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ABSTRACT

Locust protein hydrolysates (LoProHs) pre-processed with microwave and ultrasonication were developed and evaluated for their potential for enhancing the quality of the stored meat emulsion (MEmul). Locust protein (LoPro) samples pre-processed with ultrasonication (Ult) or microwave (Mic) or with no treatment (Not) were hydrolysed with alcalase enzyme (3%). The microwave pre-processed (Mic-LoProHs) and ultrasonicated (Ult-LoProHs) hydrolysates showed significantly ($P < 0.05$) higher antioxidant [FRAP (ferric reducing antioxidant power) and ABTS and DPPH radical scavenging activities] and antimicrobial [minimum inhibitory concentration (MIC) and inhibitory halos (mm)] potential. The MEmul samples incorporated with Mic-LoProHs and Ult-LoProHs at the maximum level of 1.5% exhibited significantly ($P < 0.05$) improved results for all the quality parameters such as antioxidant potential (FRAP, ABTS and DPPH), protein oxidation (total carbonyl content), lipid stability, and microbial quality during refrigerated storage (4 ± 1 °C) of two-weeks compared to the control MEmul without any LoProHs. A positive ($P < 0.05$) impact of the LoProHs was found on the sensory quality of MEmul samples after one week of storage. The digestion simulation improved ($P < 0.05$) the antioxidant potential of the MEmul samples.

1. Introduction

Climate change and other pressing environmental issues, such as pollution, environmental degradation, and resource depletion, have led to an impetus in the quest for sustainable protein sources both in the Western and Eastern worlds to feed the expanding human and livestock population in future [1]. Among the various viable options, protein production through insect farming seems highly attractive due to its association with a lower carbon footprint and environmental inputs (such as feed, water, and land) compared to the current livestock farming systems and its ability to produce cheap and high-quality protein alternatives for human consumption [2,3].

While the consumption of insects is common in many cultures in Africa, Asia, and South America and >2000 different edible insects are already consumed in over 113 countries by more than two billion people, the industry is gaining access to new markets in many developed countries [4]. More and more insect protein-based food products or novelties are being introduced commercially in Western countries, such as spreads based on mealworms (Belgium), cricket protein-based air-puffed chips (the USA), and locust protein-based crackers (France), with success and social acceptance [5]. The edible insects market is predicted to boom in the near future and is estimated to grow at over 47% compound annual growth rate and reach from a global market size of 112 million (US$) in 2019 to 710 billion in 2026 (US$) [6]. The main attractions for using insects as a food source are the ease of availability, high protein content and quality, low-calorie and fat concentration, and

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their low cost of production, all these characteristics along with shifting trends in dietary needs are likely to increase the demand and stimulate market outlook [6].

Among the edible insects, locusts and crickets are more popular and commonly consumed as dietary ingredients and have a high social acceptance as food [7,8]. With a favourable nutritional profile, locusts are considered a healthy source of food and about 10 different species have a long history of consumption by humans and animals in about 65 countries [7]. Rats consuming acute and sub-chronic levels of locust powder showed little to no signs of toxicity, indicating the safety of the protein as a dietary ingredient [9]. The locusts (*Locusta migratoria*) are a rich source of nutrients and contain high-quality protein (~51–71%, DWB) and fat (~11–35%) with a favourable ratio of omega-3: omega-6 (~0.57) and high amounts of omega-3 fatty acids (~16%) and iron (~0.008–0.015%) [10,11,12]. However, the consumption of LoPro can induce allergenicity and cause harm to sensitive people [3,13]. Pre-treatment of insect proteins, such as thermal processing of LoPro or microwave processing of cricket proteins, has been reported to significantly reduce immunoreactivity by inducing protein conformational changes and affecting the native structure of the allergen and epitopes [14,15]. Enzymatic hydrolysis is another way of reducing the allergenicity of insect proteins with no reactivity observed after 65–80% hydrolysis of cricket protein using alcalase enzyme [16]. Similar results have been reported for the hydrolysates of lesser mealworm and black soldier fly [17]. In addition to this positive effect, hydrolysis of insect proteins produces peptides with strong antioxidant and antimicrobial properties [3,15] making them a suitable candidate as a bio-preservative for animal foods, which are vulnerable to protein and lipid oxidation and microbial spoilage. Literature is silent about the use of locust protein hydrolysates (LoProHs) as a bio-preservative in foods. Therefore, this study was carried out with the objective to develop the LoProHs and evaluate the impact of pre-treatments (Mic and Ult) on the antimicrobial and antioxidant activities of the LoProHs. Another objective was to investigate the efficacy of the LoProHs as an additive in stored MEmul. MEmul samples incorporated with LoProHs were analysed for quality over 14 days of chilled storage. The impact of gastrointestinal digestion simulation was also investigated on the antioxidant activity of the MEmul samples.

2. Material and methods

2.1. Enzymes and raw materials

Analytical-grade chemicals were supplied by standard firms such as Sigma-Aldrich (Bangalore, India). The HI-Media (Mumbai, India) supplied readymade media for microbiological analysis and enzymes for *in vitro* gastrointestinal digestion [pancreatin (≥6 USP U/mg, ≥75 USP U/mg, and ≥75 USP U/mg for lipase, amylase, and protease activities, respectively and pepsin (1000 NF U/mg)]. The locust flour was purchased from the Thailand-based manufacturer JR Unique Foods Ltd. (Udon Thani, Thailand Unique Brand, Thailand) who farmed the locusts (*L. migratoria*) using grasses and vegetables and produced the flour within the factory that was HACCP (Hazard analysis and critical control points) accredited and approved by the FDA (The United States Food and Drug Administration). The flour was 100% locust powder and natural (no added preservatives, artificial colours, and flavours) and contained 71% (DMB) crude protein. After harvesting and cleaning, the locusts were dried, powdered, and vacuum packaged. The alcalase enzyme was used for preparing the LoProHs (Sigma Aldrich, Billerica MA, USA, ≥2.4 AU/g, pH 7–9 and 35–60 °C for optimum activity). This enzyme is highly effective for chitinous materials, such as insects, and has been reported to produce hydrolysates with high antioxidant and antimicrobial potential [16,18,19].

2.2. Preparation of LoProHs

Food-grade hexane was used for defatting the LoPro flour before its hydrolysis with alcalase enzyme. Studies have reported the use of hexane for the preparation of insect protein-based ingredients with superior techno-functional characteristics for food processing applications [16,19]. The method elaborated by Lone et al. [20] was employed by mixing the LoPro flour with n-hexane (1:5 ratio, w/v). The mix was stirred (30 min) and the oil was removed by centrifugation (2,000 rpm, 7 min). This was followed by filtration using Whatman filter paper No. 1 and the remained hexane was evaporated from the defatted flour during the overnight oven drying process at 70 °C. The defatted flour was vacuum packaged and stored (4 ± 1 °C) until used.

The procedure elaborated by Lone et al. [20] was followed to hydrolyse the defatted LoPro flour (200 g) by homogenizing it in a domestic blender (high speed, 2 min) in two volumes of water (w/v) and pasteurising at 90 °C (15 min) in a water bath. The hydrolysis process was allowed for 90 min at 50 °C at a pre-adjusted pH (8.0) using 3% (w/w) alcalase enzyme for maximal degree of hydrolysis. The reaction mixture was shaken during hydrolysis without any pH adjustment. The conditions followed during hydrolysis were decided on the available literature [16,19] and the preliminary trials conducted in the laboratory to produce the LoProHs with high bioactivity and excellent protein functionality. The enzyme activity was ceased by thermally treating the hydrolysed samples at 90 °C (15 min) followed by centrifugation (15 min at 5000 rpm at 4 °C,) to collect the clear supernatants containing amino acids and soluble peptides. The supernatants were lyophilized and evaluated for antioxidant and antimicrobial properties [FRAP, ABTS, and DPPH, MIC (minimum inhibitory concentration), and inhibitory halos] and employed for the development of the MEmuls at a sensorially acceptable level of 1.5%. The freeze-dried (INNOVA Bio Meditech Inc., INOFD-12S: Freeze Dryer, USA) LoProHs were stored in polystyrene tubes (−20 °C) until used.

The antimicrobial and antioxidant activities of the LoProHs were improved using two different pre-treatments (ultrasonication and microwave) and therefore, three different types of LoProHs were developed. The LoProHs developed with no treatment (as described above) were designated as 'Not-LoProHs' whereas the LoProHs prepared by pre-processing the samples with microwave or ultrasonication before hydrolysis were designated as Mic-LoProHs and Ult-LoProHs, respectively. The defatted LoPro samples were pre-processed in a microwave (IFB 30L, IFB-30BRC2, India) for 10 min at 90 °C for the production of Mic-LoProHs [21] and the rest of the hydrolysis process was the same as that described for Not-LoProHs. The Ult-LoProHs were developed by pre-processing the defatted LoPro samples in a beaker placed in an ice bath with ultrasound (15 min, 20 kHz, 500 W, pulse duration of 2/2 s on/off) using a Cole-Parmer ultrasonic processor with a flat tip probe (U.S.A make, Model WW-04711-45). The temperature did not exceed 50 °C during the processing and the rest of the procedure was the same as that discussed for Not-LoProHs. The treatment settings used during ultrasonication and microwave processing were selected on the findings of previous studies and the preliminary trials conducted in the laboratory focused to yield LoProHs with high antimicrobial and antioxidant activities.

2.3. Preparation of mutton emulsion (MEmul)

The lean meat obtained from thigh muscles (main muscles included *Semitendinosus*, *Semimembranosus, Rectus femoris, Biceps femoris, Vastus medialis, Vastus lateralis, Pectinius,* and *Gracilis*) of six carcasses (both sides, n = 6) from 3-year-old sheep was used and minced two times employing a 6 mm plate in an Italian make mincer (Sirman, Marsango). The curing ingredients [sodium tripolyphosphate (0.3% w/w), sodium nitrite (120 ppm), and NaCl (1.5% w/w)] were added to the minced meat (68.20%) and chopped in an Italian make chopper (Sirman, Marsango) for 1.5 min [22]. The next step involved the addition of ice flakes

(10% w/w, 1 min chopping) and refined soyabean oil (9% w/w, 1 min chopping). Finally, all other ingredients [condiment mixture (5% w/w), spice mixture (2% w/w), and refined wheat flour (4% w/w)] and LoProHs (replaced 1.5% meat in treated MEmuls) were added (2 min chopping) to obtain the MEmul/batter. The level of incorporation of the LoProHs (1.5%) was found sensorily during preliminary trials. Four different types of MEmuls were prepared viz. MEmul samples without the addition of LoProHs (control), MEmul samples containing Not-LoProHs (1.5%), MEmul samples with Mic-LoProHs (1.5%) and MEmul samples with Ult-LoProHs (1.5%). The MEmul samples were packaged in low-density polyethene bags separately and were kept under chilled conditions (4 ± 1 °C) for 14 days. The MEmul samples were examined for various quality characteristics (lipid and protein stability, microbiological and sensory quality, and physicochemical parameters) on days 0, 7, and 14.

2.4. Gastrointestinal digestion of MEmul samples

The *in vitro* method of digestion elaborated by [23] was followed to perform the gastrointestinal simulation using polyvinyl containers and magnetic multi-stirrer. The pepsic phase of digestion was performed for 1 h using pepsin in HCl (0.1 M, pH 1.9 ± 0.1) whereas the intestinal phase was performed for 2 h using pancreatin [phosphate buffer (0.1 M), pH 8.0]. The substrate: enzyme ratio of 100: 1 w/w was used for both phases and the digesta were continuously stirred using magnetic fleas (37 °C). The digested samples were collected after completion of the digestion, the pH was adjusted to inactivate the enzymes and were centrifuged (4000*g*, 15 min) to separate the supernatants which were examined for FRAP and ABTS and DPPH scavenging activities.

2.5. Physicochemical parameters, oxidative stability and antioxidant potential

The antioxidant capacity of the LoProHs and the MEmul samples were assessed using DPPH and ABTS free radical scavenging and FRAP assays as elaborated by Kouser et al. [24]. The methods elaborated by [25] were employed for TBARS (mg MDA/kg) and free fatty acids (% oleic acid) whereas DNPH (dinitrophenylhydrazine) method by [24] was followed for determining total carbonyl content (nmol/mg protein) of the MEmul samples. The methods described by [26] and [27] were utilized for determining the pH and moisture content of the MEmul samples, respectively.

2.6. Microbiological analysis

The stored MEmul samples were examined for microbiological quality (log_{10} CFU/g) by enumerating total plate (total plate agar), psychrophilic (total plate agar), yeast/mould (potato dextrose agar) and coliform counts (violet-red bile agar) following pour plate technique as described by [28]. The antimicrobial activity of the LoProHs was assessed by the disc agar diffusion method (inhibitory halos, mm) and the minimum inhibitory concentration (MIC) against *E. coli* and *S. aureus* [24].

2.7. Sensory analysis

The sensory analysis of the stored MEmul was performed by a trained panel comprised of five female and five male panellists (25–50 years old) [29]. The 8-point descriptive scale (1 anchored to 'disliked extremely' and 8 anchored to 'liked extremely') was used to evaluate the samples thrice (10 panellists × 03 replications for each treatment) for four sensory attributes (overall acceptability, texture, flavour, and colour and appearance). The training for sensory panellists included tests for four basic tastes and other routine sensory tests (such as descriptive and hedonic tests). The MEmul (30 g samples) was oven cooked (20 min at 180 °C) and served at 40 °C as coded samples along with potable water. The study complied with ethical guidelines and regulations.

2.8. Statistical analysis

The data was collected for storage quality and other parameters while performing the experiments (six replications, n = 6, days 0, 7, and 14) and examined by ANOVA (one-way or two-way) using version 21.0 of SPSS. The significance between the pair of means was determined by DMRT (Duncan's multiple range test, 0.05 significance level) and the results as means ± standard errors are presented in figures and tables.

3. Results and discussion

3.1. Antioxidant and physicochemical properties

Figs. 1A and 2(A, B, C) present the data about the antioxidant potential (FRAP ABTS, and DPPH) of the LoProHs and MEmul samples during refrigerated storage. The LoProHs showed a strong capacity to scavenge ABTS and DPPH radicals and the capacity to reduce Fe^{3+} ions. The LoProHs processed with microwave (Mic-LoProHs) and ultrasonication (Ult-LoProHs) exhibited higher ($P < 0.05$) values for FRAP, DPPH, and ABTS compared to the untreated samples (Not-LoProHs). The antioxidant potential of the LoProHs followed a significant order viz. Ult-LoProHs > Mic-LoProHs > Not-LoProHs and was reflected in the antioxidant potential of the MEmul. The MEmul samples enriched with Ult-LoProHs and Mic-LoProHs exhibited significantly ($P < 0.05$) higher means for the antioxidant parameters in comparison to the Not-LoProHs and MEmul without LoProHs throughout the 14 days of storage. The results for all the antioxidant parameters of the MEmul samples followed a significant ($P < 0.05$) order viz. Ult-LoProHs > Mic-LoProHs > Not-LoProHs > control. Our results indicated the strong antioxidant potential of LoProHs and their addition significantly improved the antioxidant capacity of the stored MEmul. Research papers have reported a significant increase in the antioxidant properties of the insect proteins on hydrolysis with alcalase enzyme. For example, a recent study [30] reported a significant rise in the ORAC (oxygen radical absorbance capacity) and ABTS values of alcalase hydrolysed cricket (*G. sigillatus*) protein compared to unhydrolyzed protein. Similarly, Hall et al. [16] reported a strong antioxidant potential (FRAP, ABTS and DPPH) of the hydrolysates produced from cricket protein with alcalase enzyme. The presence of the peptides and amino acids with antioxidant properties was suggested to be responsible for this radical scavenging and antioxidant potential of the hydrolysates.

Our results also indicated the effect of microwave and ultrasonication as pre-processing to improve the antioxidant potential of the LoProHs. Both these technologies can induce protein conformational and microstructural alterations and modulate the hydrolysis of proteins by facilitating the ingress of the enzymes to the cleavage sites [31]. Both ultrasonication and microwave have been reported to enhance the antioxidant activities of insect proteins without affecting the protein quality, increasing their suitability for use as an ingredient for food applications. A recent study [15] recorded a significant increase in the degree of hydrolysis and the production of bioactive peptides from alcalase hydrolysed cricket protein pre-processed with the microwave. While the use of alcalase as a hydrolysing enzyme has been found to increase the production of antioxidant amino acids during the hydrolysis of silk moth [33], thermal pre-treatments (100 or 150 °C for 10 min) enhanced the antioxidant activity of three different InsecProHs including the LoProHs (*S. gregaria*) [34]. Recent papers have investigated the impact of ultrasonication on the antioxidant potential of insect proteins. For example, Kingwascharapong et al. [35] reported a significant rise in the FRAP, ABTS, and DPPH values of LoPro pre-processed with ultrasound (10–30 min, 20 kHz, 750 W). Mintah et al. [36] reported a significant rise in the ABTS, FRAP, and superoxide scavenging activities of the hydrolysates prepared from ultrasonicated protein extracted from *H. illucens* (600 W, 40 kHz) and observed alterations in

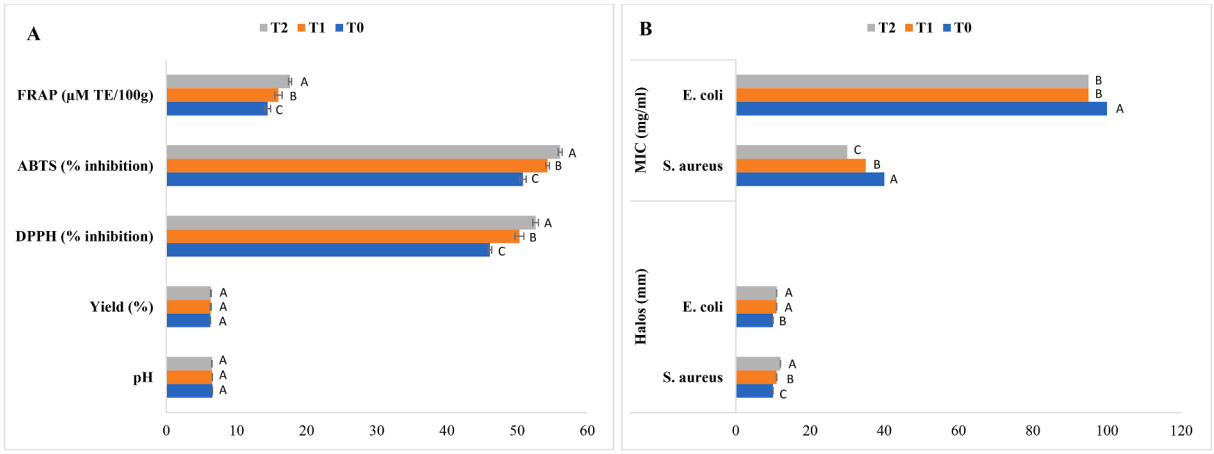


Fig. 1. Effect of pre-treatments on physicochemical, antioxidant (A) and antimicrobial (B) properties of the hydrolysates. Mean ± SE with different superscripts for each parameter differ significantly. n = 6 (for each treatment), n = 3 for MIC, TE = Trolox equivalents. One-way ANOVA was used at a 0.05 level of significance. T0 = locust protein hydrolysates with no treatment. T1 = locust protein hydrolysates pre-treated with microwave. T2 = locust protein hydrolysates pre-treated with ultrasonication

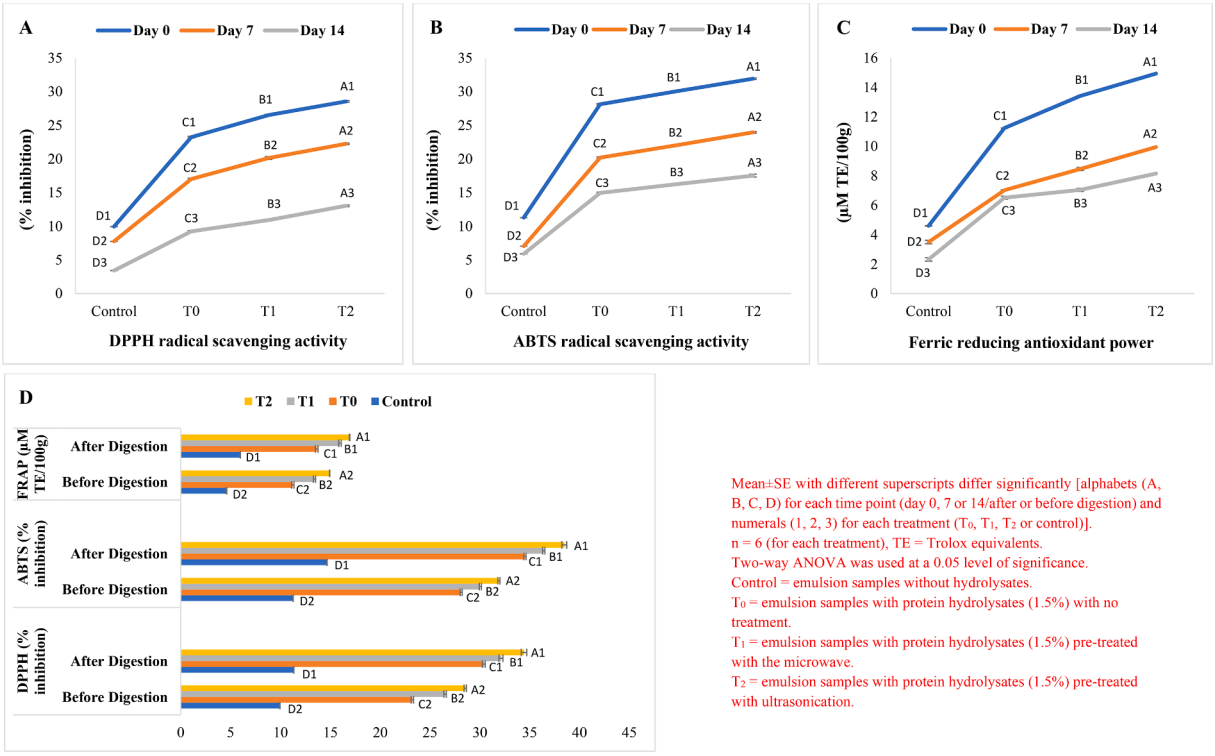


Fig. 2. Effect of hydrolysates (A, B, C) and *in vitro* gastrointestinal digestion (D) on the antioxidant potential of the meat emulsion. Mean ± SE with different superscripts differ significantly [alphabets (A, B, C, D) for each time point (day 0, 7 or 14/after or before digestion) and numerals (1, 2, 3) for each treatment (T_0, T_1, T_2 or control)]. n = 6 (for each treatment), TE = Trolox equivalents. Two-way ANOVA was used at a 0.05 level of significance. Control = emulsion samples without hydrolysates. T_0 = emulsion samples with protein hydrolysates (1.5%) with no treatment. T_1 = emulsion samples with protein hydrolysates (1.5%) pre-treated with the microwave. T_2 = emulsion samples with protein hydrolysates (1.5%) pre-treated with ultrasonication.

the microstructure, protein secondary structure, and the particle size.

No significant ($P > 0.05$) impact of the processing (ultrasonication and microwave) was recorded on the pH and yield of the LoProHs (Fig. 1A). Enrichment of the MEmul with LoProHs exhibited no significant ($P > 0.05$) impact on the moisture content throughout the storage time (Days 0, 7, and 14) (Table 1). However, the pH of the MEmul enriched with LoProHs exhibited significantly ($P < 0.05$) lower values on days 7 and 14 in comparison to MEmul without LoProHs. The addition of the LoProHs significantly reduced the oxidative changes and microbial spoilage of the stored MEmul and might have reduced the

Table 1
Effect of locust protein hydrolysates (LoProHs) on lipid stability, protein oxidation and physicochemical properties of meat emulsion (MEmul).

Treatment	Storage period (days)		
	0	7	14
pH			
Control	6.21 ± 0.03^{Aa}	6.46 ± 0.01^{Ab}	6.64 ± 0.03^{Ac}
Not	6.23 ± 0.05^{Aa}	6.32 ± 0.02^{Bb}	6.41 ± 0.05^{Bc}
Mic	6.25 ± 0.04^{Aa}	6.30 ± 0.01^{Bb}	6.39 ± 0.07^{Bc}
Ult	6.26 ± 0.05^{Aa}	6.28 ± 0.01^{Bb}	6.37 ± 0.04^{Bc}
Moisture (%)			
Control	63.21 ± 0.32^{Aa}	62.81 ± 0.33^{Aa}	61.21 ± 0.34^{Ab}
Not	63.18 ± 0.33^{Aa}	62.88 ± 0.38^{Aa}	61.26 ± 0.47^{Ab}
Mic	63.16 ± 0.34^{Aa}	62.94 ± 0.30^{Aa}	61.29 ± 0.30^{Ab}
Ult	63.14 ± 0.44^{Aa}	62.96 ± 0.37^{Aa}	61.31 ± 0.46^{Ab}
TBARS [mg malondialdehyde (MDA)/kg]			
Control	0.39 ± 0.06^{Aa}	0.68 ± 0.01^{Ab}	0.92 ± 0.01^{Ac}
Not	0.38 ± 0.04^{Aa}	0.61 ± 0.01^{Bb}	0.84 ± 0.01^{Bc}
Mic	0.37 ± 0.06^{Aa}	0.52 ± 0.01^{Cb}	0.71 ± 0.01^{Cc}
Ult	0.37 ± 0.06^{Aa}	0.46 ± 0.01^{Db}	0.65 ± 0.01^{Dc}
	FFA (% oleic acid)		
Control	0.097 ± 0.004^{Aa}	0.156 ± 0.003^{Ab}	0.226 ± 0.008^{Ac}
Not	0.096 ± 0.003^{Aa}	0.142 ± 0.002^{Bb}	0.205 ± 0.006^{Bc}
Mic	0.094 ± 0.006^{Aa}	0.131 ± 0.004^{Cb}	0.193 ± 0.004^{Cc}
Ult	0.093 ± 0.004^{Aa}	0.120 ± 0.005^{Db}	0.181 ± 0.007^{Dc}
Total carbonyl content (nmol/mg protein)			
Control	1.09 ± 0.03^{Aa}	1.89 ± 0.02^{Ab}	2.18 ± 0.02^{Ac}
Not	1.08 ± 0.03^{Aa}	1.70 ± 0.02^{Bb}	1.99 ± 0.02^{Bc}
Mic	1.07 ± 0.04^{Aa}	1.59 ± 0.02^{Cb}	1.83 ± 0.02^{Cc}
Ult	1.07 ± 0.03^{Aa}	1.46 ± 0.01^{Db}	1.27 ± 0.05^{Dc}

Mean ± SE with different superscripts in a row (lower case alphabet) and column (upper case alphabet) differ significantly ($P < 0.05$).
Two-way ANOVA was used at a 0.05 level of significance.
n = 6 for each treatment.
Control = MEmul samples without hydrolysates.
Not = MEmul samples with LoProHs (1.5%) with no treatment.
Mic = MEmul samples with LoProHs (1.5%) pre-treated with microwave.
Ult = MEmul samples with LoProHs (1.5%) pre-treated with ultrasonication.

production of breakdown compounds produced during the microbial spoilage and oxidative damage of the muscle proteins [37]. Enrichment of muscle foods with additives with a preservative potential, such as herbal extracts, has been documented to reduce the rate of spoilage during storage and a consequent increase in pH [37].

3.2. Lipid stability

Enrichment of MEmul with LoProHs (1.5%) exhibited a significant positive impact on the lipid stability of stored MEmul (Table 1). The MEmul enriched with LoProHs exhibited a significant ($P < 0.05$) decline in TBARS and FFA values on days 7 and 14, displaying the efficacy of the LoProHs in retarding the rate of lipid oxidation and lipolytic changes in stored MEmul. The MEmul enriched with Mic-LoProHs and Ult-LoProHs exhibited significantly ($P < 0.05$) lower means and the results followed a significant order viz. Ult-LoProHs < Mic-LoProHs < Not-LoProHs < control. Due to their high-fat content and unsaturated fatty acid profile, muscle foods are prone to oxidative and lipolytic changes and require measures to maintain acceptable quality during storage. Several workers have evaluated the application of food hydrolysates to control lipid oxidation in fat-rich foods such as fish meat emulsion. Li et al. [38] successfully used hydrolysates from zein protein to retard the oxidation of lipids and lipolytic changes in common carp protein emulsions during storage.

The significantly higher antioxidant potential of the processed (Mic and Ult) LoProHs might be responsible for the higher lipid stability of the MEmul containing Mic-LoProHs and Ult-LoProHs during storage. Both these technologies (Mic and Ult) have been reported to improve the antioxidant potential of InsecProHs and insect proteins. Processing with ultrasound (600 W, 40 kHz) significantly improved the antioxidative properties of InsecProHs and isolates [36]. Combining ultrasound (10–30 min, 750 W, 20 kHz) with the extraction process of protein from Bombay locusts yielded a product with the highest electron-donating ability and radical scavenging properties [35]. The ultrasonication processing induced protein unfolding by altering the surface hydrophobicity, changes which can accelerate the hydrolysis process and release of antioxidative peptides [32]. Similar to ultrasonication, the microwave pre-treatment of the cricket proteins has been recorded to catalyse the hydrolysis process, reduce the immunoreactivity of the hydrolysates, and enhance the production of peptides with higher bioactivities [15]. This positive effect was attributed to the microwave-induced conformation changes which led to protein unfolding and increased access of enzymes to hydrolytic sites on proteins.

3.3. Protein oxidation

Enrichment with LoProHs (1.5%) exhibited a significant ($P < 0.05$) positive impact on protein oxidation (total carbonyl content) of stored MEmul (Table 1). The MEmul enriched with LoProHs exhibited a significant ($P < 0.05$) decline in total carbonyl content (nmol/mg protein) on days 7 and 14, displaying the efficacy of the LoProHs in retarding the rate of protein oxidation in stored MEmul. The MEmul enriched with Mic-LoProHs and Ult-LoProHs exhibited significantly ($P < 0.05$) lower means and the results followed a significant order viz. Ult-LoProHs < Mic-LoProHs < Not-LoProHs < control. Oxidation of proteins in muscle foods during storage leads to an increase in their carbonyl content [39]. The capacity of the processed LoProHs to scavenge the free radicals and reduce metal ions necessary to inhibit oxidation might be ascribed to the significant decline in the carbonyl content of the MEmul samples containing Mic-LoProHs and Ult-LoProHs [40]. Previous works have examined the efficacy of food protein hydrolysates as bio-preservatives to inhibit the oxidation of proteins in muscle foods. While Mukherjee and Haque [41] used casein protein hydrolysates to reduce protein oxidation in catfish fillet/beef steak during storage, Li et al. [42] and Jónsdóttir et al. [43] successfully used whey protein hydrolysates and cod protein hydrolysates/peptides to retard the lipid and protein oxidation in common carp surimi and minced cod, respectively.

3.4. Microbiological characteristics

A significant positive impact of the processing (Mic and Ult) was recorded on the antimicrobial properties (MIC and Inhibitory halos) of the LoProHs and the processed LoProHs viz. Ult-LoProHs and Mic-LoProHs exhibited significantly ($P < 0.05$) lower MIC values (mg/ml) against the examined microbes (*E. coli* and *S. aureus*) (Fig. 1B). The values of 100, 95, and 95 mg/ml were observed to inhibit the growth of *E. coli* for Not-LoProHs, Mic-LoProHs, and Ult-LoProHs whereas 40, 35, and 30 mg/ml were found to inhibit the growth of *S. aureus,* respectively. A similar trend was found for inhibitory halos and the pre-processed LoProHs viz. Ult-LoProHs and Mic-LoProHs exhibited significantly ($P < 0.05$) large-sized halos against both the examined microbes compared to Not-LoProHs. Both these parameters (MIC and inhibitory halos) indicated the antimicrobial activity of the LoProHs and their suitability as a food bio-preservative. A limited number of published papers have evaluated the antimicrobial activities of insect proteins and InsecProHs which needs scientific attention. Park et al. [44] studied the efficacy of the hydrolysates and the ethanol-extracted fraction of fly maggots (*M. domestica*) and found a minimum concentration of 60–40 μg/ml against various *S. aureus* strains.

Enrichment with LoProHs significantly ($P < 0.05$) enhanced the microbiological quality of stored MEmul (Table 2). The MEmul enriched

Table 2
Effect of locust protein hydrolysates (LoProHs) on the microbiological quality of meat emulsion (MEmul).

Treatments	Storage period (days)		
	0	7	14
Total plate count (log_{10} CFU/g)			
Control	1.53 ± 0.04^{Aa}	2.52 ± 0.02^{Ab}	3.68 ± 0.02^{Ac}
Not	1.51 ± 0.05^{Aa}	2.32 ± 0.02^{Bb}	3.54 ± 0.01^{Bc}
Mic	1.49 ± 0.05^{Aa}	2.22 ± 0.02^{Cb}	3.41 ± 0.01^{Cc}
Ult	1.48 ± 0.04^{Aa}	2.06 ± 0.02^{Db}	3.28 ± 0.01^{Dc}
Psychrophilic count (log_{10} CFU/g)			
Control	ND	ND	0.98 ± 0.01^{A}
Not	ND	ND	0.87 ± 0.01^{B}
Mic	ND	ND	0.69 ± 0.01^{C}
Ult	ND	ND	0.44 ± 0.01^{D}
Coliform count (log_{10} CFU/g)			
Control	ND	ND	1.18 ± 0.05^{A}
Not	ND	ND	1.03 ± 0.01^{B}
Mic	ND	ND	0.89 ± 0.01^{C}
Ult	ND	ND	0.71 ± 0.01^{D}
Yeast and mould count (log_{10} CFU/g)			
Control	ND	ND	1.98 ± 0.04^{A}
Not	ND	ND	1.83 ± 0.01^{B}
Mic	ND	ND	1.70 ± 0.01^{C}
Ult	ND	ND	1.58 ± 0.03^{D}

Mean ± SE with different superscripts in a row (lower case alphabet) and column (upper case alphabet) differ significantly ($P < 0.05$).
Two-way ANOVA was used at a 0.05 level of significance.
n = 6 for each treatment.
ND = Not detected (Detection limit < 10 cfu/g).
Control = MEmul samples without hydrolysates.
Not = MEmul samples with LoProHs (1.5%) with no treatment.
Mic = MEmul samples with LoProHs (1.5%) pre-treated with microwave.
Ult = MEmul samples with LoProHs (1.5%) pre-treated with ultrasonication.

with LoProHs exhibited a significant ($P < 0.05$) decline in total plate counts on days 7 and 14 and the lowest ($P < 0.05$) counts were recorded for the MEmul enriched with Ult-LoProHs followed by Mic-LoProHs and Not-LoProHs. While psychrophiles, coliforms, and yeast/moulds appeared on day 14, the counts exhibited a similar pattern viz. Ult-LoProHs < Mic-LoProHs < Not-LoProHs < control. This favourable impact of the LoProHs on microbial quality during storage might be attributed to the antimicrobial peptides present in the LoProHs. A large number of published papers have employed alcalase to hydrolyse food proteins to yield peptides and soluble hydrolysates with antimicrobial properties [45]. The peptides with antimicrobial activity are typically small in size (<10 kDa, ≤50 amino residues) and operate through varied mechanisms and can disrupt the microbial cell membranes through electrostatic interactions or act through iron chelating activity [40]. While a large number of antimicrobial peptides occur naturally in the humoral immune system of insects with broad-spectrum activities, InsecProHs, such as LoProHs (*L. migratoria*), have been reported to contain high levels of small peptides and hydrophobic amino acids [30].

3.5. Effect of digestion simulation on the antioxidant potential of MEmul

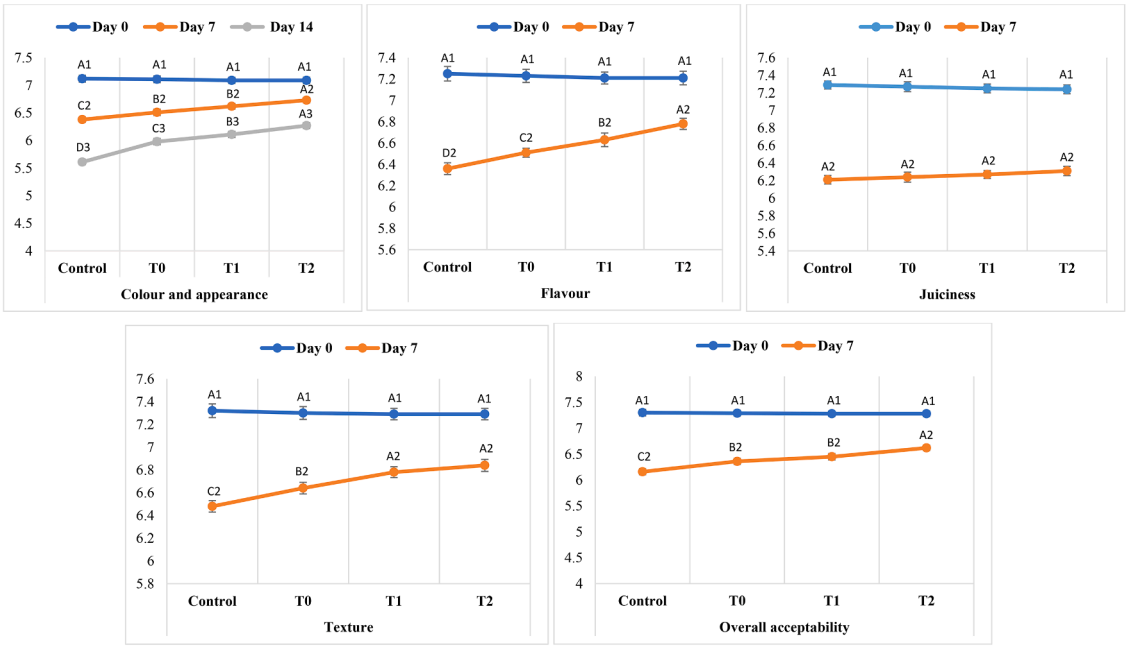
The gastrointestinal simulation was performed to evaluate the impact of digestion on the antioxidant activity of stored MEmul which can have a favourable impact on consumer health [30]. The digestion process exhibited a favourable impact on the antioxidant activity of the MEmul and a significant ($P < 0.05$) hike was recorded in the values of FRAP, DPPH, and ABTS for all MEmul samples digested using pepsin and pancreatin (Fig. 2D). The presence of the LoProHs also exhibited a favourable impact and the digested MEmul samples enriched with LoProHs exhibited significantly ($P < 0.05$) higher means for DPPH, ABTS, and FRAP in comparison to the MEmul without LoProHs. The strong radical scavenging ability of the LoProHs processed with Ult or Mic (Ult-LoProHs and Mic-LoProHs) also exhibited an effect and significantly ($P < 0.05$) higher means were observed for MEmul containing Ult-LoProHs and Mic-LoProHs followed by Not-LoProHs and control. The LoPro (*L. migratoria*) is susceptible to hydrolysis by digestive proteases, such as trypsin and chymotrypsin, released in the intestine and produce peptides and hydrophobic amino acids responsible for the antioxidant activity of the LoProHs. However, LoPro is mostly resistant to gastric pepsin due to a high level of branched-chain amino acids [9]. The published research has documented an increase in the antioxidant activity of InsecProHs after digestion simulation. The digestion of Mexican grasshopper (*S. purpurascens*) protein produced hydrolysates with a wide range of antioxidant peptides and hydrophobic amino acids ascribed to their high antioxidant potential measured in terms of ABTS and DPPH radical scavenging activities [46]. The cricket protein hydrolysates produced using alcalase enzyme showed a significant hike in FRAP, DPPH, and ABTS activities after digestion simulation due to further hydrolysis and production of hydrophobic peptides and amino acids [30,16]. Intake of InsecProHs and peptides with high radical scavenging and ion-reducing ability can show a favourable impact on consumer health and have been reported to increase the lifespan of a stress-induced nematode (*C. elegans*) [30].

3.6. Sensory analysis

Enrichment with LoProHs (1.5%) exhibited a significant ($P < 0.05$) positive impact on the sensory quality of stored MEmul (Table 1). The MEmul enriched with LoProHs exhibited significant ($P < 0.05$) higher scores for overall acceptability, texture, flavour, and colour and appearance on day 7 (Fig. 3). The MEmul enriched with Mic-LoProHs and Ult-LoProHs exhibited significantly ($P < 0.05$) higher scores for all sensory attributes (except juiciness) and the results followed a significant order viz. Ult-LoProHs > Mic-LoProHs > Not-LoProHs > control. The MEmuls were not assessed for sensory attributes (except colour and appearance) on day 14 due to the presence of coliforms. No effect ($P > 0.05$) of the LoProHs was observed on all sensory attributes on day 0 and juiciness throughout the storage time. The positive effect of the LoProHs on MEmul's sensorial quality might be ascribed to their preservative potential which helped to retard oxidative and microbial changes during storage as indicated by several parameters discussed above. The oxidation and microbial spoilage cause the degradation of lipids and proteins and produce off-flavour compounds, fatty acids and metabolites. In a recent study, Althwab et al. [47] reported high overall acceptability for bread samples containing up to 4% locust (*L. migratoria*) flour.

4. Conclusions

The present study revealed a method for the production of LoProHs with antioxidant and antimicrobial activities using alcalase enzyme. Processing of the LoPro with Mic or Ult significantly enhanced the preservative potential of the LoProHs as indicated by the results of inhibitory halos, minimum inhibitory concentration, FRAP, ABTS, and DPPH. The efficacy of the LoProHs was evaluated for their suitability as a bio-preservative in the MEmul. Enrichment of the MEmul with LoProHs (1.5%) successfully enhanced its storage quality. The LoProHs, especially Ult-LoProHs or Mic-LoProHs, exhibited a significant positive impact on the results of lipid and protein oxidation and the microbiological and sensory quality of MEmul during two weeks of storage. The antioxidant potential of the MEmul containing the LoProHs was significantly improved after gastrointestinal digestion simulation. Overall, it can be concluded that LoProHs have a preservative potential and can be used for enhancing the storage stability and nutritive value of fat and protein-rich food products. The influence of cooking on the preservative potential of LoProHs is unclear and future studies should investigate the



Mean±SE with different superscripts differ significantly [alphabets (A, B, C, D) for each time point (day 0, 7 or 14) and numerals (1, 2, 3) for each treatment (T_0, T_1, T_2 or control)]. Repeated measurements ANOVA was used at a 0.05 level of significance, 10 trained panellists performed the sensory evaluation thrice for each treatment at each time point (days 0, 7 and 14) using an 8-point descriptive scale, Control = emulsion samples without hydrolysates, T0 = emulsion samples with protein hydrolysates (1.5%) with no treatment, T1 = emulsion samples with protein hydrolysates (1.5%) pre-treated with microwave, T2 = emulsion samples with protein hydrolysates (1.5%) pre-treated with ultrasonication

Fig. 3. Effect of locust protein hydrolysates on sensory quality of the mutton emulsion. Mean ± SE with different superscripts differ significantly [alphabets (A, B, C, D) for each time point (day 0, 7 or 14) and numerals (1, 2, 3) for each treatment (T_0, T_1, T_2 or control)]. Repeated measurements ANOVA was used at a 0.05 level of significance, 10 trained panellists performed the sensory evaluation thrice for each treatment at each time point (days 0, 7 and 14) using an 8-point descriptive scale, Control = emulsion samples without hydrolysates, T0 = emulsion samples with protein hydrolysates (1.5%) with no treatment, T1 = emulsion samples with protein hydrolysates (1.5%) pre-treated with microwave, T2 = emulsion samples with protein hydrolysates (1.5%) pre-treated with ultrasonication.

efficacy of LoProHs in other food models to promote food sustainability and enhance food security.

CRediT authorship contribution statement

Shavinder Singh: Investigation. **Hina F. Bhat:** Writing – review & editing, Resources, Conceptualization. **Sunil Kumar:** Supervision, Methodology. **Aunzar B. Lone:** Investigation. **Rana Muhammad Aadil:** Methodology, Writing – review & editing, Validation. **Abderrahmane Aït-Kaddour:** Methodology, Validation, Writing – review & editing. **Abdo Hassoun:** Data curation, Validation, Writing – review & editing. **Charalampos Proestos:** Resources, Data curation, Methodology, Writing – review & editing. **Zuhaib F. Bhat:** Writing – original draft, Methodology, Supervision, 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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References

[1] A. Hassoun, A.E.D. Bekhit, A.R. Jambrak, C. Gardiner, F. Chemat, J.D. Morton, M. Guðjónsdóttir, M.C. Rodríguez, M.A.P. Lage, J.P. Varela, R.N. Arshad, R. M. Aadil, Z.F. Bhat, Ø. Ueland, The fourth industrial revolution in the food industry-Part II: Emerging food trends, Crit. Rev. Food Sci. Nutr. (2022), https://doi.org/10.1080/10408398.2022.2106472.

[2] D. Meshulam-Pascoviche, T. David-Birman, G. Refael, U. Lesmes, Big opportunities for tiny bugs: Processing effects on the techno-functionality and digestibility of edible insects, Trends Food Sci. Technol. 122 (2022) 265–274.

[3] A. Baiano, Edible insects: An overview on nutritional characteristics, safety, farming, production technologies, regulatory framework, and socio-economic and ethical implications, Trends Food Sci. Technol. 100 (2020) 35–50.

[4] J. Tao, Potential utilization of edible insects in extruded rice products to address malnutrition issues in developing countries. Master of Science Thesis, California State Polytechnic University, Pomona (2016) Download from https://scholarworks.calstate.edu/downloads/kk91fn64j.

[5] B. Lamsal, H. Wang, P. Pinsirodom, A.T. Dossey, Applications of insect derived protein ingredients in food and feed industry, J. Am. Oil Chem. Soc. 96 (2019) 105–123.

[6] Global Market Insights, Edible insects market size by product (beetles, caterpillars, grasshoppers, bees, wasps, ants, scale insects and tree bugs), by application (flour, protein bars, snacks), industry analysis report, regional outlook, application potential, price trends, competitive market share and forecast, 2020-2026 (2020) Report ID: GMI501, https://www.gminsights.com/industry-analysis/edible-insects-market.

[7] J.P. Egonyu, S. Subramanian, C.M. Tanga, T. Dubois, S. Ekesi, S. Kelemu, Global overview of locusts as food, feed and other uses, Global Food Security 31 (2021), 100574.

[8] A. Rowe, Insects farmed for food and feed-global scale, practices, and policy. DOI: 10.31219/osf.io/nh6k3, Center for Open Science (2020) Downloaded from: https://www.scienceopen.com/document?vid=e16e9dd0-b37c-446c-b288-4afc7f2a82b5.

[9] M. Ochiai, K. Tezuka, H. Yoshida, T. Akazawa, Y. Komiya, H. Ogasawara, Y. Adachi, M. Nakada, Edible insect *Locusta migratoria* shows intestinal protein digestibility and improves plasma and hepatic lipid metabolism in male rats, Food Chem. 396 (2022), 133701.

[10] E.N. Brogan, Y.L. Park, K.E. Matak, J. Jaczynski, Characterization of protein in cricket (*Acheta domesticus*), locust (*Locusta migratoria*), and silkworm pupae (*Bombyx mori*) insect powders, LWT-Food Sci. Technol. 152 (2021), 112314.

[11] C. Clarkson, M. Mirosa, J. Birch, Potential of extracted Locusta migratoria protein fractions as value-added ingredients, Insects 9 (2018) 20.

[12] D.G.A.B. Oonincx, A.F.B. van der Poel, Effects of diet on the chemical composition of migratory locusts (*Locusta migratoria*), Zoo Biol. 30 (2011) 9–16.

[13] Y. Wang, Y. Zhang, H. Lou, C. Wang, M. Ni, D. Yu, L. Zhang, L. Kang, Hexamerin-2 protein of locust as a novel allergen in occupational allergy, J. Asthma Allergy 15 (2022) 145–155.

[14] P. Phiriyangkul, C. Srinroch, C. Srisomsap, D. Chokchaichamnankit, P. Punyarit, Effect of food thermal processing on allergenicity proteins in Bombay locust (*Patanga succincta*), Int. J. Food Eng. 1 (2015) 23–28.

[15] F. Hall, A. Liceaga, Effect of microwave-assisted enzymatic hydrolysis of cricket (*Gryllodes sigillatus*) protein on ACE and DPP-IV inhibition and tropomyosin-IgG binding, J. Funct. Foods 64 (2020), 103634.

[16] F. Hall, P.E. Johnson, A. Liceaga, Effect of enzymatic hydrolysis on bioactive properties and allergenicity of cricket (*Gryllodes sigillatus*) protein, Food Chem. 262 (2018) 39–47.
[17] G. Leni, T. Tedeschi, A. Faccini, F. Pratesi, C. Folli, I. Puxeddu, P. Migliorini, N. Gianotten, J. Jacobs, S. Depraetere, A. Caligiani, S. Sforza, Shotgun proteomics, in-silico evaluation and immunoblotting assays for allergenicity assessment of lesser mealworm, black soldier fly and their protein hydrolysates, Sci. Rep. 10 (2020) 228.
[18] S. Khammuang, R. Sarnthima, K. Sanachai, Purification and identification of novel antioxidant peptides from silkworm pupae (*Bombyx mori*) protein hydrolysate and molecular docking study, Biocat. Agric. Biotechnol. 42 (2022), 102367.
[19] F.G. Hall, O.G. Jones, M.E. O'Haire, A.M. Liceaga, Functional properties of tropical banded cricket (*Gryllodes sigillatus*) protein hydrolysates, Food Chem. 224 (2017) 414–422.
[20] A.B. Lone, H.F. Bhat, A. Aït-Kaddour, A. Hassoun, R.M. Aadil, B.N. Dar, Z.F. Bhat, Cricket protein hydrolysates pre-processed with ultrasonication and microwave improved storage stability of goat meat emulsion, Innov. Food Sci. Emerg. Technol. 86 (2023), 103364.
[21] H. Uluko, S. Zhang, L. Liu, M. Tsakama, J. Lu, J. Lv, Effects of thermal, microwave, and ultrasound pretreatments on antioxidative capacity of enzymatic milk protein concentrate hydrolysates, J. Funct. Foods 18 (2015) 11381146.
[22] I.K. Kalem, Z.F. Bhat, S. Kumar, L. Wang, R.J. Mudiyanselage, H.F. Bhat, *Tinospora cordifolia*: A novel bioactive ingredient for edible films for improved lipid oxidative and microbial stability of meat products, J. Food Process. Preser. 42 (11) (2018), e13774.
[23] Z.F. Bhat, J.D. Morton, S.L. Mason, A.E.A. Bekhit, Pulsed electric field improved protein digestion of beef during *in vitro* gastrointestinal simulation, LWT-Food Sci. Technol. 102 (2019) 45–51.
[24] F. Kouser, S. Kumar, H.F. Bhat, A. Hassoun, A.E.A. Bekhit, Z.F. Bhat, *Aloe barbadensis* based bioactive edible film improved lipid stability and microbial quality of cheese, Foods 12 (2022) 229.
[25] S. Kaur, S. Kumar, Z.F. Bhat, Utilization of pomegranate seed powder and tomato powder in the development of fiber-enriched chicken nuggets, Nutr. Food Sci. 45 (2015) 793–807.
[26] I.K. Kalem, Z.F. Bhat, S. Kumar, A. Desai, *Terminalia arjuna*: A novel natural preservative for improved lipid oxidative stability and storage quality of muscle foods, Food Sci. Hum. Well. 6 (2017) 167–175.
[27] P.K. Singh, S. Kumar, Z.F. Bhat, P. Kumar, Effect of clove oil on the storage quality of aerobically packaged fiber-enriched chevon cutlets, J. Meat Sci. 10 (2014) 36–43.
[28] F.A. Zargar, S. Kumar, Z.F. Bhat, P. Kumar, Effect of incorporation of carrot on the quality characteristics of chicken sausages, Indian J. Poult. Sci. 52 (2017) 91–95.
[29] S.R. Ahmed, V. Pathak, Z.F. Bhat, S.A.A. Bukhari, Effect of sorbic acid on the storage quality of *kaladhi*, an acid-coagulated milk product, J. Food Sci. Technol. 51 (12) (2014) 4040–4046.
[30] N. Mudd, F.S. Martin-Gonzalez, M. Ferruzzi, A.M. Liceaga, *In vivo* antioxidant effect of edible cricket (*Gryllodes sigillatus*) peptides using a *Caenorhabditis elegans* model, Food Hydrocoll. Health 2 (2022), 100083.
[31] Z.F. Bhat, J.D. Morton, A.E.A. Bekhit, S. Kumar, H.F. Bhat, Emerging processing technologies for improved digestibility of muscle proteins, Trends Food Sci. Technol. 110 (2021) 226–239.
[32] Z.F. Bhat, J.D. Morton, S. Kumar, H.F. Bhat, R.M. Adil, A.E.A. Bekhit, Ultrasonication as an emerging technology for processing of animal derived foods: a focus on *in vitro* protein digestibility, Trends Food Sci. Technol. 124 (2022) 309–322.
[33] Y. Liu, S. Wan, J. Liu, Y. Zou, S. Liao, Antioxidant activity and stability study of peptides from enzymatically hydrolyzed male silkmoth, J. Food Process. Preser. 41 (1) (2017), e13081.
[34] E. Zielinska, B. Baraniak, M. Karas, E. Zielinska, B. Baraniak, M. Karas, Antioxidant and anti-inflammatory activities of hydrolysates and peptide fractions obtained by enzymatic hydrolysis of selected heat-treated edible insects, Nutrients 9 (2017) 970.
[35] P. Kingwascharapong, M. Chaijan, S. Karnjanapratum, Ultrasound-assisted extraction of protein from Bombay locusts and its impact on functional and antioxidative properties, Sci. Rep. 11 (2021) 17320.
[36] B.K. Mintah, R. He, M. Dabbour, J. Xiang, A.A. Agyekum, H. Ma, Techno-functional attribute and antioxidative capacity of edible insect protein preparations and hydrolysates thereof: Effect of multiple mode sonochemical action, Ultrason. Sonochem. 58 (2019), 104676.
[37] S. Dua, Z.F. Bhat, S. Kumar, Effect of lemon peel extract on the oxidative stability and storage quality of *tabak-maz*, traditional fried mutton ribs, Nutr. Food Sci. 45 (2015) 662–676.
[38] Y. Li, B. Kong, Q. Liu, X. Xia, H. Chen, Improvement of the emulsifying and oxidative stability of myofibrillar protein prepared oil-in-water emulsions by addition of zein hydrolysates, Process Biochem. 53 (2017) 116–124.
[39] M. Estévez, Y.L. Xiong, Protein oxidation in foods: mechanisms, consequences, and antioxidant solutions, Foods 10 (2021) 2346.
[40] J. Tkaczewska, Peptides and protein hydrolysates as food preservatives and bioactive components of edible films and coatings-A review, Trends Food Sci. Technol. 106 (2020) 298–311.
[41] D. Mukherjee, Z.Z. Haque, Reduced protein carbonylation of cube steak and catfish fillet using antioxidative coatings containing cheddar whey, casein hydrolysate and oolong tea extract, Annals Food Sci. Technol. 17 (2) (2016) 529–536.
[42] Y. Li, B. Kong, X. Xia, Q. Liu, P. Li, Inhibition of frozen storage-induced oxidation and structural changes in myofibril of common carp (*Cyprinus carpio*) surimi by cryoprotectant and hydrolysed whey protein addition, Int. J. Food Sci. Technol. 48 (9) (2013) 1916–1923.
[43] R. Jónsdóttir, M. Geirsdóttir, P.Y. Hamaguchi, P. Jamnik, H.G. Kristinsson, I. Undeland, The ability of *in vitro* antioxidant assays to predict the efficiency of a cod protein hydrolysate and brown seaweed extract to prevent oxidation in marine food model systems, J. Sci. Food Agric. 96 (6) (2016) 2125–2135.
[44] S.O. Park, J.H. Shin, W.K. Choi, B.S. Park, J.S. Oh, A. Jang, Antibacterial activity of house fly-maggot extracts against MRSA (Methicillin-resistant *Staphylococcus aureus*) and VRE (Vancomycin-resistant *enterococci*), J. Env. Biol. 31 (2010) 865–871.
[45] V.G. Tacias-Pascacio, R. Morellon-Sterling, E.-H. Siar, O. Tavanoe, A. Berenguer-Murcia, R. Fernandez-Lafuente, Use of Alcalase in the production of bioactive peptides: A review, Int. J. Biol. Macromol. 165 (2020) 2143–2196.
[46] V.M. Villasenor, J.N. Enriquez-Vara, J.E. Urías-Silva, E.D.C. Lugo-Cervantes, D. A. Luna-Vital, L. Mojica, Mexican grasshopper (*Sphenarium purpurascens*) as source of high protein flour: Techno-functional characterization, and *in silico* and *in vitro* biological potential, Food Res. Int. 162 (2022), 112048.
[47] S.A. Althwab, R.M. Alhomaid, R.F.M. Ali, A.M. El-Anany, H.M. Mousa, Effect of migratory locust (*Locusta migratoria*) powder incorporation on nutritional and sensorial properties of wheat flour bread, British Food J. 123 (2021) 3576–3591.