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Locust protein hydrolysates have the potential to enhance the storage stability of cheese

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ABSTRACT

The study evaluated the efficacy of locust protein hydrolysates (LoPHs) to enhance the quality of Cheddar cheese (ChCh) during storage. The locust protein (LoP) was pre-treated [microwave (Mic) or ultrasonication (Ult) or no treatment (Not)] before hydrolysis using alcalase enzyme (3% w/w). The ChCh samples containing LoPHs at the maximum level of 1.5% were evaluated for quality for 3 months (4 ± 1 °C) and subjected to gastrointestinal simulation. Both pre-treatments (Mic and Ult) significantly ($P < 0.05$) enhanced the antimicrobial and antioxidant activities of the LoPHs (Ult > Mic > Not). The ChCh samples with LoPHs exhibited significantly ($P < 0.05$) lower means for lipid oxidation (TBARS and free fatty acids), protein oxidation (total-carbonyl content) and microbial counts (psychrophilic, total plate and yeast/moulds) during the storage. A positive effect was found on the sensory quality of ChCh samples after one month of storage. The gastrointestinal simulation improved the antioxidant capacity of the stored ChCh samples. LoPHs can be used as a novel bio-preservative for cheese.

1. Introduction

Compared to the current livestock farming systems, large-scale production of insects is associated with a lower carbon footprint and requires less environmental resources and inputs (such as water, land, and feed) to produce cheap and alternative proteins for human consumption (Meshulam-Pascoviche et al. (2022); Baiano (2020); Around 2000 different edible insects are consumed by more than two billion people in over 113 countries mainly in Asia, Africa, and South America (Tao, 2016). Among these edible insects, locusts have been widely studied as a dietary ingredient and are already a regular part of many peoples' diets in several countries in Asia, Latin America, and Africa (Baiano, 2020; Rowe, 2020). With its superior nutritional composition and a palatable taste (Althwab et al., 2021), locust (*Locusta migratoria*) is considered a healthy source of food and have been farmed recently in many countries such as Canada, China, Thailand, the USA, and South Africa (Baiano, 2020; Rowe, 2020). A complete package for muscle growth, the locusts contain all the essential amino acids and have been reported to contain a high amount of protein (~51–71%, dry weight basis) and fat (~11–35%) in addition to high amounts of iron (~0.008–0.015%), ω-3 fatty acids (~16%) and a desirable ratio for ω-3: ω-6 (~0.57) (Brogan et al., 2021; Clarkson et al., 2018).

To meet the protein requirements of expanding human population and animals in future, the Food and Agriculture Organization (FAO) has suggested the use of alternative proteins such as edible insects as sources of protein in food and feed (van Huis et al., 2013; Bhat et al., 2014). While locusts can provide an attractive option as a source of high-quality protein and other nutrients if acceptance issues could be overcome, direct consumption of locust proteins (LoP), such as tropomyosin, can induce allergenicity in sensitive people and cause harm to some

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consumers (Baiano, 2020). However, studies have reported a significant impact of food thermal processing on the allergenicity of insect proteins which can induce conformational alterations and affect the native structure of the allergen and epitopes (Meshulam-Pascoviche et al., 2022; Baiano, 2020). Hall and Liceaga (2020) reported a significant favourable effect of microwave processing on ACE and DPP-IV (Dipeptidyl peptidase IV) inhibitory properties of the cricket protein hydrolysates in addition to the reduction of the immunoreactivity. Recent studies have also reported that enzymatic hydrolysis can significantly decrease the allergenicity of insect proteins. For example, human shrimp-allergic sera showed no reactivity with cricket protein hydrolysed (65–80%) by alcalase enzyme (Hall et al., 2018). Similar findings were also reported by Leni et al. (2020) who observed the loss of immunoreactivity for the hydrolysates of lesser mealworm and black soldier fly prepared using protease obtained from *Bacillus licheniformis*. Enzymatic hydrolysis has been reported to produce peptides of different bioactivities, such as antioxidant, antimicrobial, and ACE-inhibitory, which contribute these properties to the insect protein hydrolysates (InPHs) (Nongonierma and FitzGerald, 2017). The hydrolysis of the LoP (*L. migratoria*) flour using enzymes, such as alcalase, enhanced the techno-functional properties (Purschke et al., 2018). The superior characteristics of the InPHs make them suitable tailored ingredients for various food applications such as improving the functional properties and storage stability of food products, especially fat-rich animal foods. While studies have evaluated the use of insects as a source of nutrients in the composition of various food products and assessed their impact on food quality characteristics (Meshulam-Pascoviche et al., 2022), none of these studies has used InPHs as a bio-preservative and explored their potential for enhancing the microbial quality and oxidative stability of the foods.

Previous investigations have documented the use of emerging and minimal processing technologies, such as ultrasonication, and heat-based efficient technologies, such as microwave, to enhance the food protein hydrolysis that leads to an increase in their bioactive properties and digestibility (Bhat et al., 2019a, 2022). However, these technologies have not been evaluated for their use in the production of InPHs for the preservation of foods. Therefore, the objective of this study was to develop LoPHs for enhancing the quality of fat-rich foods during storage using Cheddar cheese (ChCh) as a model of study. The preservative potential of LoPHs was further attempted to improve using thermal (microwave) and non-thermal (ultrasonication) pre-treatments. The LoPHs were incorporated into the ChCh samples and evaluated for quality for three months during refrigeration storage. The effect of gastrointestinal digestion simulation was also evaluated on the antioxidant potential of the ChCh samples.

2. Material and methods

2.1. Raw materials and enzymes

A *Bacillus licheniformis*-based protease (alcalase enzyme) was purchased from Sigma Aldrich (Billerica MA, USA) to hydrolyse the LoP and had an activity of ≥2.4 Anson units per gram (2.4 AU/g) (optimum conditions for activity: pH 7–9 and temperature 35–60 °C). The alcalase enzyme was used based on the available literature as it has been reported to be effective for chitinous materials and produce hydrolysates with high antimicrobial and antioxidant activities (Tacias-Pascacio et al., 2020; Lone et al., 2023a). The HI-Media (Maharashtra, India) supplied the pancreatin (≥6, 75, and 75 USP U/mg lipase, protease, and amylase activities, respectively) and pepsin (1000 NF U/mg protease activity) enzymes for gastrointestinal digestion simulation. The chemicals and reagents purchased for evaluating all the parameters were of analytical grade supplied by standard Indian suppliers, such as Sigma, Merck, and Qualigens. The commercially available LoP flour (natural and 100% LoP powder) was purchased from JR Unique Foods Ltd. (Udon Thani, Thailand Unique Brand, Thailand) and contained 71% crude protein (approx. on a dry weight basis). Studies have reported the protein content of LoP flour (*L. migratoria*) in a similar range (Brogan et al., 2021; Purschke et al., 2018). The LoP flour was developed using farmed locusts (*L. migratoria,* raised on grasses and vegetables) within a HACCP-accredited and FDA-approved factory. The locusts were ground to a powder form after cleaning and drying and vacuum packaged and contained no added preservatives, artificial colours and flavours.

2.2. Defatting and processing of LoP flour

The defatting of the LoP flour was done using hexane. Hexane has been widely used for defatting insect flours for the production of insect protein-based ingredients for food processing applications without affecting their techno-functional properties. The solvent extraction method used by Lone et al. (2023a) was followed for defatting the LoP flour that was mixed with food-grade n-hexane (1:5 ratio, w/v), stirred (30 min), and centrifuged (2000 rpm, 7 min) at room temperature to remove oil from the hexane/oil miscella. This was followed by filtration using Whatman filter paper No. 1 and the residual hexane was allowed to evaporate from the wet cakes which were dried overnight at 70 °C in a dry oven. The flour was vacuum-packaged (laminates: aluminium/polyethylene) after defatting and stored (4 ± 1 °C) until used.

The protein hydrolysis process described by Lone et al. (2023a) was used and the LoP flour (defatted, 200 g) was homogenized with water (w/v) [two volumes (400 ml) for 2 min] in a domestic blender. After pasteurising the slurry at 90 °C for 15 min using a water bath, the pH was changed to 8.0 (using NaOH solution) and the temperature was maintained at 50 °C for optimal activity of alcalase enzyme [3% (w/w), enzyme: substrate (E/S) of the protein content (72 mU/g protein)] that was allowed to hydrolyse the protein for 90 min for the maximal degree of hydrolysis. The mixture was continuously shaken during the hydrolysis without any pH adjustment. The E/S ratio and other conditions were selected based on the results of Hall et al. (2018) and Lone et al. (2023b) and the preliminary trials performed in the laboratory to yield LoPHs with high bioactivity and superior protein functionality. The samples of hydrolysed LoP were thermally treated (15 min, 90 °C) to deactivate the enzyme and centrifuged (5000 rpm, 4 °C, 15 min) to collect the clear supernatants which were lyophilized (INNOVA Bio Meditech Inc., INOFD-12S: Freeze Dryer, USA) and kept within polystyrene tubes (−20 °C). This freeze-dried powder (Not-LoPHs) of the soluble peptides and amino acids was analysed for antimicrobial and antioxidant activities (MIC, inhibitory halos, ABTS, DPPH, and FRAP) and incorporated into the ChCh samples at 1.5% level to evaluate its preservative potential during storage.

Three types of LoPHs were developed viz. control hydrolysates with no treatment (Not-LoPHs) as described above, microwave-processed LoPHs (Mic-LoPHs) and ultrasonicated LoPHs (Ult-LoPHs). For Mic-LoPHs, the slurry prepared above using defatted LoP and distilled water was heated at 90 °C for 10 min in a microwave (India, IFB-30BRC2, IFB 30L) (Singh et al., 2023). This was followed with a hydrolysis process following the procedure described above for Not-LoPHs. For Ult-LoPHs, defatted LoP slurry was ultrasonicated for 15 min [500 W (power), 20 kHz (frequency), pulse duration of 2/2 s (on/off), 0.125 kWh (energy)] using a Cole-Parmer ultrasonic processor with an attached 1.5 cm flat tip probe (U.S.A, WW-04711-45 Model). The LoP samples were processed in a beaker placed in an ice bath to maintain the temperature below 50 °C. This was followed with a hydrolysis process described above for Not-LoPHs.

2.3. Cheddar cheese

The ChCh of the brand "Amul" (Gujrat, India) was locally bought and used to evaluate the preservative potential of the LoPHs. As per the information available on the product label, the ChCh contained 374 kcal energy, 30 g total fat (saturated fat-19 g, cholesterol-84 mg, trans-fat-0 g), 25 g protein, 5 g ash, 1 g total carbohydrates (0 g added sugar),

and 620 mg sodium per 100 g. The preliminary trials were performed by incorporating an increasing concentration of LoPHs (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3% w/w) in cheese and the samples were subjected to sensory evaluation to determine the optimum level of incorporation without affecting the sensory quality of the products. The mixing was done by homogenizing the ChCh samples with the freeze-dried LoPHs (1.5% w/w) for 2 min at high speed using a home blender. Based on the outcome of these trials, a maximum level of incorporation of 1.5% (w/w) was found. Higher levels of LoPHs negatively affected the overall acceptability and flavour of the product. Four different types of samples were prepared by pressing 180 g of cheese into the cube and disk-shaped steel moulds viz. ChCh samples without any LoPHs (control), ChCh samples with LoPHs (1.5%) processed with microwave (Mic-LoPHs), ChCh samples with ultrasonicated LoPHs (Ult-LoPHs, 1.5%), and ChCh samples with LoPHs (1.5%) with no treatment (Not-LoPHs). All the samples of ChCh packaged within low-density polyethylene bags were kept under chilled (4 ± 1 °C) conditions and evaluated for quality for three months (days 0, 30, 60, and 90) for microbiological and sensory quality, protein and lipid stability, and physicochemical properties.

2.4. Digestion simulation

The ChCh samples containing LoPHs were subjected to gastrointestinal digestion simulation following the protocol described by Bhat et al. (2019b) using pepsin (E/S: 1: 100 w/w, pH 1.9, 1 h, 37 °C) and pancreatin (E/S: 1: 100 w/w, pH 8.0, 2 h, 37 °C). The aliquots of the digested samples were assessed for antioxidant potential (FRAP, ABTS, and DPPH). The ChCh samples were chopped finely and digested within polyvinyl containers using pepsin in 0.1 M HCl. The digesting samples were constantly stirred using a magnetic flea within the containers placed on a magnetic multi-stirrer. The pH was changed to 8.0 during the intestinal phase of the digestion using 0.1 M sodium phosphate buffer. All the aliquots were centrifuged (4000×*g*, 15 min) to obtain the clear supernatants for antioxidant analysis.

2.5. Antioxidant activity

The antioxidant potential of LoPHs and ChCh samples enriched with LoPHs were determined by analysing ABTS and DPPH radical scavenging activities (% inhibition) and the ability to reduce ferric (Fe^{3+}) ions (FRAP) (μmol Trolox equivalent/100 g) following the procedures elaborated by Kouser et al. (2023) and Lone et al. (2023a). The water-soluble extracts of ChCh enriched with LoPHs with a concentration of 15 mg/ml were prepared for FRAP whereas the extracts of LoPHs and ChCh with a concentration of 50 mg/ml were prepared for the measurement of ABTS and DPPH activities. The ChCh samples were grated and taken in double the volume of distilled water (w/v) and homogenized at 25000 rpm for 5 min using a Cole Parmar tissue homogenizer (Ultra-turrax, India). The extracts of LoPHs and ChCh with antioxidant properties reduced the free radicals present in ABTS and DPPH solutions and changed them to a non-radical form which initiated a change in the colour of these solutions. The working solution for ABTS was developed by reducing the absorbance (at 734 nm) of the stock solution to 0.70 ± 0.02 by diluting it with an aqueous methanol solution (80%). The reagent for FRAP was maintained in a water bath at 37 °C after preparing it by mixing 1 ml of both $FeCl_3{\cdot}6H_2O$ (20 mM) and 2,4,6-tri (2-pyridyl)-s-triazine in a tube and adding 10 ml of 300 mM sodium acetate buffer to it. The working solutions of DPPH prepared in ethanol (0.1 mM) as well as ABTS and FRAP were mixed with the extract samples (ChCh and LoPHs) and placed in the dark and analysed for absorbance. The samples were evaluated using a UV-Vis spectrophotometer at 517, 734, and 593 nm for DPPH, ABTS, and FRAP, respectively.

2.6. Oxidative stability and physicochemical properties

The oxidative stability of ChCh samples enriched with LoPHs was assessed by measuring the TBARS values (mg malondialdehyde/kg), free fatty acids (% oleic acid) and total carbonyl content (nmol of carbonyl/mg protein) during the entire storage time (3 months) following the protocols elaborated by Dua et al. (2015), Zargar et al. (2017) and Lone et al. (2023a). For estimation of free fatty acids, the ChCh samples were homogenized in chloroform (5g in 30 ml), filtered through Whatman filter paper No. 1, and the filtrate was titrated against 0.1 N alcoholic KOH. For TBARS, the ChCh samples were homogenized with 20% trichloroacetic acid (10g in 25 ml), filtered through Whatman filter paper No. 1, and the filtrate (3 ml) was mixed with 3 ml of 2-thiobarbituric acid in test tubes. The tubes were heated for 30 min and the absorbance was measured at 532 nm. The pH and moisture content of ChCh samples enriched with LoPHs were determined during the period of 3 months using a digital pH meter and hot air oven following the protocols elaborated by Ahmed et al. (2014) and Bukhari et al. (2012) for cheese, respectively.

2.7. Microbiological evaluation

The microbiological quality of the stored ChCh samples enriched with LoPHs was evaluated by enumerating microbial counts viz psychrophilic, total plate, yeast/mould, and coliform (log_{10} cfu/g) using the spread plate technique as elaborated by American Public Health Association (Kouser et al., 2023). The media used for the analysis were total plate agar, potato dextrose agar, and violet-red bile agar for total plate and psychrophilic, yeast/moulds, and coliforms, respectively, and were purchased from Sigma Aldrich (Bangalore, India). The minimum inhibitory concentration (MIC) of the LoPHs was determined using a Gram -ve (*E. coli*) and a Gram + ve (*S. aureus*) microbe following microdilution technique using a 96-well plate and Muller Hinton broth (37 °C for 24 h). The LoPHs were inoculated in triplicate in an increasing concentration manner and the MIC was determined by noticing the last well in the plate that totally inhibited the growth of the microbe and did not show any visible growth. The disc agar diffusion method was also performed to determine the diameter of halos around the discs of filter paper (10 mm) infused with 1.5% solutions of LoPHs against *E. coli* or *S. aureus* (10^6 cfu/ml) grown on Muller Hinton agar at 37 °C for 24 h (Kalem et al., 2017).

2.8. Sensory analysis

A panel of 10 trained people composed of five males and five females (age group of 25–45 years) carried out the sensory analysis of stored ChCh samples thrice [03 replications x 10 panellists for each treatment for each storage point (days 0, 30, 60, and 90)] employing an 8-point descriptive scale for four sensory characteristics viz. overall acceptability, texture, flavour, and colour and appearance (1 anchored 'disliked extremely' and 8 anchored 'liked extremely') (Kouser et al., 2023). The panellists had years of experience and training in routine sensory tests such as threshold, recognition, hedonic, and descriptive tests. Informed consent was acquired from the panellists and all the ethical guidelines and regulations were followed. The samples were presented using codes and water was used to cleanse the palate.

2.9. Texture profile analysis

The stored ChCh samples were subjected to texture profile analysis (TPA) at the beginning and end of the storage (days 0 and 90) using a Shimadzu EZ-SX Texture Analyser (Japan) attached with a 25 kg load cell and a compression platform (5 cm diameter probe) (Kouser et al., 2023). The ChCh samples (20 × 20 mm) were compressed to 50% of their original height and two compression cycles were allowed with a time gap of 5 s and a crosshead speed of 2 mm/s to analyse the various textural properties viz. firmness, springiness, cohesiveness, adhesiveness, resilience and chewiness.

2.10. Statistical analysis

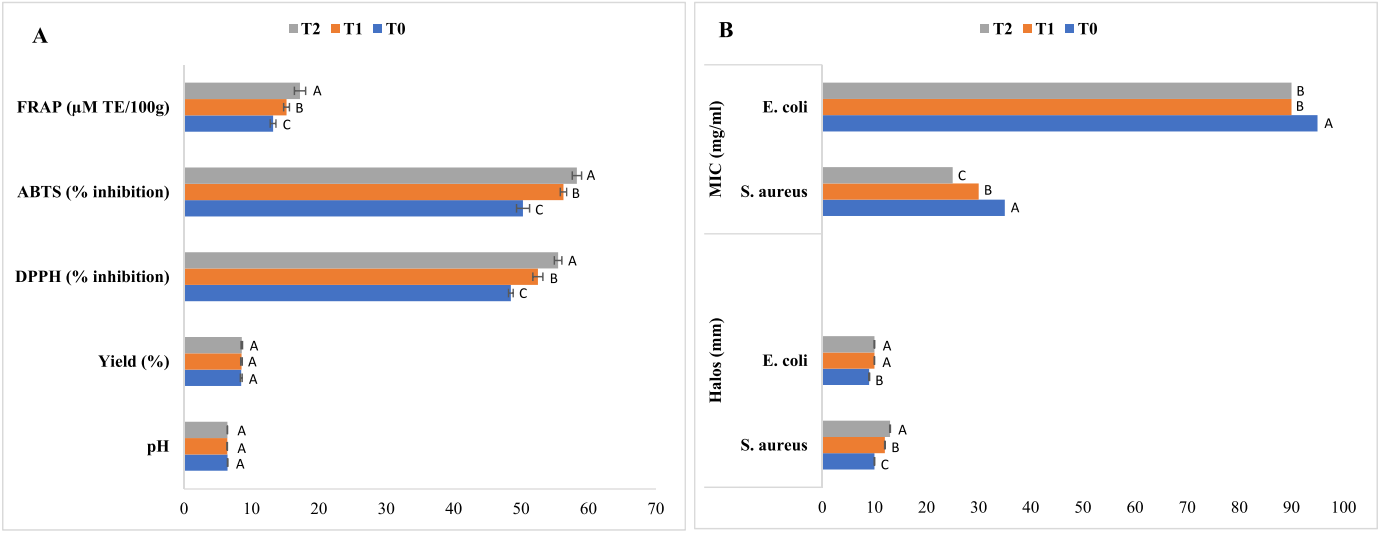
The experiments were performed (six replications, n = 6) and data were collected and analysed using SPSS-20 (ANOVA one-way or two-way). DMRT (Duncan's multiple range tests) were applied to compare the means of measured variables at a 0.05 level to find out the effects of the pre-treatments, time, and interactions. The analysed data is displayed in the form of tables and figures as standard errors±means.

3. Results and discussion

3.1. Physicochemical and antioxidant properties

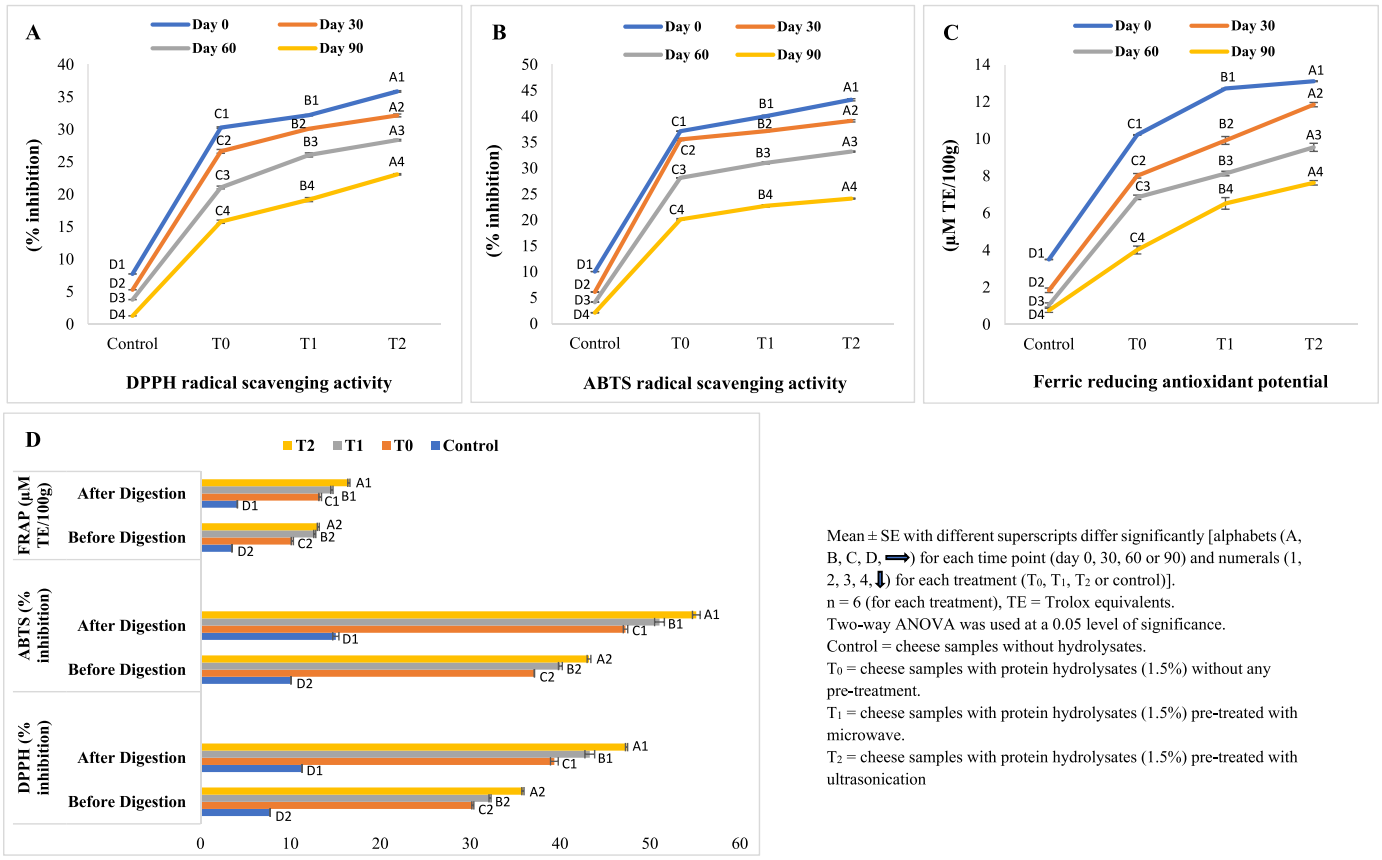
The data related to the antioxidant properties (FRAP, DPPH and ABTS) of the LoPHs and ChCh samples containing LoPHs are presented in Figs. 1 and 2 (A, B and C), respectively. A significant ($P < 0.05$) positive effect of the processing was observed on the antioxidant properties of the LoPHs and the highest values were observed for Ult-LoPHs for DPPH, ABTS, and FRAP followed by the Mic-LoPHs. The results followed a significant order viz. Ult-LoPHs > Mic-LoPHs > Not-LoPHs. The antioxidant properties of the stored ChCh samples also followed a similar significant order (Ult-LoPHs > Mic-LoPHs > Not-LoPHs > control) for DPPH, ABTS, and FRAP throughout the 90 days storage and the ChCh samples incorporated with Ult-LoPHs exhibited significantly ($P < 0.05$) higher values than the ChCh samples incorporated with Mic-LoPHs and Not-LoPHs. The ChCh samples exhibited a significant decline in antioxidant activities (DPPH, ABTS, and FRAP) with the storage time (from day 0–90) as reported previously (Farrag et al., 2020). Kouser et al. (2023) found a similar decline in the antioxidant potential of *kalari* cheese during storage. The results of the DPPH, ABTS, and FRAP assays showed strong antioxidant activities of LoPHs containing soluble peptides and amino acids and also indicated the impact of processing (Mic and Ult) to improve the antioxidant activities. Incorporation of the LoPHs significantly ($P < 0.05$) improved the antioxidant stability of the ChCh samples during the entire storage time. Earlier studies have reported strong antioxidant properties for the InPHs and indicated their potential for improving the storage quality of stored foods. Compared to unhydrolyzed cricket protein (*G. sigillatus*), hydrolysis with alcalase enzyme resulted in a significant increase in the antioxidant properties as indicated by a significant increase of ORAC and ABTS values (Mudd et al., 2022). A similar improvement in the antioxidant properties (FRAP, DPPH, and ABTS) has been reported on the hydrolysis of cricket protein with alcalase enzyme (Hall et al., 2018). The production of the peptides with antioxidant properties during hydrolysis was suggested to be the cause of improved radical scavenging and antioxidant potential.

The traditional enzymolysis of proteins is believed to limit the hydrolysis process due to the conformational incompatibilities and microstructural obstructions that affect the ingress of protease molecules to cleavage sites on proteins (Bhat et al., 2021c). Pre-treatment with emerging or current technologies, such as ultrasonication or microwave, can overcome these limitations by inducing various microstructural and protein conformational alterations (Bhat et al., 2021c; Mintah et al., 2019). A favourable impact of both these processing technologies has been reported on the antioxidant potential and techno-functional characteristics of InPHs. The treatment of cricket protein with microwave before its hydrolysis with alcalase enzyme has been reported to increase the hydrolysis degree as well as the production of peptides with bioactive properties (Hall and Liceaga, 2020). While silk moth hydrolysis with alkaline and alcalase protease has been reported to increase the production of antioxidant amino acids (Liu et al., 2017), a positive effect of thermal processing, such as boiling (10 min, 100 °C) or baking (10 min, 150 °C), have been reported on antioxidant activity of InPHs including the LoPHs (*S. gregaria*) (Zielinska et al., 2017). Similarly, processing of LoP with ultrasound (10–30 min, 750 W, 20 kHz) has been reported to cause a significant improvement in the antioxidant properties (ABTS, FRAP, and DPPH) in comparison to unprocessed samples (Kingwascharapong et al., 2021). An increase in the antioxidant activities of the InPHs (*H. illucens*) pre-processed with ultrasound (600 W, 40 kHz) has been reported by Mintah et al. (2019) who



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 = protein hydrolysates without any pre-treatment, T1 = protein hydrolysates pre-treated with microwave, T2 = protein hydrolysates pre-treated with ultrasonication

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 = protein hydrolysates without any pre-treatment, T1 = protein hydrolysates pre-treated with microwave, T2 = protein hydrolysates pre-treated with ultrasonication.



Mean ± SE with different superscripts differ significantly [alphabets (A, B, C, D, ⟹) for each time point (day 0, 30, 60 or 90) and numerals (1, 2, 3, 4, ⇩) 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 = cheese samples without hydrolysates.
T_0 = cheese samples with protein hydrolysates (1.5%) without any pre-treatment.
T_1 = cheese samples with protein hydrolysates (1.5%) pre-treated with microwave.
T_2 = cheese samples with protein hydrolysates (1.5%) pre-treated with ultrasonication

Fig. 2. Effect of hydrolysates (A, B, C) and *in vitro* gastrointestinal digestion (D) on the antioxidant potential of the cheddar cheese.
Mean ± SE with different superscripts differ significantly [alphabets (A, B, C, D) for each time point (day 0, 30, 60 or 90) and numerals (1, 2, 3, 4) 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 = cheese samples without hydrolysates.
T_0 = cheese samples with protein hydrolysates (1.5%) without any pre-treatment.
T_1 = cheese samples with protein hydrolysates (1.5%) pre-treated with microwave.
T_2 = cheese samples with protein hydrolysates (1.5%) pre-treated with ultrasonication.

recorded the highest values of 1.49%, 45.96%, and 85.00% for FRAP, superoxide scavenging, and ABTS compared to untreated samples (1.29%, 38.08%, and 71.05%, respectively). In other similar studies (such as Mintah et al., 2020), the same group reported higher anti-oxidation activity (DPPH and hydroxyl radical scavenging activity and ion chelating activity) for LoPHs pre-treated with ultrasonication compared to untreated samples and also observed that the treatment affected the microstructure, protein secondary structure, thiol value, surface charge, and the particle size of the hydrolysates in addition to their techno-functional properties.

The data relating to the physicochemical parameters of the LoPHs and ChCh samples are presented in Fig. 1A and Table 1, respectively. The pre-treatments exhibited no effect ($P > 0.05$) on the pH and yield of the LoPHs. While the incorporation of the LoPHs exhibited no effect ($P > 0.05$) on the moisture content of the ChCh samples throughout 90 days of storage, the ChCh samples incorporated with LoPHs exhibited significantly ($P > 0.05$) lower pH values on days 60 and 90 in comparison to control ChCh. This effect of the LoPHs might be ascribed to their antioxidant and antimicrobial properties which retarded the microbial growth and lipid and protein oxidation of the stored ChCh, mitigating the build-up of alkaline metabolites and a hike in the pH. Addition of the food bio-preservatives with antimicrobial and antioxidant properties, such as phytoextracts, has been recorded to reduce the rate of spoilage and increase the pH of cheese during storage (Kouser et al., 2023; Kalem et al., 2018; Singh et al., 2015). Similar results have been reported by Lone et al. (2023a, b) and Singh et al. (2023) who evaluated the effect of insect protein hydrolysates on the quality of food products including cheese during storage.

3.2. Lipid stability

Enrichment of ChCh samples with LoPHs (1.5%), especially the pre-processed samples (Ult-LoPHs and Mic-LoPHs), exhibited a significant favourable impact on the stability of the lipids during storage (Table 1). The presence of the LoPHs in the ChCh (Ult-LoPHs, Mic-LoPHs, and Not-LoPHs) retarded the lipid oxidation and lipolytic changes as indicated by a significant ($P < 0.05$) decline in TBARS and FFA values compared to the control ChCh (without LoPHs) on days 30, 60 and 90. The lowest means for both FFA and TBARS were observed for the ChCh enriched with Ult-LoPHs followed by the ChCh containing Mic-LoPHs. A significant order was recorded in the results of TBARS and FFA viz. Ult-LoPHs < Mic-LoPHs < Not-LoPHs < control. Fat-rich food products, such as cheese, are susceptible to lipolytic changes and lipid oxidation and require interventions, such as the use of natural/synthetic antioxidants or bio-preservatives, to maintain the quality during storage (Kouser et al., 2023; Kaur et al., 2015; Singh et al., 2014a, 2014b). Research papers have documented the use of food hydrolysates as bio-preservatives, such as zein protein (10–1.25 mg/ml) and casein protein hydrolysates (0.2–0.4 mg/ml, Carrillo et al., 2017; Li et al., 2017), to retard the lipid oxidative and lipolytic changes (as indicated by

Table 1
Effect of locust protein hydrolysates on lipid stability, protein oxidation and physicochemical properties of cheddar cheese during refrigerated storage.

Treatments	Storage period (days)			
	0	30	60	90
pH				
Control	5.56 ± 0.01^{Aa}	5.28 ± 0.03^{Ab}	6.08 ± 0.02^{Ac}	6.27 ± 0.02^{Ad}
T_0	5.56 ± 0.01^{Aa}	5.26 ± 0.01^{Ab}	5.95 ± 0.01^{Bc}	6.15 ± 0.01^{Bd}
T_1	5.56 ± 0.01^{Aa}	5.24 ± 0.01^{Ab}	5.84 ± 0.01^{Cc}	6.04 ± 0.03^{Cd}
T_2	5.57 ± 0.01^{Aa}	5.23 ± 0.01^{Ab}	5.76 ± 0.01^{Dc}	5.94 ± 0.02^{Dd}
Moisture (%)				
Control	38.18 ± 0.21^{Aa}	37.58 ± 0.22^{Ab}	37.13 ± 0.22^{Ac}	36.58 ± 0.32^{Ad}
T_0	38.18 ± 0.11^{Aa}	37.60 ± 0.30^{Ab}	37.16 ± 0.34^{Ac}	36.60 ± 0.21^{Ad}
T_1	38.17 ± 0.32^{Aa}	37.61 ± 0.10^{Ab}	37.18 ± 0.41^{Ac}	36.61 ± 0.41^{Ad}
T_2	38.17 ± 0.20^{Aa}	37.63 ± 0.20^{Ab}	37.19 ± 0.20^{Ac}	36.63 ± 0.41^{Ad}
TBARS (mg malondialdehyde/kg)				
Control	0.25 ± 0.003^{Aa}	0.95 ± 0.004^{Ab}	1.91 ± 0.001^{Ac}	2.54 ± 0.003^{Ad}
T_0	0.24 ± 0.004^{Aa}	0.88 ± 0.003^{Bb}	1.75 ± 0.004^{Bc}	2.39 ± 0.005^{Bd}
T_1	0.24 ± 0.002^{Aa}	0.83 ± 0.002^{Cb}	1.64 ± 0.002^{Ca}	2.27 ± 0.004^{Cd}
T_2	0.24 ± 0.005^{Aa}	0.78 ± 0.001^{Db}	1.52 ± 0.002^{Dc}	2.15 ± 0.003^{Dd}
FFA (% Oleic acid)				
Control	0.15 ± 0.008^{Aa}	0.46 ± 0.002^{Ab}	0.68 ± 0.001^{Ac}	0.88 ± 0.001^{Ad}
T_0	0.15 ± 0.001^{Aa}	0.41 ± 0.003^{Bb}	0.59 ± 0.001^{Bc}	0.72 ± 0.003^{Bd}
T_1	0.15 ± 0.002^{Aa}	0.37 ± 0.002^{Cb}	0.53 ± 0.002^{Cc}	0.67 ± 0.002^{Cd}
T_2	0.14 ± 0.002^{Aa}	0.35 ± 0.002^{Db}	0.49 ± 0.001^{Dc}	0.62 ± 0.002^{Dd}
Carbonyl content (nmol/mg protein)				
Control	0.28 ± 0.01^{Aa}	0.68 ± 0.02^{Ab}	0.87 ± 0.02^{Ac}	1.09 ± 0.02^{Ad}
T_0	0.27 ± 0.03^{Aa}	0.60 ± 0.01^{Bb}	0.80 ± 0.02^{Bc}	0.99 ± 0.01^{Bd}
T_1	0.27 ± 0.01^{Aa}	0.51 ± 0.01^{Cb}	0.71 ± 0.01^{Cc}	0.90 ± 0.01^{Cd}
T_2	0.26 ± 0.02^{Aa}	0.41 ± 0.01^{Db}	0.62 ± 0.01^{Dc}	0.79 ± 0.01^{Dd}

Mean ± SE with different superscripts in a row (lower case alphabet) and column (upper case alphabet) differ significantly ($P < 0.05$).
n = 6 for each treatment.
Control = cheese samples without hydrolysates.
T0 = cheese samples with protein hydrolysates (1.5%) without any pre-treatment.
T_1 = cheese samples with protein hydrolysates (1.5%) pre-treated with microwave.
T_2 = cheese samples with protein hydrolysates (1.5%) pre-treated with ultrasonication.

TBARS and peroxide values) of fat-rich foods, such as oil-in-water emulsions of myofibrillar protein and olive oil, respectively.

The higher lipid stability of ChCh samples containing Ult-LoPHs and Mic-LoPHs compared to other samples might be attributed to the pre-treatments which significantly increased the antioxidant properties of LoPHs. Ultrasound-assisted (10–30 min, 750 W, 20 kHz) extraction has been reported to yield LoP with the highest electron-donating and radical scavenging activities from Bombay locusts (Kingwascharapong et al., 2021) and also changed the surface hydrophobicity which induced protein structural alterations and conformational changes leading to favourable conditions for enzymatic hydrolysis and release of anti-oxidative peptides. In a similar study, Mintah et al. (2019) reported the highest antioxidative properties for ultrasonicated (40 kHz, 600 W) InPHs and isolates over a wider pH (2–12) range. Like ultrasonication, microwave treatment (10 min) of cricket proteins has also been reported to enhance the hydrolysis rate and production of peptides with bioactive properties (Hall and Liceaga, 2020). The treatment was effective in reducing the immunoreactivity of the InPHs and significantly increased their ACE and DPP-IV inhibition properties. It was suggested that microwave-induced conformational changes and protein unfolding facilitated enzymatic hydrolysis by enhancing the ingress of enzymes to cleavage sites.

3.3. Protein oxidation and interaction of InPHs with cheese

The incorporation of the LoPHs (1.5%) exhibited a significant positive impact on the protein oxidation of the stored ChCh samples (Table 1). The carbonyl content (nmol/mg protein) of the ChCh incorporated with LoPHs (Ult-LoPHs, Mic-LoPHs and Not-LoPHs) were significantly ($P < 0.05$) lower than the ChCh without LoPHs on days 30, 60 and 90 and the lowest values were observed for the ChCh containing Ult-LoPHs followed by Mic-LoPHs and Not-LoPHs. The oxidation of the proteins in dairy foods, which are vulnerable to oxidation, increases the carbonyl content during storage (Kouser et al., 2023). The antioxidant activities of the LoPHs might be attributed to this reduction of protein oxidation in stored ChCh as they have the ability to chelate the metal ions and scavenge the free radicals to retard oxidation. No study has evaluated the efficacy of InPHs as preservatives in foods or in cheeses. However, studies have investigated the application of hydrolysates derived from non-insect proteins as a bio-preservative to reduce the protein oxidation of protein-rich animal foods. For example, Jónsdóttir et al. (2016) investigated the use of cod fillet-based hydrolysates/peptides (0.7%) to retard the protein and lipid oxidation of minced cod with positive results. Li et al. (2013) and Mukherjee and Haque (2016) successfully used whey and casein protein hydrolysates to retard the protein oxidation in common carp surimi and catfish fillet/-beef steak, respectively. Li et al. (2013) developed hydrolysates from whey protein isolates using alcalase enzyme and used it to control protein oxidation in common carp surimi during frozen storage (−25 °C). The carbonyl content of the control samples increased from an initial value of 4.02–7.25 nmol/mg after 180 days whereas the addition of the hydrolysates (4%) reduced the rate of oxidation in the treated samples which showed a significantly lower value of 5.26 nmol/mg after 180 days.

The InPHs (soluble proteins, peptides, and amino acids) can interact with different nutritional components of cheese, such as protein (casein and whey proteins), fat, lactose, and minerals, and modify its techno-functional and sensory properties. Studies have reported the suitability of InPHs as a means to increase the techno-functional characteristics of foods such as water-holding capacity and solubility (Nongonierma and FitzGerald, 2017). Dairy proteins undergo a variety of complex interactions during storage and processing, leading to a wide range of conformational and structural changes and promoting protein-protein interactions and consequently affecting protein functionality (Bhat et al., 2021d). These interactions are mainly due to non-covalent forces, such as hydrophobic interaction, hydrogen bonds, van der Waals forces, and electrostatic interactions, and are affected by the processing conditions such as temperature and pH. The interactions between denatured and unfolded proteins can result in different outcomes including cross-linking and aggregation. While mild processing conditions can induce protein denaturation of milk proteins and increase protein functionality by exposing hydrophobic residues and thiol groups, excessive denaturation due to intense processing can result in the aggregation of food proteins (Bhat et al., 2021d). Disulphide cross-linking is the most frequent protein-protein interaction that occurs in denatured proteins during food processing (Kim et al., 2022). Processing can also induce interaction between InPHs and carbohydrates in cheese, which may lead to nonenzymatic and nonoxidative browning through the Maillard reaction (Lone et al., 2023a). Further, the lipid peroxides produced during the oxidation of unsaturated fats in cheese can interact with InPHs to produce lipid-protein complexes, which can affect their nutritive value (Lone et al., 2023a).

3.4. Microbiological characteristics

Both additions of the LoPHs and the processing exhibited a significant positive impact on the microbiological quality of the stored ChCh (Table 2). The total plate counts of the ChCh enriched with the LoPHs (Ult-LoPHs, Mic-LoPHs and Not-LoPHs) were significantly ($P < 0.05$) lower than the ChCh without LoPHs on days 30, 60 and 90 and the lowest counts were observed for the ChCh containing Ult-LoPHs followed by Mic-LoPHs and Not-LoPHs. While psychrophiles and yeast and moulds were detected on days 60 and 90, respectively, the counts followed a similar pattern. This favourable impact of the LoPHs on the microbial quality of stored ChCh samples might be due to the antimicrobial peptides present in these hydrolysates. The alcalase enzyme has been widely employed to hydrolyse different protein sources to produce peptides, amino acids, and soluble hydrolysates with antimicrobial activities (Tacias-Pascacio et al., 2020). The peptides with antimicrobial activity are typically small sized generally lower than 10 kDa and contain 50 or fewer amino acids (Tkaczewska, 2020). The hydrolysates developed from insect proteins, such as locust (*L. migratoria*) and cricket proteins, have been observed to contain high levels of small peptides and hydrophobic amino acids (Mudd et al., 2022; Purschke et al., 2018). These peptides operate through different mechanisms to affect the microbes and can act through iron-chelating activity or disrupt the microbial cell membranes (Tkaczewska, 2020). Characterized by their membrane permeabilizing action, these peptides can affect microbial membranes through electrostatic interactions (Tkaczewska, 2020). The studies have investigated the application of hydrolysates/peptides with antimicrobial properties as additives in different food matrices. For example, workers have investigated the use of peptides derived from whey acidic protein and potato tubers to retard microbial growth in milk and beverages (Shwaiki et al., 2020; Yang et al., 2020). The coliform bacteria went undetected in our study throughout the 90 days of storage in all the samples of ChCh.

The effect of the pre-treatments on the inhibitory halos and MIC (minimum inhibitory concentration) of the LoPHs was determined (Fig. 1B). These assays were done against one commonly available Gram-positive (*S. aureus*) and a Gram-negative (*E. coli*) bacteria. The pre-processing exhibited a significant impact and the mean MIC values of 95, 90, and 90 mg/ml were recorded to prevent the growth of *E. coli* for Not-LoPHs, Mic-LoPHs, and Ult-LoPHs whereas 35, 30, and 25 mg/ml were found to inhibit the growth of *S. aureus,* respectively. Previous investigations have analysed the antimicrobial activity of both InPHs and food protein hydrolysates in terms of MIC. While a MIC value of 60-40 μg/ml was found against various *S. aureus* strains for protein hydrolysates and the ethanol-extracted fraction from fly maggots (*Musca domestica* L., Park et al., 2010), a MIC value of 0.15 and 0.3 mg/ml were found for bleached chitosan from *Hermetia illucens* against *M. favus* and *E. coli*, respectively (Guarnieri et al., 2022). Peptides derived from the whey protein of camel milk have been recorded to inhibit the growth of *E. coli* and *S. aureus* at a minimum concentration of 65 and 130 mg/ml, respectively (Wang et al., 2020). The pre-processing also showed a significant ($P < 0.05$) impact on the inhibitory halos and the Mic-LoPHs and Ult-LoPHs showed bigger-sized halos against both *E. coli* and *S. aureus* compared to the Not-LoPHs. The results of MIC and inhibitory halos confirmed the results of microbiological counts and indicated the antimicrobial activities of the LoPHs and their potential as a food bio-preservative.

Table 2
Effect of locust protein hydrolysates on microbiological quality of cheddar cheese during refrigerated storage.

Treatments	Storage period (days)			
	0	30	60	90
Total plate count (log_{10} cfu/g)				
Control	0.79 ± 0.01^{Aa}	1.51 ± 0.01^{Ab}	2.34 ± 0.01^{Ac}	2.91 ± 0.02^{Ad}
T_0	0.77 ± 0.01^{Aa}	1.46 ± 0.01^{Bb}	2.01 ± 0.01^{Bc}	2.63 ± 0.01^{Bd}
T_1	0.78 ± 0.01^{Aa}	1.29 ± 0.01^{Cb}	1.79 ± 0.02^{Cc}	2.49 ± 0.01^{Cd}
T_2	0.78 ± 0.01^{Aa}	1.19 ± 0.01^{Db}	1.50 ± 0.01^{Dc}	2.30 ± 0.01^{Dd}
Psychrophilic count (log_{10} cfu/g)				
Control	ND	ND	0.84 ± 0.02^{Ab}	1.44 ± 0.01^{Ac}
T_0	ND	ND	0.70 ± 0.01^{Bb}	1.30 ± 0.01^{Bc}
T_1	ND	ND	0.59 ± 0.01^{Cb}	1.14 ± 0.01^{Cc}
T_2	ND	ND	0.45 ± 0.03^{Db}	1.06 ± 0.01^{Dc}
Yeast and mould count (log_{10} cfu/g)				
Control	ND	ND	ND	1.24 ± 0.019^{A}
T_0	ND	ND	ND	1.13 ± 0.013^{B}
T_1	ND	ND	ND	1.00 ± 0.026^{C}
T_2	ND	ND	ND	0.80 ± 0.016^{D}
Coliform count (log_{10} cfu/g)				
Not detected in any sample during entire storage period				

Mean ± SE with different superscripts in a row (lower case alphabet) and column (upper case alphabet) differ significantly ($P < 0.05$), n = 6 for each treatment, ND = not detected (Detection limit <10 cfu/g).
Control = cheese samples without any protein hydrolysates.
T_0 = cheese samples incorporated with protein hydrolysates without any pre-treatment.
T_1 = cheese samples incorporated with protein hydrolysates pre-treated with microwave.
T_2 = cheese samples incorporated with protein hydrolysates pre-treated with ultrasonication.

3.5. Digestion simulation of ChCh

The impact of digestion on antioxidant and radical scavenging activities of the ChCh was evaluated which can provide an additional health benefit (Mudd et al., 2022). The gastrointestinal digestion simulation showed a favourable impact on the antioxidant activities of ChCh and significantly ($P < 0.05$) higher means were recorded for ABTS, DPPH, and FRAP for all the ChCh samples after completion of the digestion (Fig. 2D). The presence of the LoPHs and pre-processing exhibited a significant ($P < 0.05$) impact and the ChCh enriched with the LoPHs exhibited significantly ($P < 0.05$) higher means for ABTS, DPPH, and FRAP in comparison to ChCh without any LoPHs. The highest ($P < 0.05$) values were recorded for the ChCh enriched with Ult-LoPHs followed by Mic-LoPHs and Not-LoPHs. With a high branched-chain amino acids content, the LoP (*L. migratoria*) is mostly resistant to the gut pepsin but susceptible to intestinal proteases, such as trypsin and chymotrypsin, and therefore digestible in the small intestine (Ochiai et al., 2022). The protein is hydrolysed in the intestine and produces small peptides and hydrophobic amino acids mostly responsible for its antioxidant and radical scavenging activities (Bhat et al., 2021a,b). Villasenor et al. (2022) reported a high antioxidant potential (IC_{50} value of 0.63 and 0.78 mg/ml for ABTS and DPPH, respectively) for hydrolysates produced from Mexican grasshopper (*S. purpurascens*) during *in vitro* gastrointestinal digestion due to the production of a wide range of antioxidant and hydrophobic peptides and amino acids. Both Mudd et al. (2022) and Hall et al. (2018) employed alcalase enzyme for the hydrolysis of cricket protein and reported a significant improvement in the bioactive properties (FRAP, ABTS, and DPPH) of the hydrolysates after simulated gastrointestinal digestion which was attributed to the high concentration of hydrophobic amino acids and peptides present in the digests. This improved radical scavenging ability and antioxidant activity of the InPHs and peptides after gut digestion can have health-beneficial effects and have been demonstrated to increase the lifespan of a nematode (*C. elegans*) under oxidative stress conditions (Mudd et al., 2022). The peptides obtained from hydrolysis of tropical banded crickets (*Gryllodes sigillatus*) using alcalase enzyme were subjected to gastrointestinal digestion and used to incubate L4 nematodes (*C. elegans*) in a solution (0.125–1.0 mg/ml) (Mudd et al., 2022). The peptides increased the lifespan of the larval parasites under acute and chronic oxidative stress conditions induced using tert-butyl hydroperoxide. The larvae fed on the peptides showed significantly lower levels of reactive oxygen species and increased expression of the stress-related gene gst-4 compared to the control group.

3.6. Sensory and texture profile analysis

The effect of the enrichment of LoPHs and pre-processing was evaluated on sensory quality and texture profile analysis (TPA) of the stored ChCh (Figs. 3 and 4). The ChCh samples enriched with the LoPHs (Ult-LoPHs, Mic-LoPHs and Not-LoPHs) obtained significantly ($P < 0.05$) higher scores on days 30, 60, and 90 for all the sensory attributes compared to the ChCh without LoPHs. The ChCh incorporated with Ult-LoPHs exhibited significantly ($P < 0.05$) higher scores followed by Mic-LoPHs and Not-LoPHs. The addition of the LoPHs might be attributed to this positive effect on sensory quality which have strong antimicrobial and antioxidant activities and the ability to retard oxidative changes and microbial spoilage and thereby minimizing off-flavour production. Studies, such as Althwab et al. (2021) and Bawa et al. (2020), have elucidated the impact of the addition of locust (*L. migratoria*) and cricket (*A. domesticus*) flours on the sensorial characteristics of bread and reported high scores for overall acceptability up to a maximum incorporation level of 4% for locust flour and 10% for cricket flour, respectively. As expected, storage time exhibited an effect ($P < 0.05$) on the TPA of the ChCh, however, the presence of the LoPHs or pre-processing did not show any impact ($P > 0.05$) on the TPA of the stored ChCh.

4. Conclusions

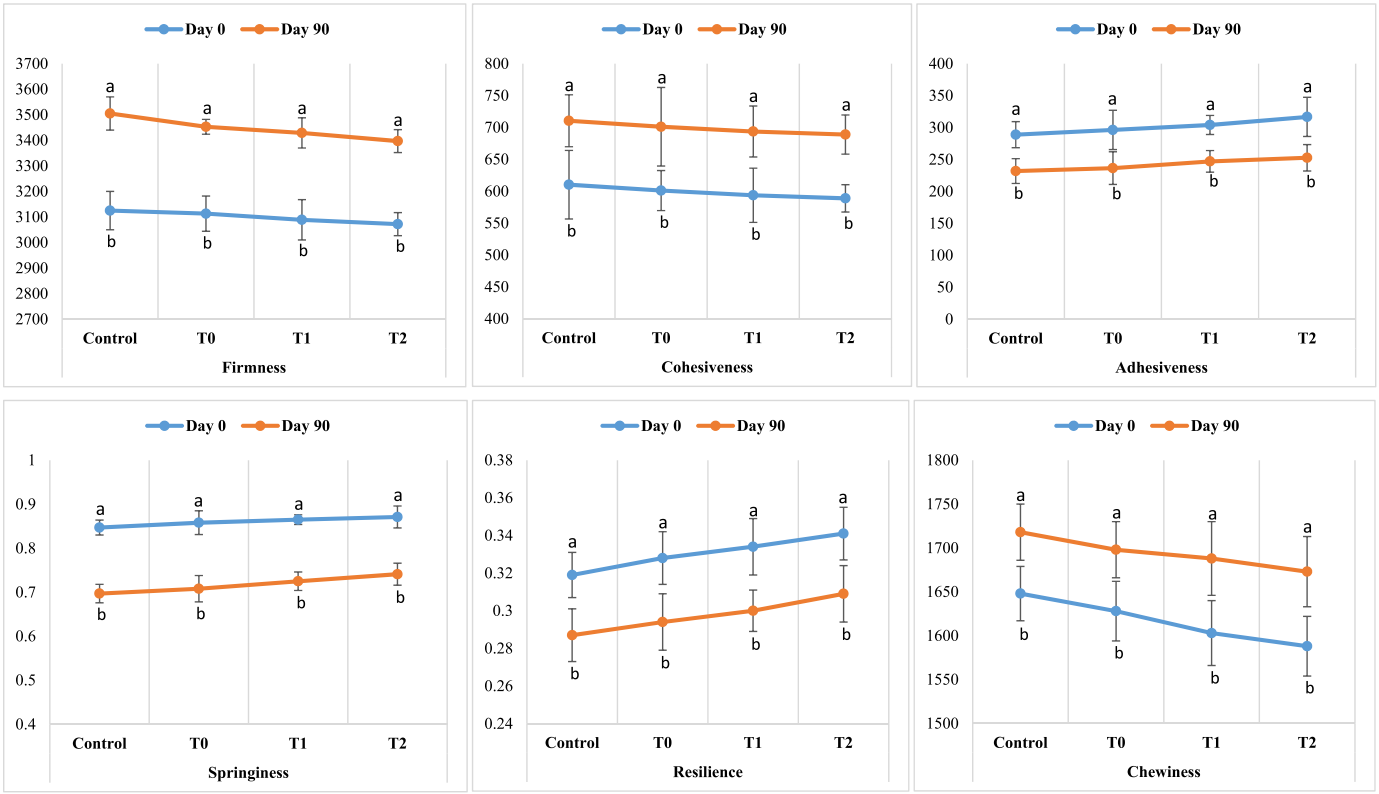
Our results indicate the antimicrobial and antioxidant activities of the LoPHs and their suitability as a food bio-preservative to enhance the stability of fat and protein-rich animal-derived foods such as ChCh. The preservative potential of the LoPHs was further enhanced through the application of ultrasonication or microwave as pre-treatments. Enrichment of ChCh with the LoPHs enhanced the quality during refrigerated storage of 3 months and a significant impact of pre-processing (Ult and Mic) was recorded on all the storage quality parameters. A positive effect of digestion was found and significantly enhanced the antioxidant activity and radical scavenging ability of the ChCh, indicating its health-promoting and functional value. Future studies should investigate the impact of cooking on the preservative potential of LoPHs and investigate the possibility of its use in other food products to promote food sustainability and enhance food security.

CRediT authorship contribution statement

Shubam Singh: Investigation, Methodology. **Hina F. Bhat:** Conceptualization, Writing – review & editing, Resources. **Sunil Kumar:** Supervision, Methodology, Data curation. **Mehnaza Manzoor:** Writing – review & editing, Data curation, Validation. **Aunzar B. Lone:** Investigation, Methodology. **Pawan Kumar Verma:** Writing – review & editing, Validation, Data curation. **Rana Muhammad Aadil:** Writing – review & editing, Software, Visualization. **Konstadina Papastavropoulou:** Writing – review & editing, Software, Visualization. **Charalampos Proestos:** Writing – review & editing, Software, Resources, Visualization. **Zuhaib F. Bhat:** Conceptualization, Supervision, Methodology, Data curation, Validation, Writing – original draft.

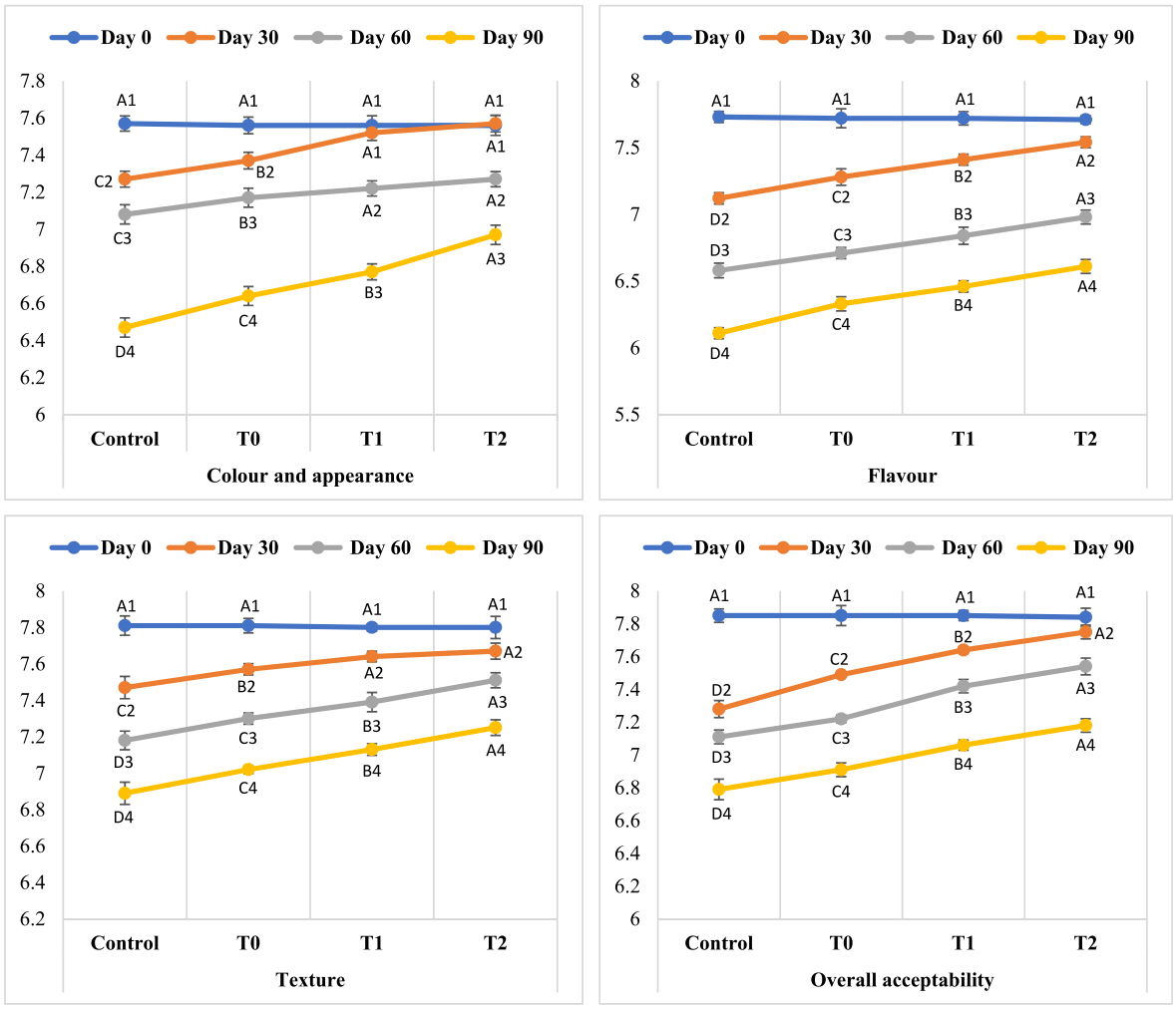
Declaration of competing interest

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.



Mean ± SE with different superscripts differ significantly, n = 10 (for each treatment), Two-way ANOVA was used at a 0.05 level of significance, Control = cheese samples without hydrolysates, T0 = cheese samples with protein hydrolysates (1.5%) without any pre-treatment, T1 = cheese samples with protein hydrolysates (1.5%) pre-treated with microwave, T2 = cheese samples with protein hydrolysates (1.5%) pre-treated with ultrasonication

Fig. 3. Effect of locust protein hydrolysates on texture profile analysis of the cheddar cheese.
Mean ± SE with different superscripts differ significantly, n = 10 (for each treatment), Two-way ANOVA was used at a 0.05 level of significance, Control = cheese samples without hydrolysates, T0 = cheese samples with protein hydrolysates (1.5%) without any pre-treatment, T1 = cheese samples with protein hydrolysates (1.5%) pre-treated with microwave, T2 = cheese samples with protein hydrolysates (1.5%) pre-treated with ultrasonication.



Mean ± SE with different superscripts differ significantly [alphabets (A, B, C, D, ⟹) for each time point (day 0, 30, 60 or 90) and numerals (1, 2, 3, 4,⇓) for each treatment (T_0, T_1, T_2 or control)].
Repeated measurements ANOVA was used at a 0.05 level of significance.
10 semi-trained panellists performed the sensory evaluation thrice for each treatment at each time point (days 0, 30, 60, and 90) using an 8-point descriptive scale (1 denoted 'disliked extremely' and 8 denoted 'liked extremely').
Control = cheese samples without hydrolysates
T0 = cheese samples with protein hydrolysates (1.5%) without any pre-treatment
T1 = cheese samples with protein hydrolysates (1.5%) pre-treated with microwave
T2 = cheese samples with protein hydrolysates (1.5%) pre-treated with ultrasonication

Fig. 4. Effect of locust protein hydrolysates on sensory quality of the cheddar cheese.
Mean ± SE with different superscripts differ significantly [alphabets (A, B, C, D) for each time point (day 0, 30, 60 or 90) and numerals (1, 2, 3, 4) for each treatment (T_0, T_1, T_2 or control)].
Repeated measurements ANOVA was used at a 0.05 level of significance.
10 semi-trained panellists performed the sensory evaluation thrice for each treatment at each time point (days 0, 30, 60, and 90) using an 8-point descriptive scale (1 denoted 'disliked extremely' and 8 denoted 'liked extremely').
Control = cheese samples without hydrolysates.
T0 = cheese samples with protein hydrolysates (1.5%) without any pre-treatment.
T1 = cheese samples with protein hydrolysates (1.5%) pre-treated with microwave.
T2 = cheese samples with protein hydrolysates (1.5%) pre-treated with ultrasonication.

Data availability

Data will be made available on request.

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