvae and silkworm pupae flours

2.2.1. Raw materials

Whole freeze-dried mealworm larvae and silkworm pupae were obtained from an edible insect farm/producer (Chubby Meal Worms, Kennesaw, GA; Exotic Nutrition, Newport News, VA). The whole insects were cleaned with distilled water (DW) and selected with SWECO 10-mesh vibrating screen to remove dust and small contaminants (Florence, KY). The selected insects were ground using a hammer mill (Fitzpatrick, The Standard FitzMill® Comminutor, Elmhurst, IL) and passed through a 20-mesh sieve (Seedburo Equipment Company, Chicago, IL) to obtain untreated mealworm larvae and silkworm pupae flours. The untreated insect flours were used for further pre-treatment processes.

2.2.2. Pre-treatments of insect flours (defatting and acid hydrolysis)

To remove fat contained in untreated insect flours, solvent extraction using n-hexane was performed. The untreated insect flours were mixed with hexane (1:5 ratio, v/w) and stirred for 30 min. The hexane/oil miscella was centrifuged at 2000 rpm for 7 min and filtered

with filter papers (Whatman No. 1). The residual hexane in wet cake was evaporated, and then the wet cake was dried in a 70 °C dry oven overnight. The dried insect flours were used as defatted insect flours in this study. The defatted insect flours were mixed with water (1:10 ratio, v/w) for 1 h, then adjusted to pH 3.5 with 1 N HCl solution and stirred for 2 h. The acid-hydrolyzed insect flours were centrifuged at 6000 rpm for 10 min. After discarding the supernatant, the residual wet cake was collected and dried in the dry oven (70 °C), and the dried insect flours were used as defatted and acid-hydrolyzed insect flours. The untreated insect flours and each of the pre-treated insect flours were vacuum-packaged individually and stored at $-20\,^{\circ}\mathrm{C}$ until analysis and preparation of emulsion sausages.

2.3. Emulsion sausage manufacturing

Meat emulsions were manufactured with the method described by Kim, Lee, and Kim (2015) with slight modification on different pork muscle (M. semitendinosus instead of M. biceps femoris) and formulations with insect flours. Fresh pork ham muscle and pork back fat at 72 h postmortem were obtained from the Purdue University meat laboratory, vacuum-packaged, and stored at -18 °C until use. Before manufacture, the frozen pork muscle and back fat were thawed in a 2 °C cooler for 24 h; then all subcutaneous and intramuscular fat and visible connective tissue were removed. The lean pork and back fat were ground through a 3/8-in. plate using a meat grinder (M-12-FS, Torrey, Monterrey, NL, Mexico). Regular formulation containing 60% lean pork, 20% back fat, and 20% ice was prepared as a control. Each insect treatment was formulated with the replacement of 10% lean pork by each insect flour prepared (1:1 ratio based on weight), while there was a difference in moisture contents between lean pork (approximately 26% dry matter, Kim et al., 2008) and the insect flours (93–95% dry matter). All treatments were individually emulsified with 2.0% sodium chloride, 0.3% sodium tripolyphosphate, 0.012% sodium nitrite, and 0.05% L-ascorbic acid in a bowl cutter (Cutter C4, Sirman, Marsango, Italy). The internal temperature of meat emulsion was confirmed by a digital thermometer (OctTemp2000, MadgeTech, Inc., Warner, NH) equipped with a data logger (T-type, Omega Engineering, Stamford, CT), which was below 10 °C during manufacture. Approximately 20 g of each meat emulsion was stuffed into an edible collagen casing (edible clear collagen casing, 21 mm in diameter, Smokehouse Chef, TX) by using a hand stuffer. The stuffed sausages were placed in a vacuum bag and cooked in a 80 °C water bath until reaching a core temperature of 71 °C, which was monitored using the digital thermometer. After cooking, the sausages were cooled at room temperature for 1 h. To determine the extent of lipid oxidation, the cooked sausages were placed on Styrofoam trays, over-wrapped with a commercial PVC film, and displayed for 7 days under continuous fluorescent natural white light (the intensity of illumination = 1600 lx and color temperature = 3500 K) in a 2 °C cooler. A total of three independent batches was performed.

2.4. Analyses of pre-treated mealworm larvae and silkworm pupae flours

2.4.1. Extraction yield

Extraction yield of pre-treated insect flours was calculated as a percentage of the weight of pre-treated insect flour in comparison with the weight of untreated insect flour before each pre-treatment. The extraction yield of each insect flour was calculated as follows; extraction yield (%) = (weight of pre-treated insect flour (g) / weight of untreated insect flour (g)) \times 100.

2.4.2. Proximate composition

Moisture (oven air-drying method), lipid (Soxhlet extraction), and ash (muffle furnace), and total fiber contents of each insect flour were analyzed by the AOAC method (AOAC, 2000). Protein content was analyzed by the high temperature combustion process according to the

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