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Improving vitamin D stability and antioxidant activity in imitation mozzarella cheese by conjugated cricket protein with fructooligosaccharide

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A R T I C L E I N F O

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Vitamin D

A B S T R A C T

Conjugated cricket protein with fructooligosaccharide (FOS), called CPF, is a new alternative protein ingredient with great potential for food industry applications. This study aimed to investigate the effect of CPF as a partial substitute for rennet casein on the composition, appearance, color, antioxidant activities, sensory characteristics, and functional characteristics of imitated mozzarella cheese. The effect of CPF on vitamin D retention of cheese after processing and 28 subsequent days of storage was also studied. Incorporation of CPF by 10–40 g/100g resulted in significantly increased FOS as prebiotic fibers by about 0.1–4.2 g/100g in imitation mozzarella cheese, but slightly decreased the protein content and shifted color balance darker, with more yellow and red. The addition of CPF reduced hardness, cohesiveness, springiness, and stretchability (25.0–3.6 cm) but increased meltability, adhesiveness, and free oil release (2.0–9.3 cm) of cheeses. In addition, CPF enhanced vitamin D stability in the cheese matrix by about 3 times after heat treatment and 2.8 times after 28 days of storage (4 °C) when compared to the control. It is concluded that adding 10–20 g/100g CPF in imitation cheese improved vitamin D stability and provided acceptable sensory scores for consumers (like moderately, 7.1–7.3).

1. Introduction

The Food and Agriculture Organization (FAO) estimates that the world's population will reach nine billion people in 2050 (Melgar-Lalanne et al., 2019). To cater to all, the world needs to increase food production by 60%. However, global agriculture's current structure requires an additional 11% of land space and 70% of water. In other words, there will be a shortage of natural resources and agricultural land, and there will not be enough food to feed the entire population. Conventional protein sources such as meat, fish, eggs, and milk may not be sustainable to meet these needs (Mancini et al., 2022). Therefore, many researchers are trying to modify foods by replacing protein-rich animal products with alternative protein sources, such as those produced by plants, insects, microbial fermentation, or cell cultures (Liu et al., 2022). Among these, edible insects, primarily crickets, are a potential sustainable protein source recommended by FAO for consumption (Mancini et al., 2022).

The functional properties of cricket (*Acheta domesticus*) make it a potential insect protein source for use in the food industry (Igual et al., 2020). Crickets are becoming popular not only for their protein content (~65 g/100g) and the potential high percentage of usability of their bodies (~80 g/100g) for food production but also due to their high-quality fatty acids, dietary fiber, and dense mineral content (Udomsil et al., 2019). Moreover, they do not require much space or

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water, nor do they need drugs and chemicals in the farming process (Igual et al., 2020).

In many countries, including 113 countries in Asia, Africa, and Latin America, the consumption of insects is a tradition and people eat whole insects as a snack or as a daily meal (Melgar-Lalanne et al., 2019). However, in most Western countries, particularly in North America and Europe, edible insects are novel food. Though some Western consumers cannot eat whole insects, they can accept the insects when used as an ingredient to prepare familiar foods/products (Burt et al., 2020). However, the lower functional properties of cricket powder might limit its applications in food products (Nissen et al., 2020).

One previous study indicated that cricket protein powder conjugated with FOS (CPF) by Maillard reaction improves their functional and bioactive properties. CPF has higher total carbohydrates from FOS as prebiotic fibers (29 g/100g w/w) compared to rennet casein (0.5 g/100g w/w) and cricket protein powder (9 g/100g w/w). Moreover, CPF has better water solubility, oil-holding capacity, emulsifying properties, and 1.5–2 times higher antioxidant activities compared with unconjugated products (Chailangka et al., 2022). There are some studies where protein-saccharide conjugates can potentially encapsulate or act as a carrier for bioactive compounds in the food system and gastrointestinal tract (Nooshkam & Varidi, 2020; Sun et al., 2022). The application of conjugated cricket powder into a familiar food product is a way to promote the spread and consumption of this sustainable protein source. Some studies suggested that the solution to improve the acceptance and consumption of edible insects is to use them as active ingredients with familiar flavors and textures (Nissen et al., 2020).

Imitation cheese/processed cheese is a food product that is widely consumed in Western countries and is now becoming popular in Eastern countries (Talbot-Walsh et al., 2018). The formulation of imitation mozzarella cheese consists of a combination of dairy or nondairy protein sources, high content of vegetable fats/oils and water as key ingredients (Masotti et al., 2018). Some literature reviews reported that the strategies and trends to develop imitation cheeses are substituting dairy protein with any novel protein and using prebiotics such as inulin/FOS in the formula (Ferrão et al., 2016; Masotti et al., 2018). Protein, especially rennet casein, plays a vital role in forming a continuous network, which provides the backbone of the cheese matrix through which the fat phase is dispersed (Talbot-Walsh et al., 2018). Some studies investigated the effect of non-dairy proteins, including soy, pea, or corn, for casein substitution on cheese qualities. They found that non-dairy protein led to a softer, less rubbery texture, less compact microstructure, lower elasticity, and decreased melting properties (Mattice & Marangoni, 2020). However, more research work is still required to study milk protein replacement with other alternative proteins.

On the other hand, micronutrient deficiencies are an important global health issue related to a higher risk of infectious diseases, especially during the COVID-19 pandemic. The presence in food of beneficial compounds, such as antioxidants, vitamins, or nutraceuticals, which may boost the immune system and promote health, is attracting consumers' interest (Grosso et al., 2020). In this regard, vitamin D fortification is one method that can enhance processed cheese functionalities (Talbot-Walsh et al., 2018). Vitamin D is a fat-soluble vitamin, and its active form is 1,25-dihydroxy vitamin D_3. Vitamin D deficiency causes rickets in children and osteoporosis and fractures in adults. Although vitamin D is synthesized naturally in the skin when exposed to sunlight, it is estimated that 50–70% of Americans, 40% of Europeans, and 70% of Chinese adults lack vitamin D (Stratulat et al., 2015). Some researchers found that 18–25.5% of vitamin D was lost during cheese processing (Boivin-Piché et al., 2016; Stratulat et al., 2015). On the other hand, several researchers have reported that the preparation techniques such as entrapped vitamin D in fat phase or sodium caseinate-vitamin D complexes resulted in vitamin D preservation in dairy products (Syama et al., 2019).

However, the knowledge regarding the effect of CPF as a vitamin D encapsulator or carrier in the imitation cheese matrix on imitation mozzarella cheese quality and vitamin D stability after processing and during storage is still limited. Therefore, this study aimed to investigate the effect of 10–40 g/100g rennet casein replacement with CPF on physicochemical and functional characteristics, vitamin D stability, and sensory properties of imitation mozzarella cheese.

2. Materials and methods

2.1. Materials

Cricket powder (65 g/100g w/w protein, 9 g/100g w/w total carbohydrate) was purchased from Groove Grubb Part., Ltd., Thailand (CM, Thailand). Conjugated cricket protein (CPF; protein 66 g/100g w/w, total carbohydrate 29 g/100g w/w) was prepared by conjugating cricket protein isolate (CPI, *A. domesticus*, Groove Grubb Part., Ltd., Thailand) with fructooligosaccharide (FOS, DP = 2–9; Orafti, Bangkok, Thailand) by the wet-heating Maillard reaction (MR) method according to method of Chailangka et al. (2022).

All ingredients for cheese manufacture were provided by Foodtech Products (Thailand) Co., Ltd. (AYA, Thailand). Vitamin D_3 was kindly obtained from Vita Chemical Co., Ltd. (SKN, Thailand). The 1,1-diphenyl-2-picrylhy-drazyl (DPPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA).

2.2. Imitation mozzarella cheese preparation

The formula and preparation of imitation cheese followed the method of Bi et al. (2016) with slight modifications. Imitation mozzarella cheese was manufactured in 1 kg batch for 3 replications. The formulation per 100 g of control cheese consisted of 49.8 g water, 25.6 g rennet casein (90 g/100g w/w protein), 22 g vegetable oil, 1.5 g emulsifying salts (trisodium citrate 1.4 g/100g, citric acid, 0.1 g/100g), 1.0 g sodium chloride, 0.1 g cheese flavor, and 0.002 g vitamin D_3. Treated imitation cheeses were prepared by replacing 0, 10, 20, 30, and 40% of the casein protein in the control formulation with an equal quantity of CPF (Sample code: 0% CPF (control), 10% CPF, 20% CPF, 30% CPF, and 40% CPF, respectively), while other ingredients were not changed. Another treated imitation cheese was prepared with unconjugated cricket powder replacing 10% of the casein protein for comparison with conjugated cricket protein (Sample code: Cricket Powder 10%). The imitation mozzarella cheese preparation is shown in Fig. 1. Briefly, CPF was dispersed in water with a magnetic stirrer (C-MAG-HS-10 Ika, Staufen, Germany) at 700 rpm at 25 °C for 10 min. Then, the CPF solution was mixed with vitamin D_3 in vegetable oil and homogenized at 10,000 rpm for 2 min using an Ultra-Turrax homogenizer (T 25 digital, Ika, Staufen, Germany). After that, all ingredients were blended in a Stephan universal machine (Model UMC5, Stephan Machinery Corp., Hameln, Germany) at 750 rpm for 1 min at 35 °C and heated to 85 °C using direct steam. After 5 min of heating, the cheese mixture was packaged and cooled to 4 °C. After 24 h, the cheese samples were vacuum packed and kept in the refrigerator at 4 °C and analyzed within 3 months.

2.3. Chemical composition, DPPH radical scavenging activity and microbiological quality of imitation mozzarella cheese with CPF

The proximate analysis was determined following the methods provided by the Association of Official Analytical Chemists (AOAC) (AOAC, 2012). Total plate count bacteria, *Escherichia coli* and coliform bacteria, yeasts and molds, and bacterial pathogens were examined following the protocol previously described by Kim et al. (2018). The DPPH radical scavenging activity was determined according to the method of Chaiwong et al. (2022) with a slight modification. Briefly, the cheese sample (0.5 g) was mixed with absolute ethanol (9.5 mL). The mixture was then centrifuged at 4000×*g* for 15 min. The supernatant was collected and

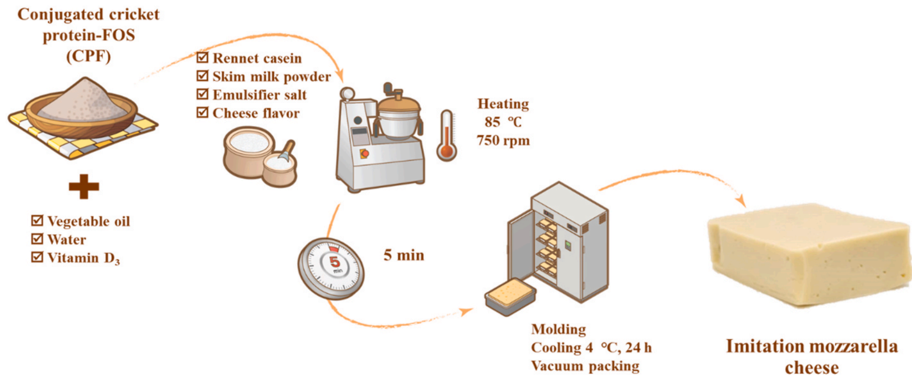


Fig. 1. Imitation mozzarella cheese preparation.

diluted to 0.5 mg/mL with absolute ethanol. Then, 1 mL of the sample was added to 1 mL of DPPH (0.2 mmol/L) in an ethanol solution, mixed thoroughly with a vortex mixer, and kept at 30 °C for 30 min in the dark. The absorbance was read at 517 nm using a UV-1800 spectrophotometer (Shimadzu UV-1601 PC, Shimadzu Corp., Kyoto, Japan). The DPPH free radical scavenging activity was expressed in terms of Trolox equivalent antioxidant capacity (TEAC) as μmol Trolox equivalents (TE)/g sample.

2.4. Color properties and visual appearance of imitation mozzarella cheese with CPF

The color of imitation mozzarella cheeses at various concentrations of CPF was determined according to the method by Phimolsiripol et al. (2017) using a Chroma Meter (CR-300, Konica Minolta Sensing, Inc., USA) to obtain the CIE L*, a*, and b* values. The total color difference between the control sample and CPF cheese expressed as the color development index (ΔE) was calculated as presented in Eq. (1):

$$\Delta E = \sqrt{\left(L_c^* - L_o^*\right)^2 + \left(a_c^* - a_o^*\right)^2 + \left(b_c^* - b_o^*\right)^2} \quad \text{Eq. 1}$$

where c defines CPF cheese and o is the control sample.

2.5. Texture profiles of imitation mozzarella cheese with CPF

Texture profile analysis (TPA) was performed using a Texture Analyzer TA-HD plus C (Stable Micro Systems, Godalming, Surrey, UK) according to the method of Grasso et al. (2021). The cheese samples were cut into a cylinder (25 mm diameter × 20 mm height) and kept inside the refrigerator (4 °C) before measurements. The cheese samples were compressed to 10 mm deformation by a 50 mm diameter cylinder aluminum probe (P/50); the rate of compression was 5 mm/s. The samples were evaluated for hardness, springiness, cohesiveness, and adhesiveness using Exponent Connect software V.7 (Stable Micro Systems, Godalming, Surrey, UK). Each measurement was repeated three times.

2.6. Meltability, free oil release and stretchability of imitation mozzarella cheese with CPF

The meltability, free oil release and stretchability were determined according to Li, Liu, et al. (2019) in triplicate with some modifications. The cheese sample was cut into a cylinder (25 mm diameter, 20 mm height, 10 ± 0.05 g weight) and placed in a Petri dish. The samples were placed horizontally in a conventional oven at 100 °C for 1 h. After cooling to room temperature, the diameter of the melted cheese, and free oil formed was detected. For the stretchability, samples were cut into a cylinder 10 mm thickness, 35 mm diameter and baked at 220 °C for 5 min. After cooling to 60 °C, the strand length of the hot cheese sample was evaluated.

2.7. Vitamin D_3 stability of imitation mozzarella cheese with CPF during storage

Vitamin D_3 was determined by an ultraviolet–visible procedure according to the method of Winuprasith et al. (2018) with slight modification. The cheese sample (0.5 g) was mixed with absolute ethanol (9.5 mL) and vortexed to extract vitamin D_3. The mixture was then centrifuged at 4000×g for 15 min. The supernatant was collected, and its absorbance was measured at 265 nm with the spectrophotometer. A standard curve (R^2 = 0.999) was used to determine the amount of vitamin D_3. The cheese sample was kept at 4 °C and measured in triplicate on days 0, 7, 14 and 28.

2.8. Sensory analysis of imitation mozzarella cheese with CPF

Sensory properties of imitation mozzarella cheese were evaluated after obtaining microbial counts and ensuring safety, with slight modifications from Talbot-Walsh et al. (2019) using 50 untrained panelists (30 females and 20 males; 20–60 years old). Panelists evaluated each sample for appearance, color, odor, texture, flavor, and overall acceptability. Cheese samples, randomly coded with three-digit numbers, were cut into 20 × 20 × 10 mm and left to equilibrate at 25 °C for 10 min before being served to panelists. The testing and scoring were based on a 9-point hedonic scale, where 1 = dislike extremely, 9 = like extremely and 5 = neither like nor dislike.

2.9. Statistical analysis

Each experiment was performed in triplicate, and all the data were presented as means ± standard deviation. Analysis of variance (ANOVA) was carried out using SPSS Version 15.0, and determination of significant differences among treatment means was done by Duncan's multiple range tests ($p < 0.05$).

3. Results and discussion

3.1. Composition, DPPH radical scavenging activity and microbiological quality of imitation mozzarella cheese

The proximate composition and antioxidant activities of mozzarella cheese with various CPF content are shown in Table 1. Rennet casein replacement with CPF slightly increased the total carbohydrate, moisture, a_w, and antioxidant activities. The addition of CPF at 10–40% casein replacement level increased moisture content (50.2–51.3 g water/100g cheese), a_w (0.969–0.986) and total carbohydrate (0.1–4.2 g/100g) of cheese. Additionally, all CPF cheese samples showed a significant ($p < 0.05$) increase in antioxidant activity by about 2–6 times compared with cricket powder and unfortified samples. However, the protein contents of mozzarella cheese decreased significantly when CPF

Table 1
Chemical composition, antioxidant activities and microbiological quality of imitation mozzarella cheese prepared with CPF.

Quality	0% CPF	10% CPF	20% CPF	30% CPF	40% CPF	10% Cricket powder
Moisture (g/100g w/w)	50.2 ± 0.3^{c}	50.4 ± 0.2^{bc}	50.7 ± 0.1^{b}	51.3 ± 0.1^{a}	51.1 ± 0.3^{a}	50.3 ± 0.4^{bc}
Protein (g/100g w/w)	23.1 ± 0.1^{a}	21.6 ± 0.3^{b}	20.3 ± 0.2^{c}	19.1 ± 0.2^{d}	18.1 ± 0.4^{e}	21.6 ± 0.1^{b}
Fat (g/100g w/w)^{ns}	22.2 ± 0.3	22.2 ± 0.3	22.2 ± 0.2	22.2 ± 0.4	22.2 ± 0.4	22.2 ± 0.1
Ash (g/100g w/w)	4.4 ± 0.1^{b}	4.4 ± 0.2^{b}	4.3 ± 0.1^{b}	4.3 ± 0.1^{b}	4.4 ± 0.2^{b}	5.1 ± 0.1^{a}
Total carbohydrate (g/100g w/w)	0.1 ± 0.01^{f}	1.4 ± 0.1^{d}	2.5 ± 0.1^{c}	3.1 ± 0.1^{b}	4.2 ± 0.1^{a}	0.8 ± 0.1^{e}
a_w	0.969 ± 0.002^{c}	0.979 ± 0.001^{b}	0.982 ± 0.001^{ab}	0.983 ± 0.002^{ab}	0.986 ± 0.004^{a}	0.980 ± 0.006^{b}
DPPH (μmol Trolox/g sample)	11.6 ± 0.6^{e}	23.1 ± 0.4^{d}	36.9 ± 0.1^{c}	50.6 ± 0.5^{b}	64.8 ± 0.7^{a}	11.9 ± 0.1^{e}
Total bacteria count (CFU/g)	<10	<10	<10	<10	<10	650 ± 60
Yeast & Mold (CFU/g)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
E. Coli & Coliform (MPN/25g)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Salmonella spp. (CFU/25g)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Bacillus cereus (CFU/g)	<10	<10	<10	<10	<10	<10
Clostridium perfringens (CFU/g)	<10	<10	<10	<10	<10	<10
Listeria monocytogenes (CFU/25g)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

Total carbohydrate = CHO + Crude fiber. N.D. = Not detect.
Mean ± standard deviation of triplicate determinations with different superscript letters in the same row are significantly different ($p < 0.05$). 0% CPF (control), 10% CPF, 20% CPF, 30% CPF and 40% CPF samples indicate the casein substitution level in the formulation by conjugated cricket protein. Cricket powder 10% indicates the casein substitution with unconjugated cricket protein.

concentration increased. The protein content of cheese samples prepared with 10% cricket powder or 10% CPF was similar. Conversely, the carbohydrate content was lower for 10% cricket powder. These results could be due to lower protein content but higher total carbohydrate from FOS as prebiotic fibers of CPF (66 g/100g w/w protein, 29 g/100g w/w total carbohydrate) compared to rennet casein (90 g/100g w/w protein, 0.5 g/100g w/w total carbohydrate) and cricket powder (65 g/100g w/w protein, 9 g/100g w/w total carbohydrate). Moreover, a previous study by Chailangka et al. (2022) indicated that conjugated cricket protein with FOS improved antioxidant activities by about 1.5–2 times. The greater antioxidant capacity of the conjugated product was probably due to the ability of the conjugate to donate electrons and the heat-induced protein to reveal more amino acid residues with strong antioxidant properties. Furthermore, the formation of intermediate and final Maillard reaction products resulted in an enhancement of the native protein's antioxidant abilities through its ability to donate hydrogen, chelate metals, and sequester oxygen radicals and functional properties. Moreover, the conjugation of cricket protein and FOS enhanced its water solubility and water-holding capacity due to the unfolding of the protein structure during the protein-saccharide

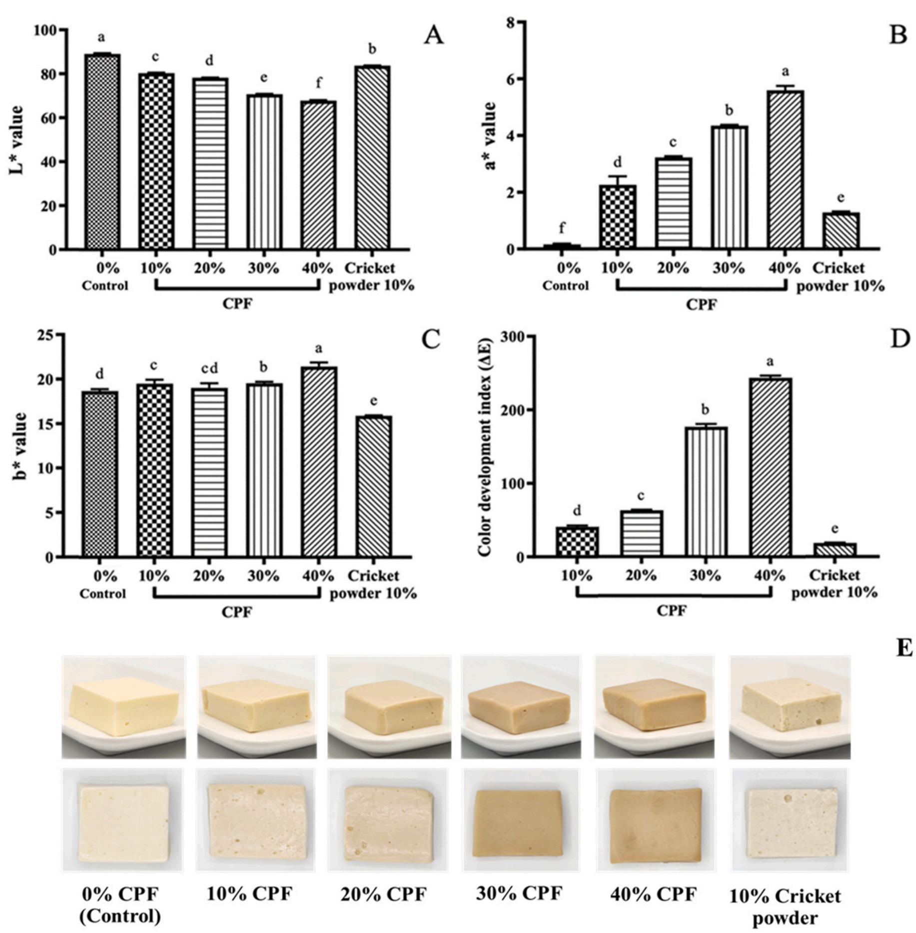


Fig. 2. L* value (A), a* value (B), b* value (C), color development index as ΔE (D) and the visual appearance (E) of imitation mozzarella cheese with CPF. 0% CPF (control), 10% CPF, 20% CPF, 30% CPF, and 40% CPF samples indicate the casein substitution level in the formulation by conjugated cricket protein. Cricket powder 10% indicates the casein substitution with unconjugated cricket protein. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

conjugation process. Additionally, the presence of hydrophilic groups from the conjugated saccharides created a hydration layer on the surface of the protein, further increasing its hydrophilicity and water solubility when added in imitation cheese. Therefore, adding more CPF content resulted in higher water content, more prebiotic fiber (FOS), and more antioxidant activities in imitation mozzarella cheese.

On the other hand, the microbiological population in imitation mozzarella cheese is shown in Table 1. As can be seen in the table, the total plate bacteria count for all samples were ~22–65 CFU/g. Yeast and mold, *E. coli* and coliform, *Salmonella* spp. and *Listeria monocytogenes* were not found. *Bacillus cereus* and *Clostridium perfringens* were lower than 10 CFU/g in all treatments of imitation mozzarella cheese. These results support that the imitation mozzarella cheese with CPF and cricket protein met the pathogenic microorganism's safety standard according to the legal international standard, and especially those of Thailand and the United States (Kim et al., 2018).

3.2. Color properties and visual appearance of imitation mozzarella cheese with CPF

The results of the color parameters and visual appearance of the control mozzarella and mozzarella fortified with CPF are presented in Fig. 2 A-E. The use of CPF and cricket powder to replace rennet casein in mozzarella cheese promoted significant ($p < 0.05$) changes in all color measurement values of the products. The lightness (L*) of cheese was significantly reduced as the CPF and cricket powder contents increased, resulting in a darker visual appearance of cheese, as shown in Fig. 2 E. The a* and b* values increased from 0.16–5.60 to 18.66–21.40 (control to CPF 40%), which indicates more red and yellow in the color formation of the cheese. This was due to the natural color of cricket powder and the brown color of conjugated cricket protein developed by the Maillard reaction (Chailangka et al., 2022). Moreover, the color development index (ΔE) of the CPF and cricket powder fortified cheese significantly increased, meaning higher CPF content in cheese more significantly affected the difference in color from the control sample. The darker, redder, and yellower color of the cheese is due to the natural color of the ingredients, especially CPF and cricket powder. These results are similar to previous reports that fortified cricket protein in muffins (Pauter et al., 2018), oat biscuits (Biró et al., 2020), and gluten-free bread (da Rosa Machado & Thys, 2019) resulted in significant changes in the color of the products, which became darker with more redness and browning.

3.3. Texture properties of imitation mozzarella cheese with CPF

The texture properties of imitation mozzarella cheeses with various CPF content are shown in Table 2. Hardness is defined as the maximum force necessary to deform the tested body. The adhesiveness value represents the attachment of food to other objects, such as gums, teeth, and lips, while eating or packaging. The cohesiveness represents the strength of the internal bonds of the cheese body before rupturing while biting, cutting, or shredding the cheese. Springiness is the flexibility of food that, when pressured, is reversible (Sołowiej et al., 2020). The level of added CPF significantly affected hardness, adhesiveness, cohesiveness, and springiness ($p < 0.05$). When CPF concentration increased from 10 to 40 g/100g casein replacement, the cheese's hardness, cohesiveness, springiness, and adhesiveness gradually declined. The cheese with 40 g/100g CPF substitution had the lowest hardness, cohesiveness, springiness, and adhesiveness.

The factors affecting the cheese texture are the formation of the structure of the casein protein, protein content, and moisture content (Kapoor & Metzger, 2008). The increase in hardness, cohesiveness, and springiness, and the low stickiness (adhesiveness) of the cheese are due to the increase in protein content and in linkage of the structure of the casein protein, and also due to the decrease in moisture content (Grasso et al., 2021; Kapoor & Metzger, 2008). Similar results were reported in

Table 2
Textural properties of imitation mozzarella cheese with CPF.

TPA Parameter	0% CPF	10% CPF	20% CPF	30% CPF	40% CPF	10% Cricket powder
Hardness (kg)	5.94 ± 0.08^{a}	4.69 ± 0.16^{b}	4.42 ± 0.15^{cd}	4.03 ± 0.13^{de}	2.71 ± 0.05^{f}	4.27 ± 0.20^{cd}
Adhesiveness (g.s)	13.81 ± 3.31^{a}	14.78 ± 0.89^{a}	18.94 ± 1.83^{a}	53.91 ± 3.17^{c}	56.4 ± 2.45^{c}	27.61 ± 8.67^{b}
Springiness (%)	0.44 ± 0.03^{a}	0.41 ± 0.04^{a}	0.27 ± 0.06^{b}	0.14 ± 0.01^{d}	0.12 ± 0.01^{d}	0.20 ± 0.01^{c}
Cohesiveness (%)	0.46 ± 0.04^{a}	0.36 ± 0.02^{b}	0.27 ± 0.07^{c}	0.13 ± 0.01^{d}	0.13 ± 0.01^{d}	0.24 ± 0.02^{c}

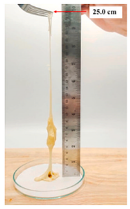
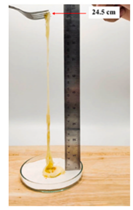
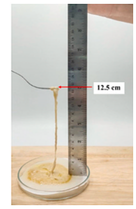
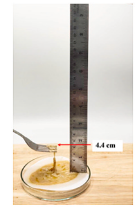
Mean ± standard deviation of triplicate determinations with different superscript letters in the same row are significantly different ($p < 0.05$). 0% CPF (control), 10% CPF, 20% CPF, 30% CPF and 40% CPF samples indicate the casein substitution level in the formulation by conjugated cricket protein. Cricket powder 10% indicates the casein substitution with unconjugated cricket protein.

sesame protein isolate replacement of milk protein in cheese, processed cheese manufactured with various starches (Talbot-Walsh et al., 2019), or plant-based block-style products (Grasso et al., 2021). When comparing the texture properties of cheese enriched with conjugated cricket protein and cricket powder, the results showed that 10% CPF cheese had significantly higher ($p < 0.05$) hardness (4.69 kg), cohesiveness (0.36%), and springiness (0.41%) but lower adhesiveness (14.78 g s) than 10% cricket powder cheese (4.27 kg, 0.24%, 0.20%, 27.61 g s). This could be due to CPF having significantly higher levels of functional properties by about 1.5–2 times compared with unconjugated products (Chailangka et al., 2022). The combination of cricket protein and FOS through conjugation changed the functional properties. The hydrophobic protein bonds to the surface of oil droplets, while the hydrophilic group of the saccharide, which is covalently linked, enhances the stability of the emulsion by thickening the water phase. This leads to the emulsion's greater stability and a better cheese structure than native protein (de Oliveira et al., 2016). Therefore, the CPF can strengthen the structure in imitation mozzarella cheese.

3.4. Meltability, free oil release, and stretchability of imitation mozzarella cheese with CPF

The meltability, free oil release, and stretchability of imitation mozzarella cheese with CPF are reported in Table 3. These qualities are related to the ability to be used in a baked goods or pizza making (Dai et al., 2019). The increased CPF content in cheese significantly increased cheese meltability, reduced stretchability, and exhibited more free oil release ($p < 0.05$). The stretchability and free oil release of the 10% CPF (24.5 cm and 2.4 cm) and 20% CPF (24.3 cm and 2.6 cm) cheese were near to that of the control sample (25.0 cm and 2.1 cm). The 40% CPF cheese showed the greatest meltability (4.4 cm), and the highest free oil release (9.3 cm), but the lowest stretchability (3.6 cm). These results were in full agreement with those reported by Sołowiej et al. (2014) and Szafrańska et al. (2020), where the authors found that a decrease in the proportion of casein proteins and increased moisture content in cheeses lead to increased meltability and free oil release. The factors influencing imitation cheese meltability, free oil release and stretchability are the casein protein network, the moisture-to-protein ratio, and the nonfat milk solid (Grasso et al., 2021). The excellent water absorption of CPF might have resulted in more moisture content in the cheese matrix, leading to increased hydrophilic protein-protein interactions and more unbound water, thus helping cheese particles to flow when heated, enhancing meltability (Chailangka et al., 2022; Sołowiej et al., 2020).

Table 3
Meltability, free oil release and stretchability of imitation mozzarella cheese with CPF.

Parameter	0% CPF	10% CPF	20% CPF	30% CPF	40% CPF	10% Cricket powder
Stretchability (cm)	 25.0 ± 0.2^{a}	 24.5 ± 0.9^{ab}	 24.3 ± 0.8^{ab}	 12.4 ± 0.4^{d}	 3.6 ± 0.3^{e}	 19.2 ± 0.3^{c}
Meltability (cm)	3.2 ± 0.2^{d}	3.6 ± 0.1^{c}	3.6 ± 0.2^{c}	3.9 ± 0.1^{b}	4.4 ± 0.2^{a}	3.7 ± 0.1^{c}
Free oil release (cm)	2.0 ± 0.3^{d}	2.4 ± 0.4^{c}	2.6 ± 0.5^{c}	8.5 ± 0.5^{b}	9.3 ± 0.3^{a}	2.8 ± 0.2^{c}

Mean ± standard deviation of triplicate determinations with different superscript letters in the same row are significantly different ($p < 0.05$). 0% CPF (control), 10% CPF, 20% CPF, 30% CPF and 40% CPF samples indicate the casein substitution level in the formulation by conjugated cricket protein. Cricket powder 10% indicates the casein substitution with unconjugated cricket protein.

However, the CPF replacement in cheese might have disrupted the casein protein structure and led to lower protein content, affecting the casein matrix, causing it to easily collapsing during heating, which allowed fat globules to coalesce, leading to more leakage and lower stretchability of cheese (Li, Liu, et al., 2019). The 10% CPF cheese had better stretchability and fat leakage resistance than 10% cricket powder cheese because CPF had higher functional property levels than cricket powder (Burt et al., 2020). Additionally, the meltability of cheese fortified with CPF and cricket powder was not significantly different. Therefore, the 10–20 g CPF/100g casein replacement in imitation mozzarella cheese showed better results than cricket powder and was similar to control mozzarella cheese.

3.5. Vitamin D_3 stability of imitation mozzarella cheese with CPF during storage

Fig. 3 shows the stability of vitamin D_3 in imitation mozzarella cheese incorporated with various CPF content during 28 days of storage. The initial value (50 μg/100g) was calculated from vitamin D_3 addition before the heating stage of imitation cheese processing. As can be seen in Fig. 3, all cheese samples showed a reducing trend of vitamin D_3 content after processing and 28 days of storage. Vitamin D_3 is sensitive to aspects of processing and storage conditions such as oxygen, light, high temperature, and low pH (Khan et al., 2020). Vitamin D_3 in cricket powder 10% and control cheese samples had the highest reduction rate at 22% after cheese processing (day 0) and reached 29% at 28 days. These results agreed with those found by Boivin-Piché et al. (2016) and Stratulat et al. (2015), who found that ~18–25.5% of vitamin D was lost during cheese processing. However, vitamin D_3 incorporated with various CPF contents in the cheese matrices was more stable than in cricket powder and control cheese. In 10–40 g CPF/100g casein replacement cheese, only a slight decrease in the amount of vitamin D_3 took place after cheese processing (2–6%) and after 28 days (5–10%). The 20–40 g CPF/100g casein replacement in cheese did not have significant differences in vitamin D_3 content (47.07–47.12 μg/100g) at 28 days and showed higher contents than 10% CPF (44.8 μg/100g), 10% cricket powder (35.04 μg/100g), and the control sample (35.11 μg/100g). These results might reveal that CPF is a good vitamin D carrier and improves stability in the cheese matrix after processing and storage. There are some studies that have investigated the nutraceutical-loaded ability, encapsulation, and protection of protein-polysaccharide conjugates products such as corn protein hydrolysate-carboxymethyl chitosan conjugates loaded with rutin (Han et al., 2019), bovine serum albumin-dextran Maillard-based conjugate nanoparticles loaded with curcumin (Fan et al., 2018), or fabricated casein-maltodextrin-proanthocyanidins nanoparticles (Sun et al., 2021). They proved that bioactive compounds can be applied in emulsion form as protein-saccharide conjugates products. This process resulted in acceptable stability and retention due to formation of denser and thicker viscoelastic layers around smaller emulsion droplets which were homogeneously distributed. In addition, Sadiq et al. (2021) confirmed that protein casein micelles are amphiphilic and can bind different compounds in food complexes. This probably provides interaction and protection to vitamin D and vitamin D-CPF complex from environmental stress in our case. Syama et al. (2022) also indicated that hydrophobic interaction is responsible for the binding of vitamin D with the casein complex, and casein micelle enhances the protection of vitamin D under acidity during gastric digestion. Loewen et al. (2018) found that casein micelle loaded with vitamin D can improve vitamin D stability during storage for 21 days. This was due to the surface of the casein micelle that may provide a physical barrier to vitamin D. Moreover, vitamin D mobility is limited because binding to caseins can decrease its availability for chemical degradation. Thus, the complex of casein, vitamin D, and conjugated cricket protein helps in enhancing vitamin D stability in cheese.

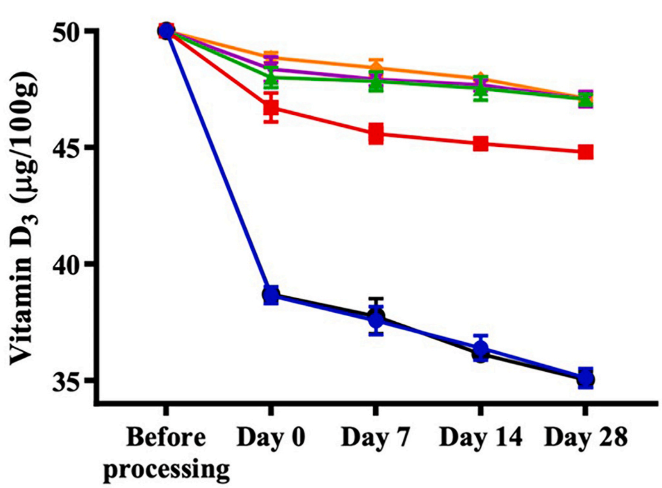


Fig. 3. Vitamin D_3 stability of imitation mozzarella cheese with various replacement contents of CPF during storage. 10% CPF (red square), 20% CPF (green triangle), 30% CPF (purple inverted triangle), and 40% CPF (orange diamond) indicate the casein substitution level in the formulation by conjugated cricket protein. 10% cricket powder (black circle) indicates the casein substitution with unconjugated cricket protein. Control sample (blue circle) indicates original imitation mozzarella cheese.

3.6. Sensory evaluation

Sensorial hedonic scores of imitation mozzarella cheese incorporated with different levels of CPF are presented in Table 4. There were significant ($p \geq 0.05$) differences of mean hedonic scores of all sensory

Table 4
Sensory properties of imitation mozzarella cheese with CPF.

Sensory attributes	0% CPF	10% CPF	20% CPF	30% CPF	40% CPF	10% Cricket powder
Appearance	7.4 ± 1.0^{a}	7.3 ± 1.0^{a}	7.2 ± 1.0^{a}	5.6 ± 1.3^{c}	5.4 ± 1.2^{c}	6.5 ± 1.0^{b}
Color	7.3 ± 1.1^{a}	7.2 ± 1.0^{a}	7.2 ± 1.0^{a}	5.2 ± 1.2^{c}	5.2 ± 1.2^{c}	7.0 ± 1.2^{b}
Odor	7.2 ± 1.0^{a}	7.2 ± 1.0^{a}	7.2 ± 1.0^{a}	6.8 ± 1.2^{bc}	6.6 ± 1.0^{c}	7.0 ± 1.0^{ab}
Texture	7.2 ± 0.8^{a}	7.1 ± 1.2^{a}	7.1 ± 0.9^{a}	5.6 ± 1.1^{c}	5.4 ± 1.2^{c}	6.2 ± 1.0^{b}
Flavor	7.2 ± 1.0^{a}	7.2 ± 1.1^{a}	7.2 ± 1.0^{a}	6.6 ± 1.2^{bc}	6.6 ± 0.8^{c}	7.0 ± 0.9^{ab}
Overall acceptability	7.3 ± 0.9^{a}	7.2 ± 1.0^{a}	7.2 ± 1.0^{a}	5.7 ± 0.8^{c}	5.9 ± 1.1^{c}	6.0 ± 1.0^{b}

Mean ± standard deviation of triplicate determinations with different superscript letters in the same row are significantly different ($p < 0.05$). 0% CPF (control), 10% CPF, 20% CPF, 30% CPF and 40% CPF samples indicate the casein substitution level in the formulation by conjugated cricket protein. Cricket powder 10% indicates the casein substitution with unconjugated cricket protein.

attributes, especially in appearance, color, and texture for all treatments. These were according to the result of the color parameters, visual appearance, and texture properties as presented in Section 3.2-3.3. The cheese with 10–20 g/100g CPF substitution received scores of "like moderately" (7.1–7.3) in all sensorial attributes and was not different from the control (no CPF). With higher CPF concentrations of 30–40 g/100g, the hedonic scores of the cheese's appearance, color, odor, texture, flavor, and overall acceptability gradually decreased. The cheese with 40 g/100g CPF substitution had the lowest all-attribute liking score, especially in appearance, color, texture, and overall acceptability, reaching the score range of "neither like nor dislike" (5.2–5.9). This is because the panelists observed a grainy and sandy texture from the cricket powder, which can be found in several food products incorporated with cricket powder (da Rosa Machado & Thys, 2019; Burt et al., 2020; Igual et al., 2020).

4. Conclusions

Replacing rennet casein by CPF in mozzarella cheese resulted in a better retention of vitamin D_3 after heat treatment and storage at 4 °C for 28 days (93% remaining) compared to cricket powder and control samples (losing about 30%). Overall, replacing 10–20% of rennet casein with CPF in mozzarella cheese can enhance antioxidant properties and improve vitamin D_3 stability, while maintaining similar physical, textural, and sensory properties to the control cheese. Using CPF in mozzarella cheese may also be a way to promote the use of this sustainable protein source. CPF and cricket powder caused the cheese to appear darker, redder, and yellower in color. Higher CPF concentrations may have disrupted the structure of rennet casein, leading to an increase in moisture content, meltability, adhesiveness, and oil release and to a decrease in hardness, cohesiveness, springiness, and stretchability of the cheese. Thus, CPF can be applied in several foods, such as noodles and pasta, or can be used as an active ingredient in functional food. Future works are required to investigate the effect of CPF in *in vitro* and *in vivo* bio-accessibility and digestion. In addition, determining antioxidant activities with different methods during the storage of cheese would be worthwhile.

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Ethical guidelines

Participants, who were involved in the sensory test, gave informed consent via the statement "I am aware that my responses are confidential, and I agree to participate in this sensory test" where an affirmative reply was required to enter the test. They were able to withdraw from the test at any time without giving a reason. The tested products were safety for consumption.

CRediT authorship contribution statement

Auengploy Chailangka: Investigation, Data curation, Conceptualization, Writing – original draft. **Noppol Leksawasdi:** Writing – review & editing. **Phisit Seesuriyachan:** Writing – review & editing. **Warintorn Ruksiriwanich:** Writing – review & editing. **Sarana Rose Sommano:** Writing – review & editing. **Kittisak Jantanasakulwong:** Writing – review & editing. **Pornchai Rachtanapun:** Writing – review & editing. **Juan Manuel Castagnini:** Writing – review & editing. **Francisco J. Barba:** Writing – review & editing. **Yuthana Phimolsiripol:** Supervision, Conceptualization, Writing – review & editing.

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.

Data availability

Data will be made available on request.

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