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Leg muscles of migratory locust (*Locusta migratoria*) as a protein source: Extraction, protein composition and foaming properties

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ABSTRACT

Migratory locust (*Locusta migratoria*) is an edible insect species that is usually consumed at the adult developmental stage and represents a valuable source of proteins. Locusts can be processed into food ingredients or directly consumed after removal of legs and wings. Legs represent a unique matrix, which comprises a chitin-based exoskeleton typical for insects and muscle tissues commonly occurring also in other animals. Muscle proteins can be easily extracted from traditional animal sources, but the efficiency of these methods for locust legs as well as the functional characteristics of the resulting protein extracts have not been previously explored. This study first compared protein extraction methods at acid, alkaline, and salt-assisted conditions. All three methods resulted in a significantly higher extraction yield from locust leg muscles (0.465–0.595 g/g) than from whole locusts (0.061–0.125 g/g). Shotgun proteomics of the protein fraction extracted at alkaline conditions assigned 310 muscle proteins, comprising components assigned to energy and carbon metabolism, as well as to skeletal, protein folding, membrane trafficking, and cell adhesion functions. The techno-functional potential of locust leg muscles was assessed by a foamability assay. Foam stability of locust leg extracts varied as a function of pH extraction and re-solubilization conditions and was significantly higher (58.3–70.8 %) than for whey proteins taken as a benchmark (38.3–51.7 %); foam capacity at t = 0 h was in the range of 48.6–68.8 %. This study demonstrates that locust legs, which can be a by-product of locust consumption or processing, should be considered a rich source of muscle proteins with promising technological functionality.

1. Introduction

Current food systems need transformation to provide the growing population with healthy diets while developing more sustainable food systems (Willett et al., 2019). The livestock industry accounts for over 70 % of all agricultural land and contributes approximately 15 % of all anthropogenic greenhouse gas emissions (Oonincx & de Boer, 2012). Shifting towards alternative protein sources, such as plant proteins, or proteins from animal species with a lower environmental impact, is considered an effective strategy to tackle this problem (Oonincx et al., 2010). In this context, during the last decades, insects have gained increasing attention as a promising emerging food source. The main advantage of edible insect farming is their low environmental footprint, as they have a higher feed conversion efficiency in comparison to conventional livestock, and their rearing results in a lower production of greenhouse gas and ammonia emissions (van Huis et al., 2013).

More than 2000 insect species are known to be edible with locusts, beetles, caterpillars, ants, and crickets being amongst the most consumed insects worldwide (Cerritos, 2009; Jongema, 2017). Migratory locust (*Locusta migratoria*) was consumed for millennia in many countries of Asia and Africa, in which adult insects are collected from the wild during outbreaks and are usually eaten after roasting, boiling, or drying (Egonyu et al., 2021). Locust legs and wings are removed before the consumption of whole insects during preparation, as they can be a choking hazard upon consumption (van Huis, 2003; van Huis et al., 2013). In Europe, migratory locust is a new food that has been authorized as safe for human consumption by the European Commission (Turck et al., 2021). To make locusts more acceptable by European consumers, these insects are processed and incorporated into familiar food products in invisible forms (Siddiqui et al., 2023).

One of the distinguished features of adult locusts compared to other insects is that they have longer hind leg length, higher elastic

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parameters, and mass increase (Mo et al., 2020). Locust leg muscles support jumping movements, while large thoracic muscles control flight (Norman, 1995; Robertson, 1988). Thus, muscles generally support the mobility of insects, including walking, flying, and jumping (Iwamoto, 2011). Insect muscle proteins have been previously studied extensively through an evolutionary and physiological perspective (Bretscher & O'Connor, 2020; Hooper & Thuma, 2005; Nave & Weber, 1990; Zhao et al., 2012). Proteomic analysis with identification of locust muscle proteins was previously done as a part of a study on potential insect allergenicity (Barre et al., 2021). From the angle of using insects as food ingredients, the use of soluble protein fractions is of special interest due to the corresponding techno-functional characteristics (Mishyna et al., 2021).

Muscle proteins occur mainly as water-insoluble components. In particular, previous studies on mealworms already demonstrated that muscle proteins, such as myosin chains, actin, and tropomyosin, are water-insoluble, that has been confirmed by proteomic analysis (Yi, Van Boekel, Boeren, et al., 2016). Therefore, extraction approaches specifically for insect muscle proteins should be still developed to use them as food ingredients. The combination of such studies with proteomic analysis will provide additional insight into the nature of extracted proteins and contribute to knowledge on insect proteins as the proteomic approach can be a powerful tool for the identification of insect proteins (Francis et al., 2020).

Extraction methods specific for muscle proteins have been studied extensively for fish and meat (Chen et al., 2017; Malva et al., 2018; Mohan et al., 2007; Nolsøe & Undeland, 2009; Pérez-Mateos & Lanier, 2007; Raghavan & Kristinsson, 2008). Those studies focused on non-denaturing extraction methods, which are efficient in terms of protein recovery, while also preserving the functionality of the recovered proteins (Xiong, 1997). Non-denaturing methods for extraction of muscle proteins are based on their good solubility in saline solutions, as well as in extreme pH values (acidic or alkaline) (Hashimoto et al., 1979). Extracted muscle proteins have promising functionality, mainly regarding their foaming, gelling, and emulsifying properties (Chan et al., 2011; Hmidet et al., 2011; Hrynets et al., 2010; Omana et al., 2010; Pacheco-Aguilar et al., 2008; Ramachandran et al., 2007). Those properties are significantly influenced by different extraction treatments, as they alter the molecular conformation of the recovered proteins. Numerous studies were done on the extraction of bulk locust proteins using different conditions (Clarkson et al., 2018; Mishyna et al., 2019; Purschke et al., 2018) with the primary focus on the assessment of the extractability and techno-functionality. However, it is still not clear to what extent the extraction methods for muscle proteins are efficient for insect biomass, how such extractions affect protein functionality, and whether the outcome of such extraction methods is comparable to traditional ones. A study on an alternative approach for myofibrillar proteins extraction from black soldier flies with ultrasound-assistant method showed its effect on conformational changes and functional properties (Ni et al., 2024). Therefore, this study aimed to investigate the efficiency of protein extraction methods already developed for muscle-rich materials from traditional animals, for locust legs. In addition, testing the corresponding foamability potential as an example of their techno-functional characteristics was done.

Locust legs represent a unique matrix that comprises muscle and exoskeletal tissues. Such matrix complexity makes locust legs different not only from the counterpart of traditional breed animals but also from the whole insect body, which comprises various organs. Besides muscle proteins, the locust body contains other protein types such as proteins related to the nervous system, respiration, digestion, haemolymph, and the cuticle (Mishyna et al., 2021). Because of this, a special interest of the current study is not only the comparison of the recovery of protein fractions from locust legs and that of the whole-body counterparts but also protein characterization using proteomics.

2. Materials and methods

2.1. Materials

Migratory locusts (*L. migratoria*) at adult stage were purchased from Kreca Ento-Feed BV (Ermelo, The Netherlands). After being starved for 24 h, they were frozen using liquid nitrogen and stored at −80 °C. Before further processing, locust wings were manually separated and discarded. For the locust leg muscle samples, legs were separated, and their muscles were extracted manually by squeezing.

2.2. Protein extraction procedures

Protein extractions were performed in triplicate according to Arnesen and Gildberg (Arnesen & Gildberg, 2006), and Hashimoto and coworkers (Hashimoto et al., 1979) with modifications. Whole locusts and locust leg muscles were homogenized with distilled water of around 4 °C (pH 7) at 1:10 wt ratio using Waring two speed blender at maximum speed, for 3 min. Then, all suspensions were centrifuged (4700 g, 20 min, 4 °C). The supernatant (water-soluble protein fraction) and the pellet (water-insoluble protein fraction) were separated using a pipette and stored at −80 °C for further analyses. The lipid fraction was discarded. The pellet fractions were used for the following extractions. For the (i) acid- and (ii) alkaline-assisted extractions, each obtained pellet was suspended by stirring in phosphate buffer (15.6 mM Na_2HPO_4, 3.5 mM KH_2PO_4, pH 7.5, 1:10 w/v), and pH was set to (i) 2 and (ii) 12, by adding (i) 2 M HCl and (ii) 2 M NaOH. For the salt-assisted extraction, each pellet was suspended in NaCl phosphate buffer (0.45 M NaCl, 15.6 mM Na_2HPO_4, 3.5 mM KH_2PO_4, pH 7.5, 1:10 w/v), and pH was set to 9, by adding 2 M NaOH. All extractions were carried out for 30 min, followed by centrifugation (4700 × g, 20 min, 4 °C). Supernatants and pellets from each method (protein extract) was stored at −80 °C for further analyses. All steps were carried out on ice. Protein solubility was determined according to Sweers and colleagues (Sweers et al., 2023).

2.3. Protein molecular mass distribution

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed to determine the molecular mass distribution of the proteins from each obtained fraction under reducing conditions, according to the method described by (Laemmli, 1970). Protein concentration for SDS-PAGE of water-insoluble fractions was not equalized between lanes. The used gels were 12 % Bis-Tris Gel (NuPAGE, Invitrogen), and the marker was Spectra™ Multicolor Broad Range Protein Ladder (ThermoFischer). Electrophoresis was done using XCell SureLock Mini-Cell using company's power settings. Proteins were stained with Coomassie blue staining (Bio-Rad) for 1 h. Then gels were destained in ethanol/acetic acid/water, 1/1/8 (v/v/v) for overnight. Gels were scanned using Image Lab Software (Bio-Rad).

2.4. Determination of protein content

The soluble protein content of all extracts was determined by the bicinchoninic acid assay (PierceTM BCA Protein Assay Kit, Thermo Scientific), according to manufacturer's instructions. The nitrogen content of whole locusts, locust legs, and locust leg muscles, as well as the corresponding extracted materials was determined by Dumas and colleagues. D-methionine was used for the calibration curve in the method of Dumas (AOAC Official Method, 2012). The nitrogen-to-protein conversion factors were 5.33 for whole locusts and locust legs (Boulos et al., 2020). This factor for locust leg muscles has not previously been reported in the scientific literature, but in this study a value equal to 5.60 was used because these tissues do not contain high amounts of chitin similarly to mealworm and black soldier fly for which the factor was previously reported (Janssen et al., 2017).

2.5. Protein extraction for proteomic analysis

Locust leg muscles were mixed with water at the ratio of 1:10 (w/w) and homogenized for 2–3 min. Then the mixture was centrifuged at 4700g for 20 min, at 4 °C and the supernatant was removed. The pellets were solubilized in water at 1:10 w/w ratio, and pH value was adjusted to pH 12 using 2 M NaOH while stirring. Next, the mixture was centrifuged at 4700g for 20 min, at 4 °C, and the supernatant was used for proteomic analysis.

2.6. Proteomic analysis

Locust leg protein extracts were analyzed in triplicate with preparative 12 % SDS-PAGE under reducing conditions, stained with colloidal Coomassie blue, and destained as reported above. The resulting gel was cut into 31 slices. These were triturated, washed with water and acetonitrile, S-alkylated with 55 mM iodoacetamide in 100 mM NH_4HCO_3, washed again and finally digested with trypsin (12.5 ng/μL) in 50 mM NH_4HCO_3, 5 mM $CaCl_2$ (Zhou et al., 2004). Peptide mixtures were desalted/concentrated using ZipTip μC18 (Merck, Darmstadt, Germany), vacuum-dried in a Savant roto-evaporator (Thermo Fisher Scientific, USA) and finally reconstituted in 0.1 % v/v formic acid. Digest samples were analyzed in technical duplicate by means of a nanoLC-ESI-Q-Orbitrap-MS/MS platform consisting of an UltiMate 3000 HPLC RSLC nano-chromatographer (Thermo Fisher Scientific, San Jose, USA) linked to a Q-ExactivePlus mass spectrometer through a Nanoflex ion source (Thermo Fisher Scientific) (Ben Abdallah et al., 2018). Peptides were loaded on an Acclaim PepMapTM RSLC C18 column (150 mm length × 75 μm ID, 2 μm particles, 100 Å pore size) (Thermo Fisher Scientific, San Jose, USA), and eluted with a gradient of solvent B (19.92/80/0.08 v/v/v water/acetonitrile/formic acid) in solvent A (99.9/0.1 v/v water/formic acid), at a flow rate of 300 nL/min. The gradient of solvent B started at 3 %, increased to 40 % over 50 min, increased to 80 % over 5 min, remained at 80 % for 4 min, and finally returned to 3 % in 1 min, with a column equilibrating step of 20 min before the subsequent chromatographic run. The mass spectrometer operated in data-dependent mode, using a full scan range (m/z 375–1500, resolution of 70,000 at m/z 200), followed by MS/MS scans of the 10 most abundant ions. MS/MS spectra were acquired in a dynamic scan m/z range, using a normalized collision energy of 28 %, an automatic gain control target of 100,000, a maximum ion target of 120 ms. The dynamic exclusion value was set at 30 s.

For protein identification, all raw mass data files associated with protein digest of slices were analyzed with Proteome Discoverer v. 2.4 software (Thermo Fisher Scientific), enabling database search with Mascot algorithm v. 2.4.2 (Matrix Science) according to a shotgun proteomic approach (Vitale et al., 2014). Database searching was performed with the following criteria: *Orthoptera* protein sequence database (downloaded from NCBI and including 257,567 entries); carbamidomethylation at Cys as fixed modification; oxidation at Met, pyroglutamate formation at N-terminal Gln, phosphorylation at Ser/Thr, and deamidation at Asn/Gln as variable modifications. Parent peptide mass tolerance was set to ± 10 ppm and to ± 0.05 Da for MS/MS fragments. Trypsin was set as the proteolytic enzyme, and the maximum number of missed cleavages was limited to 2. Proteome Discoverer peptide candidates were considered confidently identified only when the following criteria were satisfied: i) protein and peptide false discovery rate (FDR) confidence: high; ii) peptide Mascot score > 25; iii) peptide spectrum matches (PSMs): unambiguous; iv) rank of the peptide match (peptide rank): 1; v) normalized score difference between the selected PSM and the highest-scoring PSM for that spectrum (Delta CN): 0. Protein identification was considered relevant when PSMs were ≥ 2 for each gel portion.

2.7. Bioinformatic analysis

Locust protein network analysis and functional annotations were obtained with STRING software v. 12 (https://string-db.org/) (Szklarczyk et al., 2023) using *Drosophila melanogaster* orthologous genes. Result output integrated gene ontology (GO) categories [Biological Process (BP), Molecular Function (MF), and Cellular Component (CC)] (The Gene Ontology Consortium, 2017), and Reactome (Milacic et al., 2024), Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al., 2017), and BioCyc (Caspi et al., 2020) annotations. Identification of putative locust allergens was obtained carrying out a Basic Local Alignment Search Tool (BLAST) analysis against 3245 known allergenic molecules present within the Allergome v. 4 database (Mari et al., 2009), through the dedicated aligner command-line application. The results of BLAST analysis were further filtered to retain only allergens showing at least a 50 % sequence identity or having the same protein name and > 25 % sequence identity.

2.8. Foam capacity and foam stability

The foam capacity of protein extracts was determined according to the method of Wilde and Clark (Wilde & Clark, 1996) with small modifications. All extracts were standardized at a protein concentration of 1.5 mg/mL using BCA assay as described above; pH was adjusted to 3 different values (5.5, 7, and 8.5), and salt concentration was not equalized between the samples. Next, 17 mL of each solution was transferred into a 25 mL graduated cylinder and aerated using an Ultra-Turrax homogenizer (T4, 25,000 rpm) for 30 s. The volume of the foam was recorded for 1 h. Foam capacity was expressed as percent volume increase after aeration, foam stability was expressed as the percentage of foam remaining after 1 h. Whey protein isolate (purchased by Bulk™, Essex, England) was used as a reference sample. All measurements were performed in triplicate.

2.9. Statistical analysis

Statistical analysis was done using analysis of variance (ANOVA), followed by Tukey's range test, to compare the mean group values of triplicate measurements, with a significance level of 0.05.

3. Results and discussion

3.1. Water-soluble and water-insoluble proteins

The crude protein content of whole locusts was 48.5 ± 0.4 % dry mass (DM). Similar values were reported by Boulos et al. (Boulos et al., 2020), while another study described higher concentrations (65.9 % DM) (Purschke et al., 2018). In the latter, a nitrogen-to-protein conversion factor of 6.25 was used, which led to an overestimation of protein content due to the presence of non-protein nitrogen in insects (Jonas-Levi & Martinez, 2017). The crude protein content of locust legs before the removal of the corresponding exoskeleton was 71.8 ± 0.3 % DM and 77.9 ± 0.2 % DM after exoskeleton removal.

The step of the separation of water-soluble and water-insoluble proteins was accomplished to ensure the efficiency of the following protein extraction procedures. Also, this step led to the partial removal of fat, that is desirable, as fat can negatively affect the extraction yield of proteins (Purschke et al., 2017). Aqueous protein extraction resulted in a higher recovery of water-soluble proteins from whole locusts (80.4 %) than from insect leg muscles (28.3 %) (Fig. 1). This is explained as a result of the well-known occurrence of highly soluble proteins, such as the haemolymph ones, in whole locusts (Kanost et al., 1990). In contrast, locust leg muscles are rich in myofibrillar proteins, which have poor solubility in water (Yi, Van Boekel, Boeren, et al., 2016). The water-soluble fraction of the locust leg muscles could comprise sarcoplasmic proteins (Volmer Hans, 1981), which account for approximately 25–30

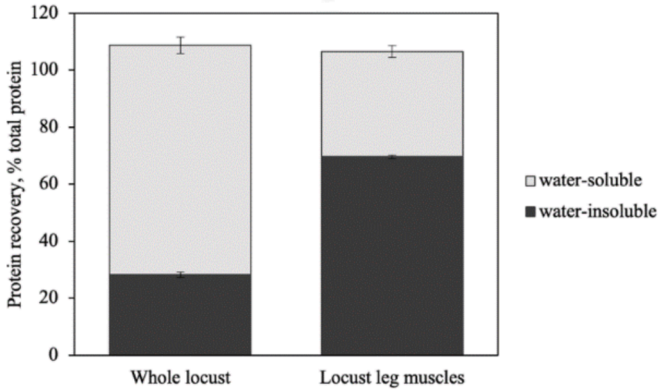

Fig. 1. Protein recovery (% total protein) of water-soluble and water-insoluble fractions extracted from whole locusts and locust leg muscles.

% of total proteins in muscle tissues of fish and livestock (Geirsdottir et al., 2007), but the ratio of this molecular class is not known for locust.

3.2. Solubility profiles

The solubility profiles of the water-insoluble protein fractions with and without the presence of salt (0.6 M NaCl) are presented in Fig. 2. When no salt was present, an increase in protein solubility was observed at extreme pH values for both water-insoluble protein extracts. For whole locusts, maximum solubility (~60 %) in alkaline conditions was determined at pH 12, and ~30 % in acidic conditions at pH 2. For locust leg muscles, the solubility curve was the typical U-shape already described for myofibrillar proteins (Abdollahi & Undeland, 2018; Geirsdottir et al., 2007; Kristinsson & Hultin, 2003a). Protein solubility of locust leg muscles was substantially higher at extreme pH values, reaching up to ~ 90 % at both pH 2 and pH 12. The lowest protein solubility was at around pH 5.5 for both whole locusts and locust leg muscles. It differed from the previously reported isoelectric point at around pH 4 (Mishyna et al., 2019; Purschke et al., 2018); this was explained as the result of the additional step of protein extraction done in this study, where water-soluble proteins were removed determining a possible shift of the isoelectric point.

The addition of salt resulted in different effects on the solubility curve of both extracts. Specifically, protein solubility of the extracts from whole locusts significantly increased at the area of isoelectric point and up to pH 8; in all other tested pH values, protein solubility was significantly lower than without salt. For proteins of locust leg muscles, the isoelectric point shifted to around pH 4 and solubility also increased between pH 5.5 and pH 10. In the latter context, the largest solubility increase associated with augmented ionic strength was observed at pH 9 (from 39.9 % to 73.1 %). An increase of protein solubility in the presence of 0.3–1.0 NaCl (salting-in effect) was previously reported for yellow mealworm (Yi, Van Boekel, & Lakemond, 2016), as well as for meat (Zhao et al., 2020) and myofibrillar proteins (Hong & Xiong, 2012; Li et al., 2022). The observed low solubility at acidic pH values and the shift in the isoelectric point was hypothetically associated with the fact that Cl^- has a stronger binding affinity for positively charged groups compared to the one of Na^+ for negatively charged moieties (Hamm, 1961).

3.3. Molecular mass distribution of proteins

Fig. 3 shows the molecular mass distribution of water-insoluble proteins from whole locusts and locust leg muscles solubilized at pH values from pH 2 to pH 12. The total intensity of the protein bands was in line with the solubility curves, with almost no components observed at pH values close to the molecular isoelectric point values, and an increased band intensity at more alkaline and acidic pH values.

The protein pattern of the whole locust extract was notably different from that of the insect leg muscle counterpart, as result of the presence in the former of components from organs located in head, thorax, and abdomen. Most of the proteins in the whole locust extracts (Fig. 3A) at pH 2–4 and pH 8–12 occurred within the range of 100–170 kDa. In the extract obtained at pH 2, additional proteins appeared at 43–45 kDa. The dominant bands in extracts from locust leg muscles were at the mass ranges of 40–45 kDa, 120–140 kDa, and 160–260 kDa (Fig. 3B). Above-mentioned electrophoretic profiles differed from the ones obtained for soluble proteins of whole locusts where, along with high-intensity bands with molecular mass of 100–170 kDa, numerous protein bands between 15 and 10 kDa were observed as well.

3.4. Extraction yield

The extraction yield obtained by three methods from water-insoluble protein fractions derived from the locust whole body and leg muscles is shown in Table 1.

The protein solubilization at alkaline conditions (pH 12) was associated with the highest fraction yield for both start materials (0.125 and 0.595 g/g for whole locusts and insect leg muscles, respectively). The lowest extraction yield was obtained in the salt-assisted (0.6 M NaCl) extraction method at pH 9 for both materials. For whole locusts, the solubility at pH 12 was significantly higher than that at pH 2, which is in line with previously reported data (Rose et al., 2023). A comparison of the results obtained for whole locusts and insect leg muscles revealed a five to nine times significantly higher recovery of proteins from locust legs at pH 2, pH 12 and pH 9 + 0.6 M NaCl. It was opposite to the recovery of water-soluble proteins from these materials; therefore, most of the soluble proteins from whole locusts were already solubilized at the step of water extraction. In addition, whole locusts contain chitin and therefore contain proteins that are bound to chitin, such as cuticular

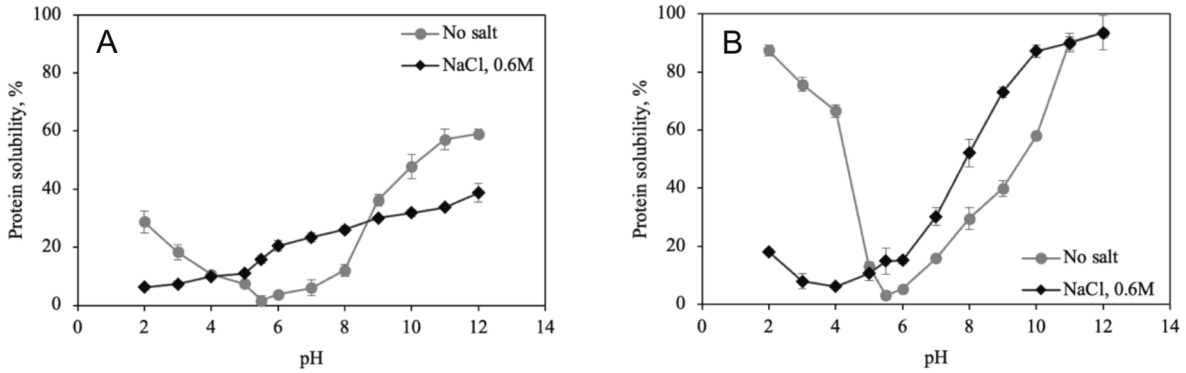

Fig. 2. Protein solubility of water-insoluble fractions of whole locusts (A) and locust leg muscles (B) as affected by pH and salt addition (0.6 M NaCl).

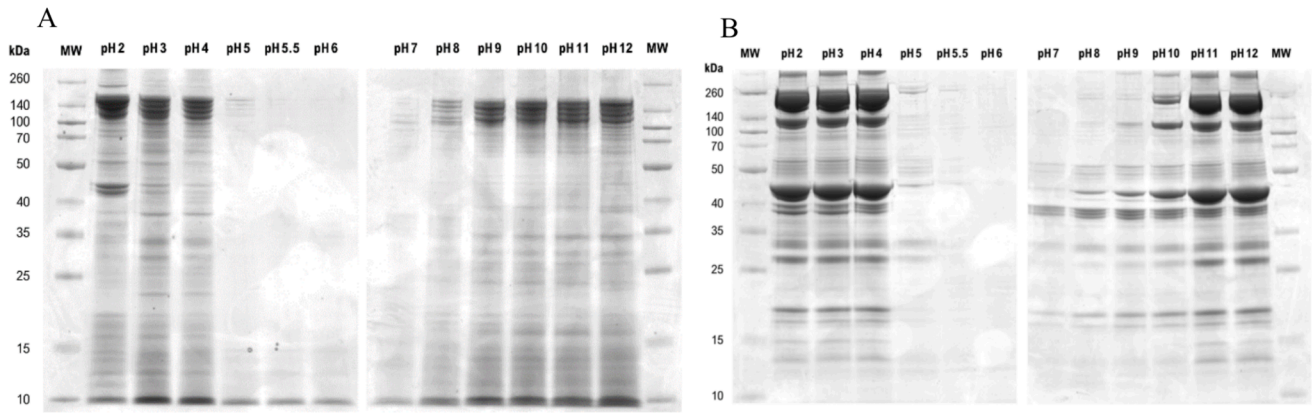

Fig. 3. SDS-PAGE of proteins extracted from water-insoluble fractions of whole locusts (A) and locust leg muscles (B) at various pH values under reducing conditions. MW (molecular weight) lanes correspond to the standard protein markers.

Table 1
Extraction yield (g/g of starting material, dry basis) of fractions from whole locust and locust leg muscles obtained under three different extraction conditions.

Extraction condition	Whole locust	Locust leg muscles
pH 2	0.061 ± 0.008^{a}	0.556 ± 0.012^{a}
pH 12	0.125 ± 0.003^{b}	0.595 ± 0.060^{a}
pH 9 + 0.6 M NaCl	0.064 ± 0.001^{a}	0.465 ± 0.011^{b}

Values are reported as mean ± S.D. (n = 3). Different superscripts in the same column denote significant differences between extraction methods ($p < 0.05$).

proteins (Muthukrishnan et al., 2020), and thus cannot be extracted by the tested methods.

3.5. Protein identification

Locust leg muscle protein extracts were analyzed with preparative SDS-PAGE under reducing conditions (Fig. 4). The resulting gel was stained with colloidal Coomassie, destained, and cut into 31 slices, which were further treated with iodoacetamide and trypsinolyzed. Derived peptide mixtures were analyzed according to a shotgun proteomic approach. A search of recorded mass spectrometric data against an updated *Orthoptera* protein sequence database (NCBI) identified 310 protein entries mostly related to various locust species (Supporting Information Table S1), among which those associated with known muscle components showed the highest values of sequence coverage (%), number of identified peptides and peptide spectrum matches. In particular, intense protein bands at 17, 27, 35, 43, 51, 73, 100, 150 and 220 kDa were associated with myosin light chain isoforms, myosin regulatory light chain, tropomyosins, actin, muscle LIM proteins, heat shock protein 70 kDa protein cognate isoforms, α-actinin, myosin heavy chain isoforms and twitchin isoforms, respectively, plus additional muscle components.

With the aim to analyze identified locust proteins according to their association network and functional annotation, the corresponding sequence entries (Supporting Information Table S2) were loaded in the STRING portal (Szklarczyk et al., 2023) and matched with the comprised *Drosophila melanogaster* database using the default minimum required interaction score (medium confidence, 0.4). This analysis allowed predicting a locust muscle protein association map including 239 components and consisting of a predominant ramified network linking together 221 molecules, plus one distinct quaternary molecular complex (Fig. 5A). The involvement of most locust proteins in the former network emphasized the occurrence of a functional assembly bridging various metabolic pathways and molecular processes associated with locust leg physiology. When a functional clustering of assigned proteins was

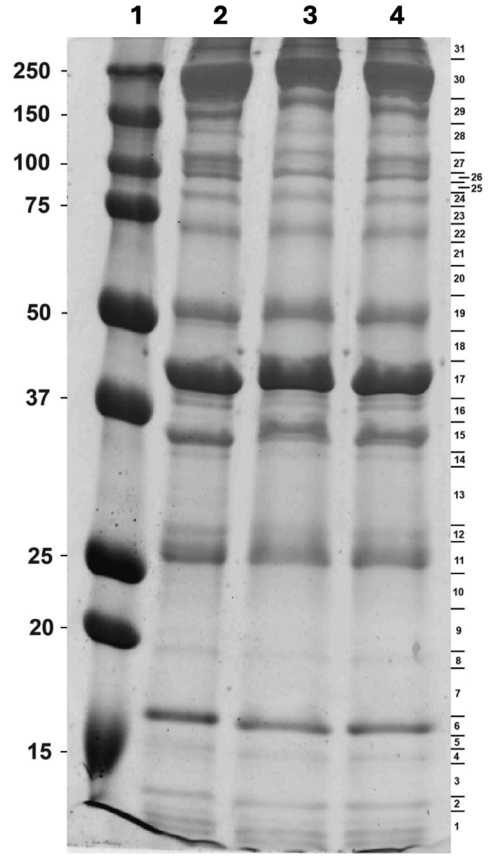

Fig. 4. SDS PAGE of protein extracts from locus legs (lane 2, 3, 4). Line 1: standard protein markers. Numbering on the right corresponds to gel cutting into 31 slices.

applied to above-reported network, 5 subnetworks were identified that included enzymes associated with energy and carbon metabolism (125 entries), skeletal proteins (54 entries), and components involved in protein folding (23 entries), membrane trafficking (9 entries), and cell adhesion (4 entries) (Fig. 5B). The subnetwork outputs describing the corresponding integrated Biological Process, Molecular Function and Cellular Component GO categories (The Gene Ontology Consortium, 2017), and Reactome (Milacic et al., 2024), KEGG (Kanehisa et al., 2017), and BioCyc (Caspi et al., 2020) annotations are reported in Supporting Information Tables S4–S8. These results were in perfect agreement with previous proteomic studies on various animal muscle

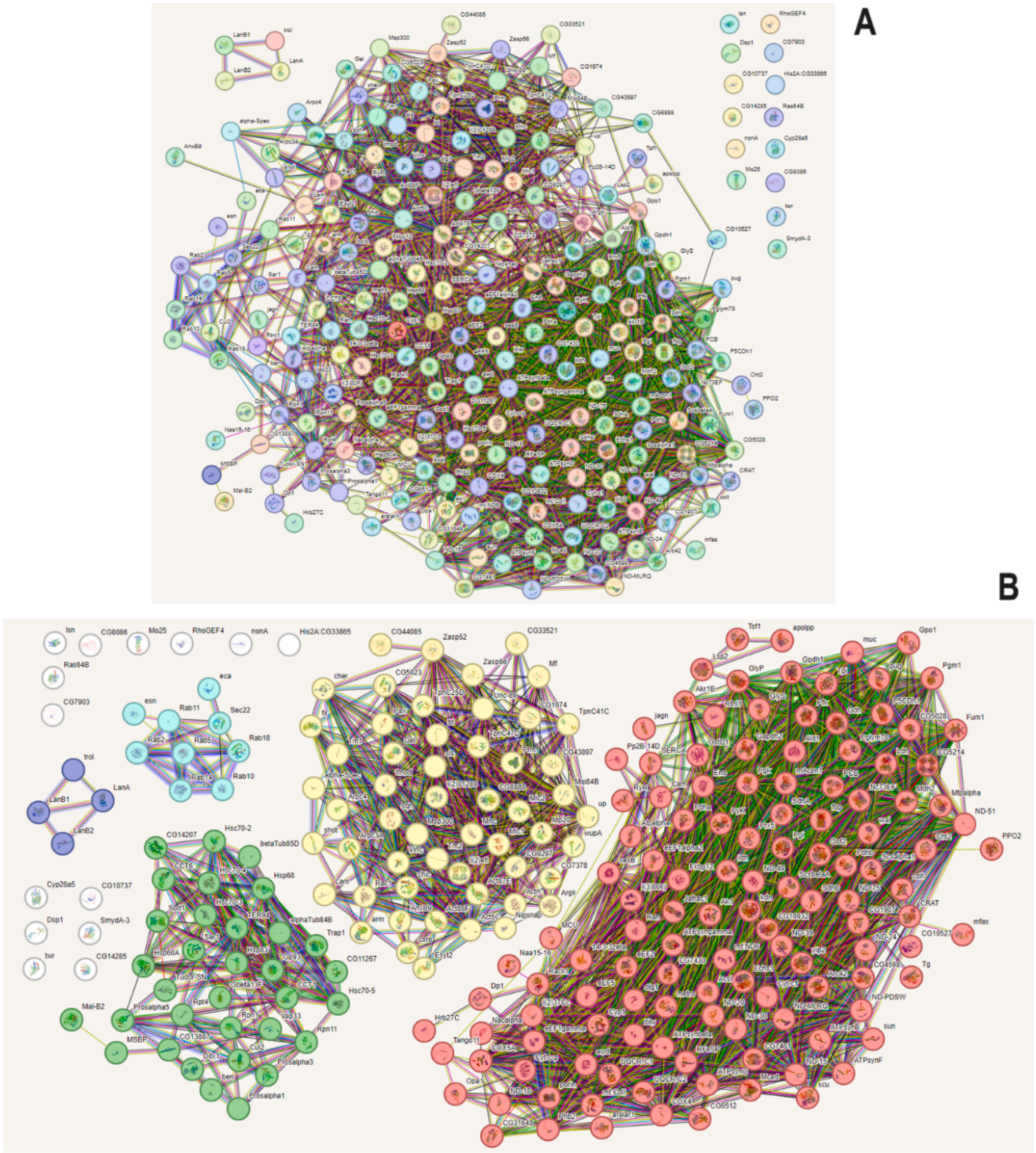

Fig. 5. STRING association map of the proteins (310 in number) identified in locust leg muscles after matching their sequence with the *D. melanogaster* database. Default median-confidence interactions are shown. Protein codes are reported in Supporting Information Table S3. (A) Association map of locust leg muscle proteins including 239 components and consisting of a predominant ramified network linking together 221 molecules, plus one distinct quaternary molecular complex. (B) Functional clustering of the assigned protein nodes within the above-reported STRING network, which identified five subnetworks including enzymes associated with energy and carbon metabolism (red), skeletal proteins (yellow), and components involved in protein folding (green), membrane trafficking (light blue), and cell adhesion (velvet).

types reporting the most important functional classes of proteins in these tissues (Murphy et al., 2016) (Supporting Information Fig. S1).

Edible insects can cause allergic reaction and possess risk for cross-reactivity of house dust mite to migratory locusts (Pali-Schöll et al., 2019). Previous studies on the identification of allergens from several insect species including migratory locust, identified both protein allergens which are phylogenetically-related to other arthropods and mollusks as well as insect-specific proteins. The latter included chemosensory protein, hexamerin, and the odorant-binding protein (Barre et al., 2021). In this study, matching sequence information of identified locust proteins with respect to insect (I), crustacean (C), fish (F), mollusk (M), bovine (B), plant (P) and fungal (Y) allergen entries (reported below in parenthesis) within the Allergome database (Mari et al., 2009) provided information on grasshopper components putatively eliciting allergenic response after ingestion, contact and/or inhalation. They included: phosphoglucomutase (Dan re PGM, muscle, F); glutathione S-transferase (Per a 5, muscle, I); glyceraldehyde-3-phosphate dehydrogenase (Per a 13, I); tubulin isoforms (Lep d 33, whole body, I); porin (Cul q 6, I); cytochrome *c* (Cur l 3, Y); heat shock protein 70 isoforms (Blo t 28 and Aed a 8, whole body, I); heat shock protein 80 isoforms (Hev b HSP80, latex, P); nucleoside diphosphate kinase (Dan re NDKB, muscle, F); myosin heavy chain isoforms (Myt g PM, muscle, M); transferrin (Bos d LF, muscle, B); actin isoforms (Sal s alpha-actin, muscle, F); troponin isoforms (Pon l 7, muscle, C, and Bla g 6, muscle, I); laminin isoforms (Bos d laminin, muscle, B); adenylate kinase (Epi co AdK, muscle, F); myosin light chain isoforms (Cra c 5, muscle, C, and Bla g 8, muscle, I); ATP synthase isoforms (Bos d OSCP, muscle, B); glucose-6-phosphate isomerase (Pan h 11, muscle, F); pyruvate kinase (Lit v PK, muscle, C); gelsolin (Der f 16, whole body, I); aspartate aminotransferase (Bos d AATr, muscle, B); arginine kinase (Per a 9, muscle, I); actinin (Der f actinin, whole body, I); triosephosphate isomerase (Pan h 8, muscle, F); superoxide dismutase (Sola l SOD, fruit, P); fructose-bisphosphate aldolase (Gal d 10, muscle, chicken); chitinase (Per a 12, whole body, I); malate dehydrogenase (Citr l MDH, fruit, P); hemocyanin isoforms (Per a 3, whole body, I); peroxiredoxin isoforms (Ory s 32, fruit, P, and Pen ma 3, Y); guanine nucleotide-binding protein isoforms (Bla g RACK1, whole body, I); phosphoglycerate kinase (cand a PGK, whole body, I); enolase (Bla g enolase, whole body, I); tropomyosin isoforms (Dro m 7, Tri ca 7, Bla ca 7, and Loc m 7, muscle, I); cyclophilin (Der f 29, whole body, I); calcium-transporting ATPase sarcoplasmic/endoplasmic reticulum type (Chi o SERCA, muscle, C); translation elongation factor 2 (Der f EF, whole body, I); nascent polypeptide-associated complex subunit alpha-like (Can f Homs2-like, dandruff, canis); profilin (Mus a 1, fruit, P); lactate dehydrogenase (Bos d LD, muscle, B); apolipophorin (Onc m 5, egg, F) (Supporting Information Table S9). The allergenic action of above-reported locust proteins was previously verified only in the case of muscle tropomyosin (Loc m 7, P31816)(Mari et al., 2009). In order to decrease potential allergic reaction, insects can be processed with use, for example enzymes or heat treatment (Pali-Schöll et al., 2019).

3.6. Foaming properties

Foaming properties were determined for leg muscle protein extracts due to higher extraction yield. Fig. 6 shows the foaming capacity of the muscle proteins extracted at pH 5.5, pH 7, and pH 8.5, when compared to reference whey proteins assayed at the same protein concentration. At pH 5.5, initial foaming capacity was the highest for whey proteins and muscle proteins extracted at pH 9, NaCl 0.6 M (57.1 %). At pH 7 and pH 8.5, foaming capacity for locust leg muscle extracts was in the range of 57.1–68.8 %, and for whey proteins 50.5–57.1 %. Incubation of foam for 60 min resulted in a noticeable decrease of the foam volume at a higher degree for whey proteins, than for locust extracts. Foam stability (Table 2) at pH 5.5 after one hour of incubation for all muscle extracts was significantly higher (66.7–70.8 %) than for whey proteins (38.3 %). A similar trend was observed at pH 7 with the highest value for locust leg muscle proteins extracted at pH 9 + 0.6 M NaCl (68.5 %). At pH 7, the foam stability of locust extracts was closer to that of whey proteins (51.1 %), than at pH 5.5, but significantly higher than whey proteins at both pH values. At pH 8.5, foam stability of whey proteins and locust muscle

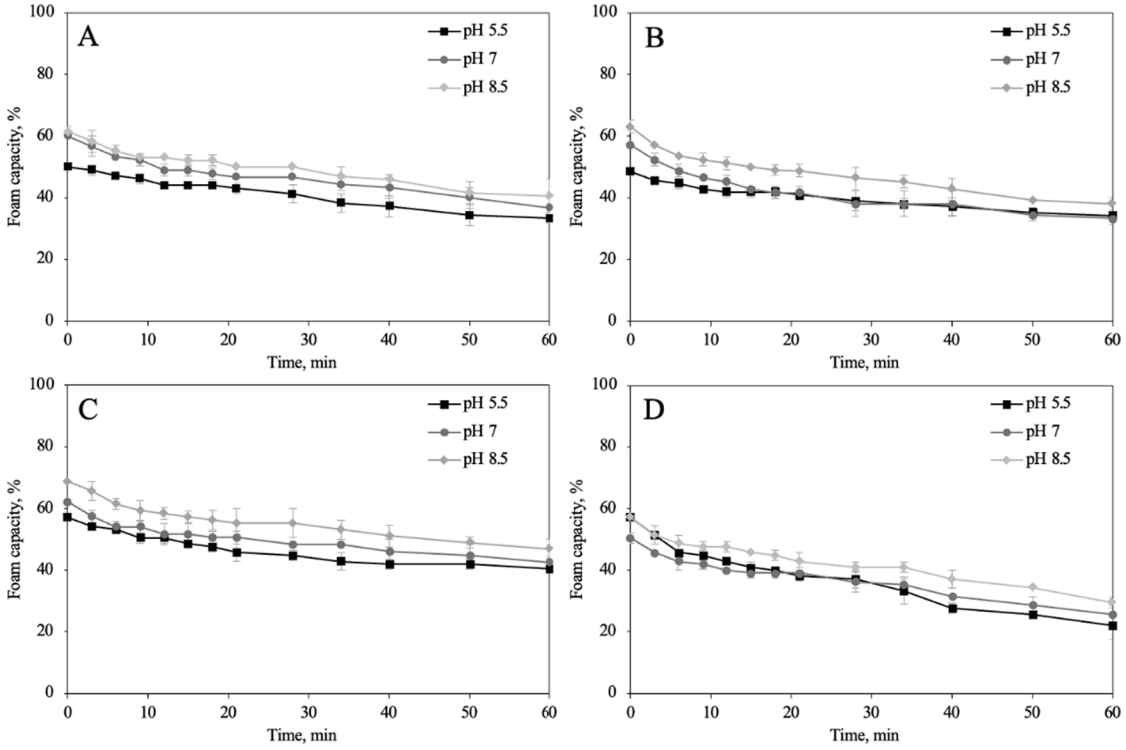

Fig. 6. Foaming capacity of locust leg muscle protein extracts obtained at pH 2 (A), pH 12 (B), pH 9 + 0.6 M NaCl (C), and of the reference whey proteins (D). Foaming capacity was evaluated as a function of pH and time (n = 3).

Table 2
Foam stability (%) of proteins extracted from locust leg muscles at pH 2, pH 12, and pH 9 + 0.6 M NaCl, and whey proteins.

Sample	Foam stability, %		
	pH 5.5	pH 7	pH 8.5
Whey proteins	38.3 ± 7.6^{a}	51.1 ± 7.3^{ab}	51.7 ± 2.9^{abc}
Locust leg muscle proteins:			
pH 2	66.7 ± 3.4^{cd}	61.1 ± 5.6^{bcd}	66.2 ± 9.9^{bcd}
pH 12	70.6 ± 0.0^{d}	58.3 ± 3.6^{bcd}	60.5 ± 4.6^{bcd}
pH 9 + 0.6 M NaCl	70.8 ± 1.4^{d}	68.5 ± 3.2^{d}	68.2 ± 4.5^{d}

Values are reported as mean ± S.D. (n = 3). Different superscripts in the same column denote significant difference between the samples ($p < 0.05$).

extracts (pH 2 and pH 12) was not significantly different (51.7 %, 66.2 %, and 60.5 %, respectively); however, this parameter for whey proteins was significantly lower than that of locust extracts obtained at pH 9 + 0.6 M NaCl (68.2 %).

According to Townsend and Nakai (Townsend & Nakai, 1983), high protein surface hydrophobicity and moderate dispersibility are ideal for foam formation. The balance between hydrophobicity and solubility allows the proteins to rapidly adsorb on the air–liquid interface and unfold, stabilizing the air bubbles. Based on the results of foam stability (Table 2), it can be assumed that the solubility of protein extracts obtained at pH 9 + 0.6 M NaCl was the highest when it was adjusted to pH 5.5, pH 7, and pH 8.5 for foam tests. In addition, it should be noted that net protein charge and charge distribution also affected foaming properties (Pacheco-Aguilar et al., 2008). The presence of 0.3 M NaCl in the buffer might therefore have a significant effect on these results.

As shown by proteomic analysis, locust leg muscle extract obtained at pH 12 contained a great number of muscle proteins. Previous studies assessed solubility, emulsifying and gelling properties of myofibrillar protein fractions from cod muscles obtained at pH 11 and 2.5 with following readjustment to 7.5 (Kristinsson & Hultin, 2003b). According to this study, the mechanism through which myosin molecules unfold at extreme pH differs between acidic and alkaline conditions. At high pH values, a fraction of myosin light chains dissociates, and heavy chains remain associated, while at low pH values, both heavy and light chains dissociate almost fully. Upon refolding at neutral pH values, the molecular structure of myosin is also different, as light chains remain partially dissociated after alkaline treatment (Kristinsson & Hultin, 2003b). It can be assumed that similar structural variations occurred in locust protein fractions obtained at extreme alkaline and acid pH values, but their occurrence should be confirmed by further studies for insect proteins. However, it should be noted that the extent of potential structural changed did not result in differences in foam stability in this study.

While the current study demonstrates extraction approaches at extreme pH or increased ionic strength, recent studies proposed other alternatives to solubilise muscle proteins. A recent review article summarized the main existing directions for the improvement of solubility and thermostability of myofibrillar proteins, which comprise glycosylation, use of amino acids, use of proteases, high-intensity ultrasound and high-pressure processing, and other methods (Wang et al., 2022). One of the most promising methods utilized for increasing the solubility of porcine myofibrillar proteins is based on the use of lysine and 0.3 M NaCl (to ensure low ionic strength) (Li et al., 2022). It should be noted that all methods have their positive and negative effects, which might impact the quality of the products where these food ingredients would be used. In this study, extracts obtained at extreme pH values had yellowish colour, which was not developed during salt-assisted extraction at milder pH values.

It should be noted that small size of locust legs possesses low feasibility of manual separation of locust leg muscles even considering the weak attachment of leg muscles to exoskeleton; accordingly, dedicated methods applicable for industrial scale should be further developed.

4. Conclusions

This study explored the applicability of protein extraction methods typically used for traditional animal sources in the case of whole locusts and insect leg muscles. Three tested extraction methods (acid-, alkaline-, and salt-assisted) were efficient in protein recovery with the highest yield at extreme pH 2 and pH 12 for locust leg muscles. Recovery of muscle proteins after extraction at pH 12 has been confirmed by proteomic analysis. The molecular mass distribution of extracted proteins varied strongly as a function of the starting material (whole locusts or insect leg muscles) and also based on pH value. It can be concluded that locust leg muscles possess similarities to muscle tissues of other animals, and the methods used for muscle extraction in the latter cases are also applicable to insects. In addition, proteins of locust leg muscles demonstrated promising results in their foaming properties. Further studies are needed to explore other techno-functional characteristics and how extracted insect materials can be used for food applications.

The process of industrial separation of legs from locust body is not yet developed. In this context, locust legs should be considered as an important by-product of locust consumption and/or processing and as a valuable source of muscle proteins. The re-use of this by-product should be highly considered, based on protein recovery and techno-functional characteristics. Thus, this study contributes to the development of knowledge and solutions for the postharvest processing of edible insects, which are important players of protein transition and future circular economy.

CRediT authorship contribution statement

Maryia Mishyna: Writing – review & editing, Writing – original draft, Visualization, Project administration, Formal analysis, Data curation, Conceptualization. **Valentina Ciaravolo:** Writing – review & editing, Writing – original draft, Visualization, Funding acquisition, Formal analysis, Data curation. **Maria Litsa:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation, Conceptualization. **Catriona Lakemond:** Writing – review & editing, Validation, Conceptualization. **Andrea Scaloni:** Writing – review & editing, Writing – original draft, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Vincenzo Fogliano:** Writing – review & editing, Resources, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2024.115228.

Data availability

Data will be made available on request.

References

Abdollahi, M., & Undeland, I. (2018). Structural, functional, and sensorial properties of protein isolate produced from salmon, cod, and herring by-products. *Food and Bioprocess Technology, 11*(9), 1733–1749. https://doi.org/10.1007/s11947-018-2138-x

AOAC Official Method. (2012). *Crude Protein in Meat and Meat Products including Pet Foods – Combustion Method. Chapter 39*, 6–7.

Arnesen, J. A., & Gildberg, A. (2006). Extraction of muscle proteins and gelatine from cod head. *Process Biochemistry, 41*(3), 697–700. https://doi.org/10.1016/j.procbio.2005.09.001

Barre, A., Pichereaux, C., Simplicien, M., Burlet-Schiltz, O., Benoist, H., & Rougé, P. (2021). A Proteomic- and Bioinformatic-Based Identification of Specific Allergens from Edible Insects: Probes for Future Detection as Food Ingredients. *Foods, 10*(2), 280. https://doi.org/10.3390/foods10020280

Ben Abdallah, M., Trupiano, D., Polzella, A., De Zio, E., Sassi, M., Scaloni, A., Zarrouk, M., Ben Youssef, N., & Scippa, G. S. (2018). Unraveling physiological, biochemical and molecular mechanisms involved in olive (Olea europaea L. cv. Chétoui) tolerance to drought and salt stresses. *Journal of Plant Physiology, 220*, 83–95. https://doi.org/10.1016/j.jplph.2017.10.009

Boulos, S., Tännler, A., & Nyström, L. (2020). Nitrogen-to-Protein Conversion Factors for Edible Insects on the Swiss Market: T. molitor, A. domesticus, and L. migratoria. *Frontiers in nutrition, 7*, 89. https://doi.org/10.3389/fnut.2020.00089

Bretscher, H., & O'Connor, M. B. (2020). The Role of Muscle in Insect Energy Homeostasis. *Frontiers in Physiology, 11*. https://doi.org/10.3389/fphys.2020.580687

Caspi, R., Billington, R., Keseler, I. M., Kothari, A., Krummenacker, M., Midford, P. E., Ong, W. K., Paley, S., Subhraveti, P., & Karp, P. D. (2020). The MetaCyc database of metabolic pathways and enzymes – a 2019 update. *Nucleic Acids Research, 48*(D1), D445–D453. https://doi.org/10.1093/nar/gkz862

Cerritos, R. (2009). Insects as food: An ecological, social and economical approach. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 4*(27), 1–10.

Chan, J. T. Y., Omana, D. A., & Betti, M. (2011). Functional and rheological properties of proteins in frozen turkey breast meat with different ultimate pH. *Poultry Science, 90*(5), 1112–1123. https://doi.org/10.3382/ps.2010-01185

Chen, X., Tume, R. K., Xu, X., & Zhou, G. (2017). Solubilization of myofibrillar proteins in water or low ionic strength media: Classical techniques, basic principles, and novel functionalities. *Critical Reviews in Food Science and Nutrition, 57*(15), 3260–3280. https://doi.org/10.1080/10408398.2015.1110111

Clarkson, C., Mirosa, M., & Birch, J. (2018). Potential of extracted *Locusta migratoria* protein fractions as value-added ingredients. *Insects, 9*(20). https://doi.org/10.3390/insects9010020

Egonyu, J. P., Subramanian, S., Tanga, C. M., Dubois, T., Ekesi, S., & Kelemu, S. (2021). Global overview of locusts as food, feed and other uses. *Global Food Security, 31*, Article 100574. https://doi.org/10.1016/j.gfs.2021.100574

Francis, F., Mazzucchelli, G., Baiwir, D., Debode, F., Berben, G., & Caparros Megido, R. (2020). Proteomics based approach for edible insect fingerprinting in novel food: Differential efficiency according to selected model species. *Food Control, 112*, Article 107135. https://doi.org/10.1016/j.foodcont.2020.107135

Geirsdottir, M., Hlynsdottir, H., Thorkelsson, G., & Sigurgisladottir, S. (2007). Solubility and Viscosity of Herring (*Clupea harengus*) Proteins as Affected by Freezing and Frozen Storage. *Journal of Food Science, 72*(7). https://doi.org/10.1111/j.1750-3841.2007.00471.x

Hamm, R. (1961). Biochemistry of meat hydration. In *Advances in food research* (Vol. 10, pp. 355–463). Academic Press. https://doi.org/10.1016/S0065-2628(08)60141-X.

Hashimoto, K., Watabe, S., Kono, M., & Shiro, K. (1979). Muscle Protein Composition of Sardine and Mackerel. *Nippon Suisan Gakkaishi, 45*(11), 1435–1441. https://doi.org/10.2331/suisan.45.1435

Hmidet, N., Balti, R., Nasri, R., Sila, A., Bougatef, A., & Nasri, M. (2011). Improvement of functional properties and antioxidant activities of cuttlefish (Sepia officinalis) muscle proteins hydrolyzed by Bacillus mojavensis A21 proteases. *Food Research International, 44*(9), 2703–2711. https://doi.org/10.1016/j.foodres.2011.05.023

Hong, G.-P., & Xiong, Y. L. (2012). Microbial transglutaminase-induced structural and rheological changes of cationic and anionic myofibrillar proteins. *Meat Science, 91*(1), 36–42. https://doi.org/10.1016/j.meatsci.2011.12.002

Hooper, S. L., & Thuma, J. B. (2005). Invertebrate Muscles: Muscle Specific Genes and Proteins. *Physiological Reviews, 85*(3), 1001–1060. https://doi.org/10.1152/physrev.00019.2004

Hrynets, Y., Omana, D. A., Xu, Y., & Betti, M. (2010). Effect of Acid- and Alkaline-Aided Extractions on Functional and Rheological Properties of Proteins Recovered from Mechanically Separated Turkey Meat (MSTM). *Journal of Food Science, 75*(7). https://doi.org/10.1111/j.1750-3841.2010.01736.x

Iwamoto, H. (2011). Structure, function and evolution of insect flight muscle. *Biophysics, 7*, 21–28. https://doi.org/10.2142/biophysics.7.21

Janssen, R. H., Vincken, J.-P., Van Den Broek, L. A. M., Fogliano, V., & Lakemond, C. M. M. (2017). Nitrogen-to-protein conversion factors for three edible insects: Tenebrio molitor, Alphitobius diaperinus, and Hermetia illucens. *Journal of Agricultural and Food Chemistry, 65*, 2275–2278. https://doi.org/10.1021/acs.jafc.7b00471

Jonas-Levi, A., & Martinez, J. J. I. (2017). The high level of protein content reported in insects for food and feed is overestimated. *Journal of Food Composition and Analysis, 62*, 184–188. https://doi.org/10.1016/j.jfca.2017.06.004

Jongema, Y. (2017). *List of edible insects of the world.*

Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y., & Morishima, K. (2017). KEGG: New perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Research, 45*(D1), D353–D361. https://doi.org/10.1093/nar/gkw1092

Kanost, M. R., Kawooya, J. K., Law, J. H., Ryan, R. O., Van Heusden, M. C., & Ziegler, R. (1990). Insect haemolymph proteins. In *Advances in insect physiology* (Vol. 22, pp. 299–396). Academic Press. https://doi.org/10.1016/S0065-2806(08)60008-9.

Kristinsson, H. G., & Hultin, H. O. (2003a). Changes in Conformation and Subunit Assembly of Cod Myosin at Low and High pH and after Subsequent Refolding. *Journal of Agricultural and Food Chemistry, 51*(24), 7187–7196. https://doi.org/10.1021/jf026193m

Kristinsson, H. G., & Hultin, H. O. (2003b). Effect of low and high pH treatment on the functional properties of cod muscle proteins. *Journal of Agricultural and Food Chemistry, 51*(17), 5103–5110. https://doi.org/10.1021/jf026138d

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227*(5259), 680–685.

Li, X., Wang, W., Wang, S., Shen, Y., Pan, J., Dong, X., & Li, S. (2022). The Solubility and Structures of Porcine Myofibrillar Proteins under Low-Salt Processing Conditions as Affected by the Presence of L-Lysine. *Foods, 11*(6), 855. https://doi.org/10.3390/foods11060855

Malva, A. D., Albenzio, M., Santillo, A., Russo, D., Figliola, L., Caroprese, M., Marino, R., & Díaz-Cruz, J. M. (2018). Methods for Extraction of Muscle Proteins from Meat and Fish Using Denaturing and Nondenaturing Solutions. *Journal of Food Quality, 2018*. https://doi.org/10.1155/2018/8478471

Mari, A., Rasi, C., Palazzo, P., & Scala, E. (2009). Allergen databases: Current status and perspectives. *Current Allergy and Asthma Reports, 9*(5), 376–383. https://doi.org/10.1007/s11882-009-0055-9

Milacic, M., Beavers, D., Conley, P., Gong, C., Gillespie, M., Griss, J., Haw, R., Jassal, B., Matthews, L., May, B., Petryszak, R., Ragueneau, E., Rothfels, K., Sevilla, C., Shamovsky, V., Stephan, R., Tiwari, K., Varusai, T., Weiser, J., & D'Eustachio, P. (2024). The Reactome Pathway Knowledgebase 2024. *Nucleic Acids Research, 52*(D1), D672–D678. https://doi.org/10.1093/nar/gkad1025

Mishyna, M., Keppler, J. K., & Chen, J. (2021). Techno-functional properties of edible insect proteins and effects of processing. *Current Opinion in Colloid & Interface Science, 56*, Article 101508. https://doi.org/10.1016/j.cocis.2021.101508

Mishyna, M., Martinez, J. I., Chen, J., & Benjamin, O. (2019). Extraction, characterization and functional properties of soluble proteins from edible grasshopper (*Schistocerca gregaria*) and honey bee (*Apis mellifera*). *Food Research International, 116*, 697–706. https://doi.org/10.1016/j.foodres.2018.08.098

Mo, X., Romano, D., Milazzo, M., Benelli, G., Ge, W., & Stefanini, C. (2020). Impact of Different Developmental Instars on *Locusta migratoria* Jumping Performance. *Applied Bionics and Biomechanics, 2020*, 1–11. https://doi.org/10.1155/2020/2797486

Mohan, M., Ramachandran, D., Sankar, T. V., & Anandan, R. (2007). Influence of pH on the solubility and conformational characteristics of muscle proteins from mullet (Mugil cephalus). *Process Biochemistry, 42*(7), 1056–1062. https://doi.org/10.1016/j.procbio.2007.04.005

Murphy, S., Dowling, P., & Ohlendieck, K. (2016). Comparative Skeletal Muscle Proteomics Using Two-Dimensional Gel Electrophoresis. *Proteomes, 4*(3), 27. https://doi.org/10.3390/proteomes4030027

Muthukrishnan, S., Mun, S., Noh, M. Y., Geisbrecht, E. R., & Arakane, Y. (2020). Insect cuticular chitin contributes to form and function. *Current Pharmaceutical Design, 26*(29), 3530–3545. https://doi.org/10.2174/1381612826666200523175409

Nave, R., & Weber, K. (1990). A myofibrillar protein of insect muscle related to vertebrate titin connects Z band and A band: Purification and molecular characterization of invertebrate mini-titin. *Journal of Cell Science, 95*(4), 535–544. https://doi.org/10.1242/jcs.95.4.535

Ni, X., Chen, C., Li, R., Liu, Q., Duan, C., Wang, X., & Xu, M. (2024). Effects of ultrasonic treatment on the structure and functional characteristics of myofibrillar proteins from black soldier fly. *International Journal of Biological Macromolecules, 278*, Article 135057. https://doi.org/10.1016/j.ijbiomac.2024.135057

Nolsøe, H., & Undeland, I. (2009). The Acid and Alkaline Solubilization Process for the Isolation of Muscle Proteins: State of the Art. *Food and Bioprocess Technology, 2*(1), 1–27. https://doi.org/10.1007/s11947-008-0088-4

Norman, A. P. (1995). Adaptive Changes in Locust Kicking and Jumping Behaviour During Development. *Journal of Experimental Biology, 198*(6), 1341–1350. https://doi.org/10.1242/jeb.198.6.1341

Omana, D. A., Moayedi, V., Xu, Y., & Betti, M. (2010). Alkali-aided protein extraction from chicken dark meat: Textural properties and color characteristics of recovered proteins. *Poultry Science, 89*(5), 1056–1064. https://doi.org/10.3382/ps.2009-00441

Oonincx, D. G. A. B., & de Boer, I. J. M. (2012). Environmental Impact of the Production of Mealworms as a Protein Source for Humans – A Life Cycle Assessment. *PLoS ONE, 7*(12), 1–5. https://doi.org/10.1371/journal.pone.0051145

Oonincx, D. G. A. B., van Itterbeeck, J., Heetkamp, M. J. W., van den Brand, H., van Loon, J. J. A., & van Huis, A. (2010). An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. *PLoS ONE, 5*(12), e14445. https://doi.org/10.1371/journal.pone.0014445

Pacheco-Aguilar, R., Mazorra-Manzano, M. A., & Ramírez-Suárez, J. C. (2008). Functional properties of fish protein hydrolysates from Pacific whiting (Merluccius productus) muscle produced by a commercial protease. *Food Chemistry, 109*(4), 782–789. https://doi.org/10.1016/j.foodchem.2008.01.047

Pali-Schöll, I., Meinlschmidt, P., Larenas-Linnemann, D., Purschke, B., Hofstetter, G., Rodríguez-Monroy, F. A., Einhorn, L., Mothes-Luksch, N., Jensen-Jarolim, E., &

Jäger, H. (2019). Edible insects: Cross-recognition of IgE from crustacean- and house dust mite allergic patients, and reduction of allergenicity by food processing. *World Allergy Organization Journal, 12*(1). https://doi.org/10.1016/j.waojou.2018.10.001

Pérez-Mateos, M., & Lanier, T. C. (2007). Comparison of Atlantic menhaden gels from surimi processed by acid or alkaline solubilization. *Food Chemistry, 101*(3), 1223–1229. https://doi.org/10.1016/j.foodchem.2006.03.026

Purschke, B., Stegmann, T., Schreiner, M., & Jäger, H. (2017). Pilot-scale supercritical CO2 extraction of edible insect oil from Tenebrio molitor L. larvae – Influence of extraction conditions on kinetics, defatting performance and compositional properties. *European Journal of Lipid Science and Technology, 119*(2), 1–12. https://doi.org/10.1002/ejlt.201600134

Purschke, B., Tanzmeister, H., Meinlschmidt, P., Baumgartner, S., Lauter, K., & Jäger, H. (2018). Recovery of soluble proteins from migratory locust (*Locusta migratoria*) and characterisation of their compositional and techno-functional properties. *Food Research International, 106*, 271–279. https://doi.org/10.1016/j.foodres.2017.12.067

Raghavan, S., & Kristinsson, H. G. (2008). Conformational and rheological changes in catfish myosin during alkali-induced unfolding and refolding. *Food Chemistry, 107*(1), 385–398. https://doi.org/10.1016/j.foodchem.2007.08.037

Ramachandran, D., Mohan, M., & Sankar, T. V. (2007). Physicochemical characteristics of muscle proteins from barracuda (Sphyraena jello) of different weight groups. *LWT – Food Science and Technology, 40*(8), 1418–1426. https://doi.org/10.1016/j.lwt.2006.09.010

Robertson, R. M. (1988). Insect Flight (Locust). In *Comparative Neuroscience and Neurobiology* (pp. 53–54). Birkhäuser Boston. https://doi.org/10.1007/978-1-4899-6776-3_23.

Rose, A., Chiu, Y.-C., Showman, C., Ku, K. M., Jaczynski, J., & Matak, K. (2023). Characterization of protein concentrates obtained by defatting cricket, locust, and silkworm powders using one-step organic solvent extraction. *LWT, 182*, Article 114876. https://doi.org/10.1016/j.lwt.2023.114876

Siddiqui, S. A., Ghisletta, M., Yunusa, B. M., Abdullahi, F. J., Saraswati, Y. R., Fernando, I., Nagdalian, A. A., Gvozdenko, A. A., Shah, M. A., Lorenzo, J. M., & Dar, B. N. (2023). Grasshoppers and locusts as human foods – a comprehensive review. *Journal of Insects as Food and Feed, 1–18*. https://doi.org/10.3920/JIFF2023.0010

Sweers, L. J. H., Mishyna, M., Boom, R. M., Fogliano, V., Keppler, J. K., & Lakemond, C. M. M. (2023). Microfiltration for effective microbiological decontamination of edible insects – Protein hydrolysis, aggregation and pH are critical for protein recovery. *Food and Bioproducts Processing, 141*, 128–138. https://doi.org/10.1016/j.fbp.2023.08.002

Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., Gable, A. L., Fang, T., Doncheva, N. T., Pyysalo, S., Bork, P., Jensen, L. J., & von Mering, C. (2023). The STRING database in 2023: Protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Research, 51*(D1), D638–D646. https://doi.org/10.1093/nar/gkac1000

The Gene Ontology Consortium. (2017). Expansion of the Gene Ontology knowledgebase and resources. *Nucleic Acids Research, 45*(D1), D331–D338. https://doi.org/10.1093/nar/gkw1108

Townsend, A., & Nakai, S. (1983). Relationships Between Hydrophobicity and Foaming Characteristics of Food Proteins. *Journal of Food Science, 48*(2), 588–594. https://doi.org/10.1111/j.1365-2621.1983.tb10796.x

Turck, D., Castenmiller, J., Henauw, S De, Hirsch-ernst, K. I., Kearney, J., Maciuk, A., Mangelsdorf, I., Mcardle, H. J., Naska, A., Pelaez, C., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vinceti, M., Cubadda, F., Frenzel, T., Heinonen, M., Marchelli, R., & Neuh, M. (2021). Safety of frozen and dried formulations from migratory locust (*Locusta migratoria*) as a Novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal, 19*(May). https://doi.org/10.2903/j.efsa.2021.6667

van Huis, A. (2003). Insects as food in sub-saharan africa. *Insect Science and Its Application, 23*(3), 163–185.

van Huis, A., Van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G., & Vantomme, P. (2013). Edible insects: Future prospects for food and feed security. *FAO*.

Vitale, A., Rocco, M., Arena, S., Giuffrida, F., Cassaniti, C., Scaloni, A., Lomaglio, T., Guarnaccia, V., Polizzi, G., Marra, M., & Leonardi, C. (2014). Tomato susceptibility to Fusarium crown and root rot: Effect of grafting combination and proteomic analysis of tolerance expression in the rootstock. *Plant Physiology and Biochemistry, 83*, 207–216. https://doi.org/10.1016/j.plaphy.2014.08.006

Volmer Hans. (1981). Temperature-dependence of the functional activities of sarcoplasmic reticulum from locust, cockroach and crayfish muscle. *Comparative Biochemistry and Physiology, 70A*, 351–358.

Wang, K., Li, Y., Zhang, Y., Luo, X., & Sun, J. (2022). Improving myofibrillar proteins solubility and thermostability in low-ionic strength solution: A review. *Meat Science, 189*, Article 108822. https://doi.org/10.1016/j.meatsci.2022.108822

Wilde, P., & Clark, D. (1996). Foam formation and stability. *Methods of Testing Protein Functionality*, 110–152.

Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., Garnett, T., Tilman, D., & Declerck, F. (2019). Food in the Anthropocene: The EAT – Lancet Commission on healthy diets from sustainable food systems. *The Lancet Commissions*. https://doi.org/10.1016/S0140-6736(18)31788-4

Xiong, Y. L. (1997). Protein denaturation and functionality losses. In *Quality in frozen food* (pp. 111–140). Boston, MA: Springer, US.

Yi, L., Van Boekel, M. A. J. S., Boeren, S., & Lakemond, C. M. M. (2016). Protein identification and in vitro digestion of fractions from Tenebrio molitor. *European Food Research and Technology, 242*, 1285–1297. https://doi.org/10.1007/s00217-015-2632-6

Yi, L., Van Boekel, M. A. J. S., & Lakemond, C. M. M. (2016). Extracting Tenebrio molitor protein while preventing browning: effect of pH and NaCl on protein yield. *Journal of Insects as Food and Feed*, 1–12. https://doi.org/10.3920/JIFF2016.0015

Zhao, D., He, J., Zou, X., Nian, Y., Xu, X., Zhou, G., & Li, C. (2020). Influence of salting process on the structure and in vitro digestibility of actomyosin. *Journal of Food Science and Technology, 57*(5), 1763–1773. https://doi.org/10.1007/s13197-019-04210-w

Zhao, D. J., Zhang, Z. Y., Harrison, J., & Kang, L. (2012). Genome-wide analysis of transcriptional changes in the thoracic muscle of the migratory locust, *Locusta migratoria*, exposed to hypobaric hypoxia. *Journal of Insect Physiology, 58*(11), 1424–1431. https://doi.org/10.1016/j.jinsphys.2012.08.006

Zhou, D., Noviello, C., D'Ambrosio, C., Scaloni, A., & D'Adamio, L. (2004). Growth Factor Receptor-bound Protein 2 Interaction with the Tyrosine-phosphorylated Tail of Amyloid β Precursor Protein Is Mediated by Its Src Homology 2 Domain. *Journal of Biological Chemistry, 279*(24), 25374–25380. https://doi.org/10.1074/jbc.M400488200