Current Research in Food Science 1 (2019) 17–23

Contents lists available at ScienceDirect

Current Research in Food Science

journal homepage: www.editorialmanager.com/crfs/

Research Paper

Contributions of protein and milled chitin extracted from domestic cricket powder to emulsion stabilization

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ARTICLE INFO

Keywords:
Cricket
Insect protein
Chitin
Emulsion
Interfacial tension
Pickering stabilizer

ABSTRACT

Interfacial and emulsifying properties of fractionated cricket powder were assessed to identify whether emulsification properties originate from protein or chitin particles. Fractions extracted in alkaline water, containing high protein and mineral contents, increased the surface pressure of heptane-water interfaces with near-saturation equilibrium surface pressure of 31 mN/m. Dynamic surface pressure profiles indicated adsorption of protein clusters to the interface. Emulsification capacity of protein fraction was 50% greater than that of the source cricket flour, although oil-in-water emulsions prepared with 1–2% (w/w) protein fraction formed a cream layer within one day of storage. Emulsified layers persisted for up to 20 days, and light scattering measurements described a stable population with surface-volume-mean diameter of approximately 3 μm. Chitin-rich fractions milled to a particle size of 0.5–200 μm contributed negligible surface pressure, and its emulsification capacity was 5% of the value for the source cricket flour. Emulsions prepared with chitin-rich fractions coexisted with an unstable precipitate layer comprising 60% of the added solid, which was attributed to larger particles with poor emulsifying capability. Stable chitin-stabilized emulsion phases were resistant to creaming, yet volume-mean droplet diameter surpassed 50 μm within 24 h of storage. Both protein and chitin fractions have emulsifying capabilities but would require further processing or secondary additives to achieve desirable storage stability.

1. Introduction

Insects are a promising alternative to traditional livestock to address forecasted demand increases for meat-like products. Livestock farming generates large quantities of greenhouse gases, produced directly by the animals (Nelson et al., 2009) or during growth of the feed-crops (Henchion et al., 2017). Insect-rearing generates much fewer greenhouse gases, and feed-per-protein output is less than half of chicken or cattle (Van Huis, 2013). Considerations for farming, preparation, and nutrition of insects as food or feed ingredients have been compiled recently by several groups (Dossey et al., 2016; Rumpold and Schlüter, 2013a; Elhassan et al., 2019). A readily accessible edible insect ingredient is dried cricket powder, which contains 45–70% of nutritionally-balanced protein, as well as 20–30% lipid, 5–20% fiber, and 5% ash (Rumpold and Schlüter, 2013b; Montowska et al., 2019). Recent studies have shown comparable emulsifying activity and foaming capabilities of cricket flours and protein isolates when compared to legume flours (Zielińska et al., 2018; Stone et al., 2019). Cricket powder has been used to supplement or replace protein-rich components in various complex foods, including meat emulsions (Kim et al., 2017), pasta (Duda et al., 2019), and bread (Osimani et al., 2018); protein-nutrition bars containing crickets are also commercially available.

Crickets contain a complex variety of proteins of widely varying molecular weight and solubility. Only ~20% of the proteins are soluble in pure water, as the water-insoluble fraction includes a large quantity of muscle proteins (Montowska et al., 2019; Yi et al., 2013). Solubility of 20–35 kDa proteins is increased in alkaline pH conditions with high ionic strength (Kim et al., 2017), allowing extraction with potentially greater yields. Aqueous protein extracts provide modest foam stabilization at pH 3 (Yi et al., 2013) and were better at producing emulsions than heptane-extracted fractions (Ndiritu et al., 2017). Boiling treatments decrease protein solubility while also decreasing protein digestibility and iron bioaccessibility (Manditsera et al., 2019). Aqueous fractions extracted at low temperature with alkali and salt are then a good model

Abbreviations: CP, fraction enriched in non-muscle cricket protein.

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https://doi.org/10.1016/j.crfs.2019.09.002

system for understanding emulsification properties of cricket protein.

Much of the crude fiber of the house cricket is in the form of chitin, which serves as a structural material (Jonas-Levi and Martinez, 2017). Chitin can be hydrolyzed with acid into nanometer-scale particles and used to prepare Pickering emulsions, which possess high resistance to droplet coalescence and oil separation (Zhang et al., 2015; Larbi et al., 2018). However, milling operations used to prepare ground powders creates micrometer-scale particles, and the larger size reduces the capability to stabilize emulsion droplets following homogenization operations (Aveyard et al., 2003). An older study found that milled chitin from shellfish could be used to prepare paraffin-oil emulsions (Magdassi and Neiroukh, 1990), yet more understanding on the relative emulsifying properties of a cricket-based milled chitin particle is required. Chitin-stabilized emulsions are especially attractive for food product development, as recent reports indicate their resistance to lipid digestion (Tzoumaki et al., 2013).

Ground crickets contain a significant amount of protein and chitin particles, and both could be contributing towards emulsification. This study determined the emulsifying properties of cricket-derived aqueous protein suspensions and finely-milled chitin particles using dynamic tensiometry and emulsion stability tests. Extracts were prepared in a manner to maintain relevance to the background media and particle sizes found in industrial ground cricket powders.

2. Materials and methods

2.1. Materials

Griopro® 100% Cricket Powder was purchased from All Things Bugs, LLC (Midwest City, OK). Petroleum ether, sodium carbonate, and Florosil were purchased from Fisher Scientific (Hampton, NH). Concentrated hydrochloric acid, sodium hydroxide, and n-heptane were purchased from Sigma-Aldrich (St. Louis, MO). Canola oil was purchased from a local market. Both n-heptane and canola oil were purified by Florosil adsorbent before use. Ultrapure water was obtained from a water filtration system (Barnstead E-pure, Thermo Scientific, Waltham, MA).

2.2. Fractionation of cricket powder

Ground cricket powder was dried overnight at 40 °C in a vacuum oven. Fat content was removed via extraction in petroleum ether using AOCS protocol 991.36 (AOAC-International and Latimer, 2012). Further lipid was extracted from the solid with hexane (20 mL/g powder) based upon the defatting procedure of Yi and others (Yi et al., 2013). Defatted powder was collected by vacuum filtration and dried overnight at 77 °C in a vacuum oven. Dry samples were sealed and stored in a desiccator at 23 °C.

A fraction enriched in non-muscle cricket protein (CP) was extracted from defatted whole cricket powder based upon the approach of Kim and others (Kim et al., 2017). In brief, dry defatted powder was dispersed in ultrapure water (50 g/L), adjusted to pH 9.5 using 1.0 M sodium hydroxide solution, and agitated for 18 h. Insoluble residue was removed by centrifugation at 35,267×*g* for 1 h (Beckman Avanti J-25I, Beckman Coulter; Brea, CA). Soluble fractions were filtered (0.45 μm pore size), adjusted to pH 7.5 with 1.0 M hydrochloric acid, lyophilized, and stored in a desiccator at 23 °C.

Chitin particles were prepared from the pellet following CP extraction. Specifically, pellet was sequentially refluxed with 1.0 M hydrochloric acid, 1.0 M sodium hydroxide, and 0.4% sodium carbonate aqueous solutions at 1% (w/v) pellet content to remove catechols, polysaccharides/pigments, and residual proteins (Zhang et al., 2000). Between each refluxing step, pellet was washed with water and dried overnight at 77 °C in a vacuum oven. After the final step, chitin powder was milled first with a Cyclotec™ 1093 Mill (Foss; Hillerød, Denmark) and next with a Retsch ball mill (Retsch; Haan, Germany). Milled chitin was stored in a desiccator at 23 °C.

Aqueous suspensions of CP and chitin were prepared from dried powders by dispersing in ultrapure water at 25 °C. Suspensions were vortexed for 30 s, sonicated in a sonicating bath for 2 min, and agitated for 15–18 h at 25 °C.

2.3. Characterization of powders

Yield of CP and chitin fractions were determined by mass in relation to initial mass of defatted ground cricket powder. Ash content was determined after incineration of powders in a 550 °C muffle furnace. Crude protein content of the powders was determined by a Tru Mac N nitrogen combustion analyzer (LECO Corporation, St. Joseph, MI); insect-specific nitrogen-to-protein conversion factors of 4.76 and 5.60 were used for the ground cricket powder and CP fraction, respectively (Janssen et al., 2017). Particle size of chitin particles was determined from >28,000 particles by an automated microscope-based particle counter with a 20× objective lens (Morphologi G3-ID; Malvern, Worcestershire, England).

2.4. Interfacial and emulsifying properties

Interfacial properties of CP or chitin were assessed using droplet shape tensiometry (Kruss DSA 30b, Kruss GMbH; Hamburg, Germany). Droplets of n-heptane were injected within aqueous CP suspensions, as n-heptane is frequently utilized as a clean model system to describe protein adsorption (Beverung et al., 1999). Chitin suspensions were excessively turbid for droplet shape measurements with n-heptane, so measurements were taken of aqueous suspensions injected in air, as previously described (Tzoumaki et al., 2011). Interfacial tension was determined from droplet shape using DSA4 software (Kruss GMbH; Hamburg, Germany). As the interfacial tensions of heptane with water (~52 mN/m), air with water (~72 mN/m), and food-grade oil with water (~30 mN/m) are dissimilar, interfacial measurements were reported as surface pressure and discussed individually. Average diffusion coefficient of adsorbing species was estimated from dynamic interfacial tension from all sample replicates using the short-time approximation for the Ward & Tordai equation, as described by Murphy and others (Murphy et al., 2016).

Emulsifying capacity of cricket flour and extracted fractions was determined by the volume of oil that could not be dispersed within aqueous mixtures using an established technique (Webb et al., 1970). Specifically, canola oil was added dropwise into 0.05% (w/w) aqueous mixtures of cricket powder and CP samples while agitated with shear at 24,000 rpm (T25 Ultra-Turrax; IKA, Breisgau, Germany). End-point of the titration was identified by > 20% change in conductivity (YSI Inc., Yellow Springs, OH, USA). Since chitin suspensions possessed initially low conductivity, emulsifying capacity of chitin-containing emulsions was determined by direct observation of separated oil volume after 10% (w/w) canola oil was emulsified with 1.8% (w/w) aqueous chitin suspensions and allowed to separate for 4 h at 23 °C (Fig. S1). Separated oil volume was measured from the top layer containing non-emulsified oil, which was removed and centrifuged at 3000×*g* for 10 min. Precipitate was observed in emulsified samples (Fig. S1), so chitin content contributing to emulsions was determined by the mass difference between total added chitin and precipitate. Precipitate mass was determined gravimetrically from fresh emulsions following centrifugation at 4000×*g* for 10 min, rinsing with ultrapure water, and drying. All emulsifying capacity values were reported as emulsified oil weight with respect to solid mass of emulsifying material (g/g).

2.5. Emulsion preparation and characterization

CP emulsions with 10% (w/w) oil content were prepared by dispersing canola oil in 0.5–2.0% (w/w) CP aqueous mixtures and shearing with a T25 Ultra-Turrax (IKA, Breisgau, Germany) for 60 s at 24,000 rpm. Chitin emulsions with 5.3% (w/w) oil and 0.72% (w/w)

chitin were taken from the stable emulsion layer following emulsifying capacity and emulsifying-chitin content measurements, as described above. CP emulsions were further homogenized by a high-pressure two-stage homogenizer (GEA Panda, GEA; Columbia, MD) operating at 10,000 PSI, whereas chitin emulsions were not further homogenized due to the potential damage to the homogenizer valve from solid particulates. All emulsions were sealed and stored at 23 °C.

Creaming index (CI) of emulsions during storage at 23 °C was measured by height of the cream layer in relation to total height of liquid from digital images using ImageJ software (National Institutes of Health; Bathesda, Maryland). Mean emulsion droplet diameters, including surface-volume mean diameter (D_{32}) and volume mean diameter (D_{43}), were determined by static light scattering (Mastersizer, 2000; Malvern Instruments, Worcestershire, England) using refractive indices of 1.335 and 1.495 for the continuous and dispersed phase, respectively. All emulsion samples were vortexed for 25 s and diluted to an obscuration of 12–13% prior to scattering measurements.

Chitin-stabilized emulsion droplets were imaged on covered glass slides using a Nikon A1-Rsi confocal laser-scanning microscope (Nikon Instruments Inc.; Melville, NY) with a 60× oil immersion objective lens.

2.6. Statistical analysis

All analyzed samples were prepared in triplicate unless otherwise specified. Significant differences ($p < 0.05$) were identified by Tukey's method.

3. Results and discussion

3.1. Characterization of extracted materials

Following removal of lipid components, water-soluble (CP) and water-insoluble, de-proteinated (chitin) extracts were obtained with yields of 20.7 ± 1.4% and 5.14 ± 0.30%, respectively. Mean particle size (D_{10}) of dry chitin particles was 68.06 ± 13.18 μm (range = 0.54–206 μm), which was comparable to particle sizes observed for finely milled whole cricket powders (Sipponen et al., 2018) but less than sizes reported for other cricket flours before and after exposure to high-temperatures (David-Birman et al., 2018). Ash content of CP was more than five times greater than ground cricket powder, indicating enrichment of water-soluble minerals (Table 1). Minerals, such as iron, zinc, calcium, and manganese, are naturally present in ground crickets (Montowska et al., 2019; Barker et al., 1998) and can increase water solubility of proteins (Kim et al., 2017); to assess the functionality of protein in cricket powders, these minerals were therefore not removed from CP.

Protein content of CP was ~50% and did not differ from the protein content of the original ground cricket powder (Table 1). Prior studies have also found approximately 50% protein content in water-soluble insect extracts (Yi et al., 2013; Ndiritu et al., 2017). This did not imply that the extraction failed, as there was significant insoluble matter removed during extraction; rather, this reflected the simultaneous extraction of salts, carbohydrates and other water-soluble organic matter. Considering the yield of ~20% dry extract, the protein content matched the expected 20% content of water-soluble proteins in ground crickets (Yi et al., 2013). Similar to these other studies, CP was not a protein isolate but rather an industrially-relevant extract in which water-insoluble muscle protein was depleted and water-soluble protein was enriched. Heat treatments applied during production of the cricket flour may also have contributed towards aggregation and reduced solubility of some of the protein (Montowska et al., 2019), lowering the relative capture of proteins in the alkaline water extract. Further steps could have been performed to enrich the content of water-soluble proteins, such as the use of trichloroacetic acid and acetone, yet this would have questionable effects on protein structure or function (Cilia et al., 2009; Gresiana et al., 2015). Surprisingly, other studies have shown that isoelectric precipitation achieves minimal enrichment of protein content among insect extracts (Yi et al., 2017; Bußler et al., 2016).

Table 1
Contents of Ash, Nitrogen, and Estimated Protein of Ground Cricket Powder and Water-Soluble Cricket Protein (CP) extracted fraction.

	Ground Cricket Powder (%)	CP (%)
Ash (%)	4.367 ± 0.017	25.71 ± 0.48
Nitrogen (%)	11.0 ± 0.3	8.95 ± 0.32
Protein (%)[a]	52.1 ± 1.4	50.1 ± 1.8

[a] Protein content for Ground Cricket Powder/CP used the conversion factor of 4.76/5.60 for whole insects/insect protein isolates based on the findings from Janssen and others, 2017.

Aqueous dispersions of CP, chitin, and cricket powder were prepared for subsequent analyses at contents of 0.5–2% (w/v). Solution pH was approximately 6.6 for chitin and cricket powder dispersions and pH ~7.2–7.5 for CP dispersions.

3.2. Interfacial activity of CP and chitin

CP demonstrated significant surface activity at heptane-water interfaces with ~40 mm^2 interfacial area (Fig. 1). Modeling of dynamic surface pressure with the Ward & Tordai equation provided estimated diffusion coefficients of 7×10^{-12} - 5×10^{-11} m^2/s for adsorbing species (Fig. S2), corresponding to hydrodynamic radii of 4.9–35.6 nm. Assuming protein molecular weight of 25 or 35 kDa (Kim et al., 2017), then these sizes would correspond to small protein clusters. Equilibrium surface pressure was ~26 mN/m at 0.01%, implying that the interface was highly saturated even at low concentration. The trend of diminishing surface pressure increases with concentration indicated full interfacial saturation of the droplets at a CP concentration of ~0.1% (Fig. S3). Observed surface pressure of ~31 mN/m at 0.08% w/w CP was comparable to saturated interfaces with whey protein (~30 mN/m) (Pérez et al., 2007). These results demonstrated CP's strong activity at oil-water interfaces and capability for emulsion formation.

Chitin caused minimal changes in surface pressure at air-water interfaces within 5 h (Fig. S4). This could be attributed to weak interaction between particles and an insufficient interfacial load during the time course of the measurement (Binks, 2002). Previous studies showed that chitin nanocrystals required 24 h to achieve significant surface pressures (Tzoumaki et al., 2011), and the larger chitin particles in cricket extracts could adsorb even slower. However, even low interfacial loads of particles can still provide significant stabilization against coalescence among emulsions (Horozov and Binks, 2006).

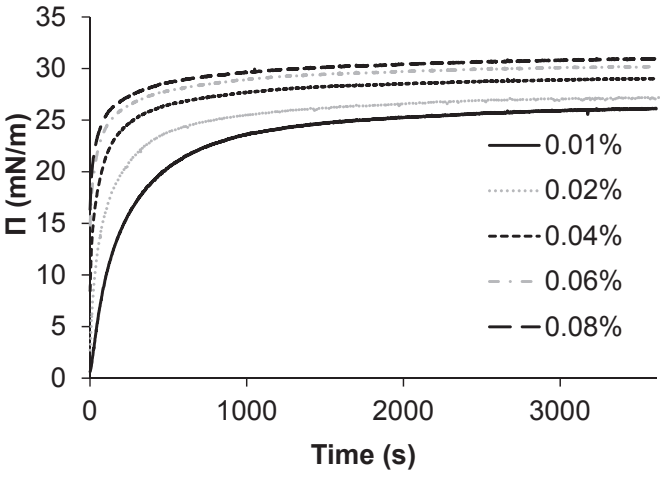

Fig. 1. Dynamic surface pressure (Π) of heptane droplets in water with increasing content of CP. Lines were chosen as representative of three separate measurements.

Table 2
Emulsifying capacity (EC) of Ground Cricket Powder, Water-Soluble Cricket Protein (CP) extracted fraction, and Chitin extracted fraction.

	EC (g oil/g)[a]
Ground Cricket Powder	129 ± 4^{A}
CP	194 ± 17^{B}
Chitin	6.96 ± 0.57^{C}

[a] Superscripted upper-case letters indicate significantly different groups.

3.3. Emulsion-stabilizing properties of CP and chitin

Emulsifying capacity measurements were performed to identify the practical ability of CP and chitin to emulsify oils in water. CP emulsifying capacity was ~1.5x greater than ground cricket powder and >20x greater than chitin (Table 2). This reinforced prior studies showing better emulsifying capacity for protein extracts when compared to the whole flour (Ndiritu et al., 2017). In comparison to literature values, CP emulsifying capacity was less than caseinates (250–700 g/g) (Mohanty et al., 1988) and comparable to egg, whey, and soybean (Stone et al., 2015), while those for ground cricket powder were comparable to peanut or sesame seed flours (50–170 g/g) (Khalid et al., 2003; Yu et al., 2007). High mineral contents and pH of the CP suspensions used in this study may have positively influenced the observed emulsifying capacity values, as prior studies have shown better emulsifying capacity of cricket protein at neutral-to-alkaline pH and with high ion contents (Ndiritu et al., 2019).

Emulsions of 10% oil content were prepared with quantities of CP (0.5–2% w/w) that were well in excess of the emulsifying capacity in order to test for resistance to storage instabilities. CP emulsions possessed a mild yellow coloration that was attributed to cricket pigments (Fig. 2a).

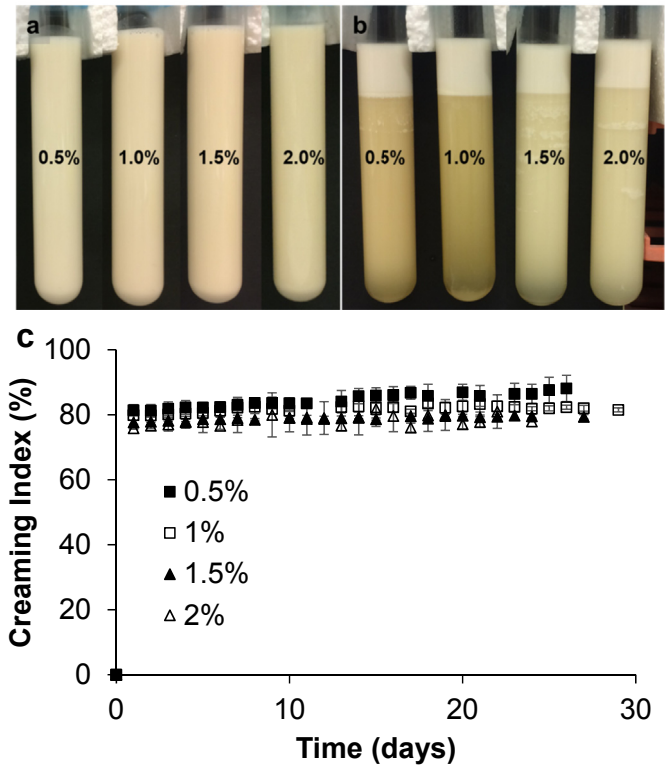

Fig. 2. Gravitational separation of 10% (w/w) oil-in-water emulsions with increasing CP based on photographs of emulsions after (a) 1 h or (b) 1 day and (c) creaming index.

After 1 day of storage, emulsions exhibited an opaque cream layer at the top, a dilute emulsion phase below, and a minor serum phase at the bottom (Fig. 2b). These phases remained stable over 24 days storage, with creaming index increasing marginally and no evidence of oil separation (Fig. 2c). Initial mean droplet diameters (both D_{32} and D_{43}) were 2–5 μm for all CP contents and indicated sufficient CP for droplet coverage (Fig. 3). D_{32} during 24 days of observation remained stable with 1–2% CP but increased with 0.5% CP during storage, indicating poor stability in the latter (Fig. 3a). Size of larger droplets in emulsions (D_{43}) increased among all samples during storage, yet growth rate was slower with greater CP concentration (Fig. 3b). For example, D_{43} after 7 days of storage increased to 7 μm, 12 μm, 15 μm, and 28 μm among emulsions prepared with 0.5%, 1%, 1.5%, and 2% CP, respectively. Particle size distributions showed formation of a droplet population with 10–100 μm diameter after storage that increased in prevalence with less CP content (Fig. S5). CP contributed negligibly to viscosity at the studied concentrations (*not shown*), so the reduction in droplet growth with more CP could be due to concentration-dependent changes in spreading/density of protein at the interface to reduce cohesion after droplet collisions (Fang and Dalgleish, 1993).

Only a fraction of the chitin with was capable of forming stable emulsions, as indicated by the persistence of precipitated chitin after emulsification at various concentrations. Precipitated chitin was attributed to larger chitin particles that were unable to participate in emulsification. However, persisting emulsions layers were formed

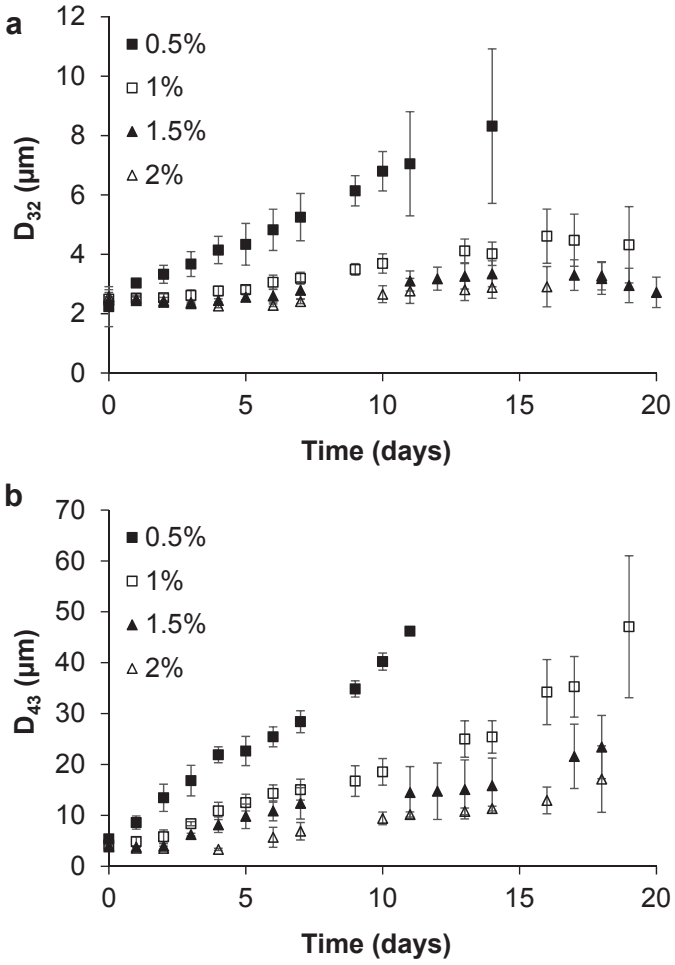

Fig. 3. Droplet size of 10% w/w oil-in-water emulsions with increasing CP during storage – (a) surface-volume mean diameter (D_{32}), (b) volume mean diameter (D_{43}).

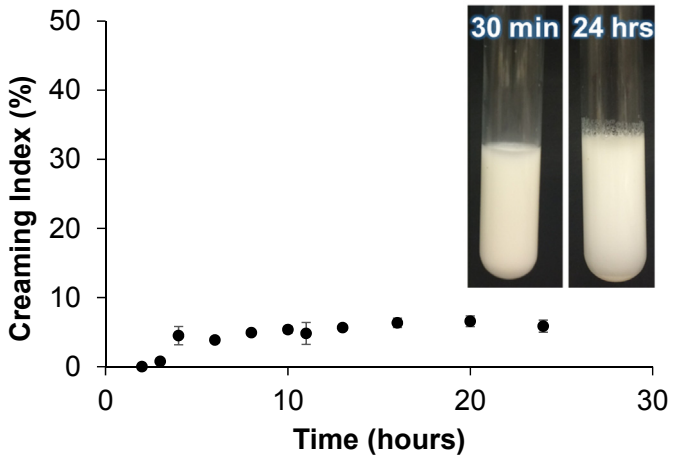

Fig. 4. Effect of storage time on gravitational separation of 5.3% (w/w) oil-in-water emulsions stabilized by 0.76% (w/w) cricket-derived chitin; inset shows photographs of emulsions at specified time points.

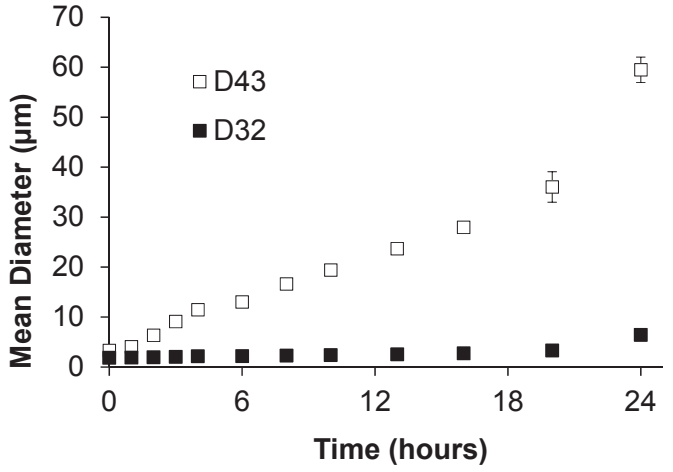

Fig. 5. Droplet size of 5.3% (w/w) oil-in-water emulsions stabilized by 0.76% (w/w) cricket-derived chitin during storage.

with the remainder of the chitin particles (~37.5% of initially added chitin); this emulsion was isolated and possessed an oil content of 5.3% (w/w) with a chitin content of 0.76% (w/w). Chitin emulsions remained white and turbid over one day of storage (Fig. 4, inset). Creaming indices of the emulsions were negligible within the first hours after preparation but increased to ~4–6% because of serum separation (Fig. 4), which was comparable to emulsions stabilized by chitin nanoparticles (Tzoumaki et al., 2011). Viscosity-based stabilization of the continuous phase cannot be discounted as a possible mechanism for emulsion stability during this storage period, although a prior study using similar concentrations of chitin nanoparticles demonstrated minor viscosification of the continuous phase and argued that emulsion stability was conferred by the ability of adsorbed chitin particles to reduce droplet mobility via interactions of the interfacial layers (Tzoumaki et al., 2011).

Mean diameters of chitin-stabilized emulsions, initially ~2–3 μm, increased substantially within 24 h of storage (D_{43} = 59.49 μm) (Fig. 5), and further measurements were stopped. Particle size distributions showed a population of small droplets and a second 10–200 μm population that was poorly stabilized (Fig. S6). Assuming that the 37.5% of chitin particles remaining within the emulsion phase represented the smallest particles (the largest particles removed with the precipitate phase), particles with diameter closer to the minimum detected size (~0.5 μm) could have acted as the stabilizing fraction. Chitin particles less than 10 μm represented a very small fraction of the total obtained powder, and the insufficient concentration of small chitin particles to cover the surface of 2–5 μm droplets would explain the rapid growth of emulsion droplets during storage.

Using a light microscope, particulate matter was observed at the interface of chitin-stabilized droplets (Fig. 6). These particles were attributed to the chitin particles that adsorbed to the emulsion droplet interfaces. Observed particles were comparable in size to structures observed in electron micrographs of chitin subjected to ball-milling and hydrothermal treatments (Aida et al., 2014). Particles were less apparent on the smaller droplets, demonstrating the difficulty for solid particles to adsorb to highly curved interfaces. Smaller droplets were frequently observed in clusters with larger droplets, and prior research has shown that surface-active particles can simultaneously stabilize two droplet interfaces by bridging interactions (Horozov and Binks, 2006).

Both micrographs and particle size distributions showed that small chitin particles could adsorb onto emulsion droplets but with insufficient coverage to prevent droplet growth. Recent studies have shown the ability to significantly improve emulsion stabilization of solid cellulose particles after reducing the particle size to less than 1 μm by extensive milling operations (Lu et al., 2018). Similar approaches could be utilized to achieve even greater emulsion-stabilizing properties of cricket-based chitin. Smaller particle sizes of chitin achieved by milling has also been shown to improve dispersion in water, susceptibility to hydrothermal treatments (Aida et al., 2014), and enzymatic reactivity (Nakagawa et al., 2013).

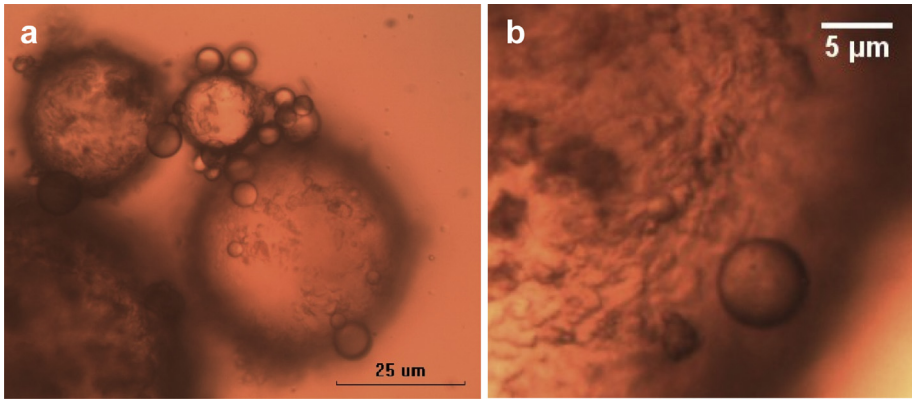

Fig. 6. Light micrographs of oil-in-water emulsion droplets stabilized by cricket-derived chitin, showing (a) general droplet morphology and (b) close-up image of particle-laden interface.

4. Conclusions

Results indicated that both water-soluble protein and milled chitin particles found in ground cricket powder could be contributing towards stabilization of oil-in-water emulsions, as both were capable of forming a stable emulsion fraction. CP fractions that possessed water-soluble proteins demonstrated classical concentration-dependent lowering of interfacial tension at droplet surfaces and better emulsifying capacity than the source cricket powder, while chitin fractions demonstrated much weaker adsorption behaviors. However, both fractions possessed a fraction of emulsion droplets that rapidly grew during storage. Further, only the smallest of the milled chitin was useful for emulsification, limiting their relevance as industrial emulsifiers. Emulsion stability for both fractions could be bolstered by arresting droplet motion via increased continuous phase viscosity or increased oil content. A potential means to improve interfacial characteristics of the whole flour or chitin fraction would be to partially deacetylate the chitin particles and increase hydrophilicity. Future studies are also required to understand how the interfacial properties of cricket protein is affected by the ion composition, which could be performed by removing ions from protein isolates and adding these back to the formulation.

Conflicts of interest and source of funding

Authors declare no conflict of interest in the presented studies, and there was no scientific contribution or direction from any corporation or industrial interest. Funding was received from HATCH project No. IND011662/228289 from the USDA National Institute of Food and Agriculture.

Acknowledgements

The authors would like to thank Dhananjay Pai, Patricia Jaynes, and Andy Schaber at Purdue University for their assistance in dry particle size characterization, nitrogen content analysis, and light microscopy, respectively.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2019.09.002.

References

Aida, T.M., Oshima, K., Abe, C., Maruta, R., Iguchi, M., Watanabe, M., Smith, R.L., 2014. Dissolution of mechanically milled chitin in high temperature water. Carbohydr. Polym. 106, 172.
AOAC-International, Latimer, G.W., 2012. Official Methods of Analysis, 19 ed. AOAC International, Gathersburg, MD.
Aveyard, R., Binks, B.P., Clint, J.H., 2003. Emulsions stabilized solely by colloidal particles. Adv. Colloid Interface Sci. 100–102, 503.
Barker, D., Fitzpatrick, M.P., Dierenfeld, E.S., 1998. Nutrient composition of selected whole invertebrates. Zoo Biol. 17, 123.
Beverung, C.J., Radke, C.J., Blanch, H.W., 1999. Protein adsorption at the oil/water interface: characterization of adsorption kinetics by dynamic interfacial tension measurements. Biophys. Chem. 81, 59.
Binks, B.P., 2002. Particles as surfactants—similarities and differences. Curr. Opin. Colloid Interface Sci. 7, 21.
Bußler, S., Rumpold, B.A., Jander, E., Rawel, H.M., Schlüter, O.K., 2016. Recovery and techno-functionality of flours and proteins from two edible insect species: meal worm (Tenebrio molitor) and black soldier fly (Hermetia illucens) larvae. Heliyon 2, e00218.
Cilia, M., Fish, T., Yang, X., McLaughlin, M., Thannhauser, T.W., Gray, S., 2009. A comparison of protein extraction methods suitable for gel-based proteomic studies of aphid proteins. J. Biomol. Tech. 20, 201.
David-Birman, T., Raften, G., Lesmes, U., 2018. Effects of thermal treatments on the colloidal properties, antioxidant capacity and in-vitro proteolytic degradation of cricket flour. Food Hydrocolloids 79, 48.
Dossey, A.T., Morales-Ramos, J.A., Guadalupe Rojas, M., 2016. Insects as Sustainable Food Ingredients. Elsevier, Cambridge, MA, p. 402.
Duda, A., Adamczak, J., Chełmińska, P., Juszkiewicz, J., Kowalczewski, P., 2019. Quality and nutritional/textural properties of durum wheat pasta enriched with cricket powder. Foods 8, 46.
Elhassan, M., Wendin, K., Olsson, V., Langton, M., 2019. Quality aspects of insects as food—nutritional, sensory, and related concepts. Foods 8, 95.
Fang, Y., Dalgleish, D.G., 1993. Dimensions of the adsorbed layers in oil-in-water emulsions stabilized by caseins. J. Colloid Interface Sci. 156, 329.
Gresiana, F., Marpaung, A., Sutanto, H., 2015. Protein Isolation from Cricket (Gryllus Mitratus). In: International Conference on Innovation, Entrepeneurship, and Technology. Swiss German University, Indonesia, BSD City, Indonesia.
Henchion, M., Hayes, M., Mullen, A.M., Fenelon, M., Tiwari, B., 2017. Future protein supply and demand: strategies and factors influencing a sustainable equilibrium. Foods 6, 53.
Horozov, T.S., Binks, B.P., 2006. Particle-stabilized emulsions: a bilayer or a bridging monolayer? Angew. Chem. 118, 787.
Janssen, R.H., Vincken, J.-P., van den Broek, L.A.M., Fogliano, V., Lakemond, C.M.M., 2017. Nitrogen-to-protein conversion factors for three edible insects: Tenebrio molitor, Alphitobius diaperinus, and Hermetia illucens. J. Agric. Food Chem. 65, 2275.
Jonas-Levi, A., Martinez, J.-J.I., 2017. The high level of protein content reported in insects for food and feed is overestimated. J. Food Compos. Anal. 62, 184.
Khalid, E.K., Babiker, E.E., El Tinay, A.H., 2003. Solubility and functional properties of sesame seed proteins as influenced by pH and/or salt concentration. Food Chem. 82, 361.
Kim, H.-W., Setyabrata, D., Lee, Y., Jones, O.G., Kim, Y.H.B., 2017. Effect of house cricket (Acheta domesticus) flour addition on physicochemical and textural properties of meat emulsion under various formulations. J. Food Sci. 82, 2787.
Larbi, F., García, A., del Valle, L.J., Hamou, A., Puiggalí, J., Belgacem, N., Bras, J., 2018. Comparison of nanocrystals and nanofibers produced from shrimp shell á-chitin: from energy production to material cytotoxicity and Pickering emulsion properties. Carbohydr. Polym. 196, 385.
Lu, X., Zhang, H., Li, Y., Huang, Q., 2018. Fabrication of milled cellulose particles-stabilized Pickering emulsions. Food Hydrocolloids 77, 427.
Magdassi, S., Neiroukh, Z., 1990. Chitin particles as O/W Emulsion stabilizers. J. Dispersion Sci. Technol. 11, 69.
Manditsera, F.A., Luning, P.A., Fogliano, V., Lakemond, C.M.M., 2019. Effect of domestic cooking methods on protein digestibility and mineral bioaccessibility of wild harvested adult edible insects. Food Res. Int. 121, 404.
Mohanty, B., Mulvihill, D.M., Fox, P.F., 1988. Emulsifying and foaming properties of acidic caseins and sodium caseinate. Food Chem. 28, 17.
Montowska, M., Kowalczewski, P.Ł., Rybicka, I., Fornal, E., 2019. Nutritional value, protein and peptide composition of edible cricket powders. Food Chem. 289, 130.
Murphy, R.W., Farkas, B.E., Jones, O.G., 2016. Dynamic and viscoelastic interfacial behavior of β-lactoglobulin microgels of varying sizes at fluid interfaces. J. Colloid Interface Sci. 466, 12.
Nakagawa, Y.S., Eijsink, V.G.H., Totani, K., Vaaje-Kolstad, G., 2013. Conversion of α-chitin substrates with varying particle size and crystallinity reveals substrate preferences of the chitinases and lytic polysaccharide monooxygenase of Serratia marcescens. J. Agric. Food Chem. 61, 11061.
Ndiritu, A.K., Kinyuru, J.N., Kenji, G.M., Gichuhi, P.N., 2017. Extraction technique influences the physico-chemical characteristics and functional properties of edible crickets (Acheta domesticus) protein concentrate. J.Food Meas.Char. 11, 2013.
Ndiritu, A.K., Kinyuru, J.N., Gichuhi, P.N., Kenji, G.M., 2019. Effects of NaCl and pH on the functional properties of edible crickets (Acheta domesticus) protein concentrate. J.Food Meas.Char. 13, 1788.
Nelson, G.C., Rosegrant, M.W., Koo, J., Robertson, R., Sulser, T., Zhu, T., Ringler, C., Msangi, S., Palazzo, A., Batka, M., 2009. Climate Change: Impact on Agriculture and Costs of Adaptation. International Food Policy Research Institute, Washington, D.C.
Osimani, A., Milanović, V., Cardinali, F., Roncolini, A., Garofalo, C., Clementi, F., Pasquini, M., Mozzon, M., Foligni, R., Raffaelli, N., Zamporlini, F., Aquilanti, L., 2018. Bread enriched with cricket powder (Acheta domesticus): a technological, microbiological and nutritional evaluation. Innov. Food Sci. Emerg. Technol. 48, 150.
Pérez, O.E., Carrera-Sánchez, C., Rodríguez-Patino, J.M., Pilosof, A.M.R., 2007. Adsorption dynamics and surface activity at equilibrium of whey proteins and hydroxypropyl–methyl–cellulose mixtures at the air-water interface. Food Hydrocolloids 21, 794.
Rumpold, B.A., Schlüter, O.K., 2013. Potential and challenges of insects as an innovative source for food and feed production. Innov. Food Sci. Emerg. Technol. 17, 1.
Rumpold, B.A., Schlüter, O.K., 2013. Nutritional composition and safety aspects of edible insects. Mol. Nutr. Food Res. 57, 802.
Sipponen, M.H., Mäkinen, O.E., Rommi, K., Heiniö, R.-L., Holopainen-Mantila, U., Hokkanen, S., Hakala, T.K., Nordlund, E., 2018. Biochemical and sensory characteristics of the cricket and mealworm fractions from supercritical carbon dioxide extraction and air classification. Eur. Food Res. Technol. 244, 19.
Stone, A.K., Karalash, A., Tyler, R.T., Warkentin, T.D., Nickerson, M.T., 2015. Functional attributes of pea protein isolates prepared using different extraction methods and cultivars. Food Res. Int. 76, 31.
Stone, A.K., Tanaka, T., Nickerson, M.T., 2019. Protein quality and physicochemical properties of commercial cricket and mealworm powders. J. Food Sci. Technol. 56, 3355.
Tzoumaki, M.V., Moschakis, T., Kiosseoglou, V., Biliaderis, C.G., 2011. Oil-in-water emulsions stabilized by chitin nanocrystal particles. Food Hydrocolloids 25, 1521.
Tzoumaki, M.V., Moschakis, T., Scholten, E., Biliaderis, C.G., 2013. In vitro lipid digestion of chitin nanocrystal stabilized o/w emulsions. Food Funct. 4, 121.
Van Huis, A., 2013. Potential of insects as food and feed in assuring food security. Annu. Rev. Entomol. 58, 563.
Webb, N.B., Ivey, F.J., Craig, H.B., Jones, V.A., Monroe, R.J., 1970. The Measurement of emulsifying capacity by electrical resistance. J. Food Sci. 35, 501.

Yi, L., Lakemond, C.M.M., Sagis, L.M.C., Eisner-Schadler, V., van Huis, A., van Boekel, M.A.J.S., 2013. Extraction and characterisation of protein fractions from five insect species. Food Chem. 141, 3341.
Yi, L., Boekel, M.A.J.S.V., Lakemond, C.M.M., 2017. Extracting Tenebrio molitor protein while preventing browning: effect of pH and NaCl on protein yield. J.Insect Food Feed 3, 21.
Yu, J., Ahmedna, M., Goktepe, I., 2007. Peanut protein concentrate: production and functional properties as affected by processing. Food Chem. 103, 121.
Zhang, M., Haga, A., Sekiguchi, H., Hirano, S., 2000. Structure of insect chitin isolated from beetle larva cuticle and silkworm (Bombyx mori) pupa exuvia. Int. J. Biol. Macromol. 27, 99.
Zhang, Y., Chen, Z., Bian, W., Feng, L., Wu, Z., Wang, P., Zeng, X., Wu, T., 2015. Stabilizing oil-in-water emulsions with regenerated chitin nanofibers. Food Chem. 183, 115.
Zielińska, E., Karaś, M., Baraniak, B., 2018. Comparison of functional properties of edible insects and protein preparations thereof. Lebensm. Wiss. Technol. 91, 168.