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Meat analogue preparation from cricket and rice powder mixtures with controlled textural and nutritional quality by freeze alignment technique

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ABSTRACT

The rising demand for sustainable protein sources has encouraged interest in alternative food products like meat analogues. This study explores formulating meat analogues using cricket powder (CP) and rice flours, comparing them with soy protein-based analogues. CP exhibited a higher soluble protein content (5.9%) than soy protein powder (4.7%), enhancing textural properties by forming fiber-like structures, increasing firmness and adhesion, and reducing chewiness. Despite having a lower water holding capacity (WHC) than soy analogues, all samples outperformed chicken breast WHC. Under freezing conditions, CP and rice flour combinations formed multi-layered structures in protein gels. The optimal formulations were CP and sticky rice flour (ratio 6:1) with respect to their texture properties, fiber structure, and nutritional value. This study highlights the potential of cricket powder and rice flour combinations as viable meat analogue ingredients, addressing the need for sustainable protein sources in the food industry.

1. Introduction

In recent times, there has been a growing global interest in nutritious dietary alternatives, with a specific focus on plant-based protein sources. Comprehensive research indicates that the production of meat carries adverse environmental implications and exerts pressure on natural resources (Henchion, Hayes, Mullen, Fenelon, & Tiwari, 2017). As a result, the development of plant-based proteins and meat substitutes has significantly increased. Consequently, there is a distinct consumer trend towards reducing meat consumption and embracing plant-based nutrition replacements (Mattice & Marangoni, 2020). Plant-based meat analogues, often known as substitute meat or fake meat, mimic muscle meat in a variety of ways, including visual appearance, color, texture, and structural features (Osen, Toelstede, Wild, Eisner, & Schweiggert-Weisz, 2014). To clarify, plant-derived ingredients create artificial meat, a food product that mimics the nutritional value and sensory characteristics of traditional meat. Plant proteins, found in well-known foods like wheat, soybeans, peas, and peanuts, serve as the primary building blocks of synthetic meat. Unconventional sources such as mung beans and lupin have also been investigated (Pietsch, Bühler, Karbstein, & Emin, 2019).

Edible insects have long been acknowledged as a feasible and sustainable food source for the human diet. A notable array of over 2000 insect species exists (Rumpold & Schlüter, 2013), encompassing categories like grasshoppers, beetles, bees, and nematodes, with the latter being prevalent among the commonly ingested insect groups (Udomsil, Imsoonthornruksa, Gosalawit, & Ketudat-Cairns, 2019). Across different nations, the consumption of insects holds cultural significance, although the specific species embraced differ in correspondence with geographic location. Remarkably, around 113 countries spanning Asia, Africa, Australia, and the Americas exhibit a general proclivity for insect consumption (Van Huis et al., 2013).

Edible insects have now emerged as a novel trend within the global food market, garnering attention due to their dual role as both a healthful and nutritious dietary option. These insects are replete with valuable nutrients, including ample healthy fats, proteins, vitamins, dietary fiber, and low carbohydrate content. Significantly, the protein content within insects averages between 10% and 70% of their dry weight, with a majority boasting protein levels as high as 60%. This substantial protein content renders them a fitting source to meet the

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Table 1
Formulation of the prepared meat analogues.

Ingredients		Cricket powder; Rice flour		Cricket powder; Sticky rice flour		Cricket powder
	Control*	**CR1**	**CR2**	**CSR1**	**CSR2**	**CP**
Soy Protein	6.0	0	0	0	0	0
Cricket	0	5.0	6.0	5.0	6.0	7.0
Rice flour	0	2.0	1.0	0	0	0
Sticky rice flour	0	0	0	2.0	1.0	0
Sodium alginate	2.0	2.0	2.0	2.0	2.0	2.0
Salt	1.0	1.0	1.0	1.0	1.0	1.0
MSG	0.1	0.1	0.1	0.1	0.1	0.1
Pepper	0.2	0.2	0.2	0.2	0.2	0.2
Oil	8.2	8.2	8.2	8.2	8.2	8.2
Water	82.5	81.5	81.5	81.5	81.5	81.5

* Modified the formula by Chantanuson et al. (2022).

Table 2
Proximate analysis and soluble protein content of raw materials.

Parameter (%)	Soy protein	Rice flour	Sticky rice flour	Cricket powder
Moisture	4.91 ± 0.13^{c}	7.69 ± 0.45^{a}	6.84 ± 0.20^{b}	2.12 ± 0.13^{d}
Ash	5.01 ± 0.35^{a}	1.31 ± 0.05^{d}	1.85 ± 0.04^{c}	3.68 ± 0.19^{b}
Protein	42.33 ± 0.43^{b}	8.35 ± 0.63^{c}	7.66 ± 0.05^{d}	59.83 ± 0.10^{a}
Fat	22.94 ± 0.97^{a}	3.69 ± 0.03^{c}	3.68 ± 0.27^{c}	20.03 ± 0.41^{b}
Fiber	13.89 ± 0.78^{a}	1.38 ± 0.03^{d}	1.71 ± 0.11^{c}	5.68 ± 0.05^{b}
Carbohydrate	10.92 ± 0.35^{c}	77.58 ± 0.11^{b}	78.26 ± 0.30^{a}	8.66 ± 0.38^{d}
Soluble protein content	4.75 ± 0.05^{b}	0.70 ± 0.04^{c}	0.50 ± 0.04^{d}	5.97 ± 0.16^{a}

Values are expressed as mean ± SD of triplicate measurements (n = 3) Means with different letters (a,b,c, …) are significantly different at p < 0.05 within the same row in the parameter.

Table 3
Pasting properties of rice flour and sticky rice flour.

Pasting properties	Rice flour	Sticky rice flour
Peak viscosity (Cp)	3136.33 ± 47.54^{b}	3629 ± 15.31^{a}
Trough viscosity (Cp)	2634.33 ± 55.05^{a}	2011.67 ± 20.81^{b}
Breakdown (Cp)	502.00 ± 8.66^{b}	1617.67 ± 6.65^{a}
Final Viscosity (Cp)	4748.33 ± 144.19^{a}	2543.00 ± 21.70^{b}
Setback (Cp)	2114.00 ± 190.53^{a}	531.33 ± 26.73^{b}
Pasting temperature (°C)	83.32 ± 3.41^{a}	69.07 ± 0.62^{b}

Values are expressed as mean ± SD of triplicate measurements (n = 3) Means with different letters (a,b) are significantly different at $p < 0.05$ within the same row in the parameter.

Table 4
color (L^*, a^* and b^*) and water holding capacity (%) of the prepared meat analogues.

Sample	color			WHC (%)
	L^*	a^*	b^*	
Control	74.30 ± 0.17^{b}	1.21 ± 0.01^{e}	17.15 ± 0.05^{a}	52.20 ± 2.57^{a}
CR1	51.87 ± 0.25^{c}	3.32 ± 0.02^{b}	13.27 ± 0.25^{c}	44.38 ± 3.91^{b}
CR2	49.86 ± 0.15^{d}	3.09 ± 0.08^{c}	12.22 ± 0.34^{e}	45.94 ± 2.55^{b}
CSR1	52.51 ± 0.88^{c}	3.11 ± 0.04^{c}	13.62 ± 0.31^{bc}	45.11 ± 3.33^{b}
CSR2	52.68 ± 0.11^{c}	2.91 ± 0.02^{d}	12.83 ± 0.07^{d}	47.47 ± 1.49^{b}
CP	47.94 ± 0.13^{e}	3.58 ± 0.01^{a}	13.80 ± 0.13^{b}	47.09 ± 2.51^{b}
Chicken breast	80.21 ± 0.74^{a}	1.18 ± 0.02^{e}	11.49 ± 0.13^{f}	23.17 ± 0.95^{c}

CR, CSR and CP is Rice flour, Sticky rice flour and cricket respectively. Values are expressed as mean ± SD of triplicate measurements (n = 3) Means with different letters (a, b, c, …) are significantly different at p < 0.05 within the same column in the parameter.

dietary recommendations for children as stipulated by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO). Additionally, insects serve as an excellent reservoir of diverse micronutrients, encompassing essential minerals like copper, iron, magnesium, manganese, phosphorous, selenium, and zinc, along with vital vitamins such as riboflavin, pantothenic acid, biotin, and folic acid (Rachman, Brennan, Morton, & Brennan, 2021; Rumpold & Schlüter, 2013). Several investigations are presently underway regarding the utilization of edible insects in diverse food formulations, including ice cream (David-Birman et al., 2022) and meat analogs such as jerky substitutes (Kim et al., 2022). Furthermore, Mishyna, Keppler, & Chen (2021) conducted a study investigating the integration of edible insect proteins into meat products as a functional food additive. This approach yielded several technical advantages, including notably enhanced emulsion and gelling properties. In this context, substituting edible insects for a portion of meat constituents emerges as a promising strategy for developing sustainable meat products that retain nutritional attributes while contributing to a more environmentally responsible and ecologically sustainable solution.

Artificial meat production encompasses various innovative methodologies aimed at manipulating soluble and/or insoluble protein fractions to imitate the fibrous textures and structures found in authentic meat. These techniques involve complicated processes, including compression, freeze alignment techniques, electric rotation, *in vitro* cultivation of animal cells, precision cell shearing, among others. The extrusion technique is widely recognized as one of the most promising industrial techniques. It is particularly valued for large-scale continuous production that maintains stable quality. However, research and development for the production of meat analogues is still in a developing stage. In order to achieve the desired texture characteristics of the final products, the processing conditions need to be improved by tuning the variations of factors. (Ferawati et al., 2021; Mateen, Mathpati, & Singh, 2023; Osen et al., 2014; Wittek, Ellwanger, Karbstein, & Emin, 2021). Alternatively, it would be important to consider processes other than the extrusion process.

The freeze-alignment technique, also denoted as the freeze structuring technique, is a potential technique for small-scale industries that cannot afford expensive investments (Yuliarti, Kiat Kovis, & Yi, 2021). This technique involves freezing plant-based protein emulsions, resulting in ice crystals. When heat removal is uniform, the resulting ice crystal arrangement exhibits isotropic characteristics, whereas when unidirectional heat removal is applied, it yields an anisotropic structure due to the alignment of ice crystals. Subsequently, these frozen products undergo thawing, thereby preserving the aligned, porous, and interconnected fiber-like microstructures formed by the ice crystals. This aligned arrangement remarkably resembles the layered structure present in animal meat muscles. Research by Chantanuson, Nagamine, Kobayashi, and Nakagawa (2022) demonstrates the widespread application of the freeze structuring methodology in shaping protein structures derived from meat, fish, and plant sources. The technoeconomic analysis results reported that meat analogue production by freeze-alignment technology is an attractive investment opportunity and that reduced processing time, labour, raw materials, and energy requirements are key to increasing project profitability (Jarunglumlert et al., 2023).

Recent advancements in meat analogues have improved aspects like product quality, aroma, and flavor. However, creating a texture

Table 5
Texture analysis results of the prepared meat analogues.

Sample	Firmness (N)	Hardness (N)	Cohesiveness	Adhesiveness (N.sec)	Springiness (%)	Chewiness (N)
Control	30.47 ± 0.59[b]	3.45 ± 0.07[e]	25.77 ± 0.64[b]	−1.70 ± 0.12[a]	29.83 ± 0.70[b]	0.27 ± 0.01[b]
CR1	28.08 ± 0.81[cd]	5.94 ± 0.17[b]	19.96 ± 1.18[cd]	−1.83 ± 0.13[ab]	18.11 ± 0.46[e]	0.21 ± 0.02[c]
CR2	29.44 ± 0.36[c]	4.76 ± 0.09[c]	21.40 ± 0.44[c]	−2.23 ± 0.20[b]	20.80 ± 0.59[d]	0.21 ± 0.01[c]
CSR1	26.90 ± 0.24[d]	4.06 ± 0.33[d]	19.31 ± 0.67[d]	−2.00 ± 0.15[ab]	21.83 ± 1.28[cd]	0.17 ± 0.00[d]
CSR2	26.14 ± 0.69[d]	4.06 ± 0.16[d]	24.36 ± 1.17[b]	−1.97 ± 0.13[ab]	22.74 ± 0.15[c]	0.23 ± 0.01[c]
CP	28.13 ± 0.74[cd]	4.00 ± 0.13[d]	20.86 ± 1.09[cd]	−2.01 ± 0.12[ab]	10.87 ± 0.67[f]	0.09 ± 0.00[e]
Chicken breast	47.45 ± 3.07[a]	12.14 ± 0.02[a]	42.70 ± 1.03[a]	−5.08 ± 0.43[c]	54.72 ± 1.08[a]	2.84 ± 0.03[a]

CR, CSR and CP is Rice flour, Sticky rice flour and cricket respectively. Values are expressed as mean ± SD of triplicate measurements (n = 3) Means with different letters (a, b, c, …) are significantly different at $p < 0.05$ within the same column in the parameter.

resembling real meat remains a challenge, prompting further exploration, especially in the choice of raw materials. This study attempts to produce meat analogues from rice flour and cricket powder by using the freeze alignment technique. The primary goal is to identify the nutritional qualities that local resources, particularly rice and crickets, can achieve, and to comprehend the characteristics and textures of meat analogues that these resources can yield. This research aims to provide new insights into meat analogs, emphasizing the potential of using unconventional ingredients for better product development.

2. Materials and methods

2.1. Materials

Rice flour from normal rice (*Oryza sativa*), sticky rice flour (*Oryza sativa* var. *glutinosa*), and cricket (*Acheta domestica*) were obtained from the local source (Roi-et, Thailand). The soy protein powder was procured from Fuji Oil Co. Ltd. (Osaka, Japan). The composition of raw materials was shown in Table 2. Sodium alginate, salt, monosodium glutamate (MSG), pepper, oil, and chicken breast were purchased from the local market (Mahasarakham, Thailand).

2.2. Preparation of meat analogues

The freezing alignment technique was employed to generate meat analogues in accordance with the methodology described by Chantanuson et al. (2022). A summary of the formulations of the samples with rice flour and cricket powders in various proportions is listed in Table 2. Each formulation was thoroughly mixed using a blender at approximately 500 rpm for 1 min at room temperature. The resulting slurry was poured into cubic silicone molds (3.5 × 3.5 × 3.5 cm). Subsequently, the slurry was frozen at −10 °C for 8 h (1st freezing step). After freezing, the frozen samples were removed from the molds, soaked in a 3% calcium chloride solution, and then stabilized at 4 °C overnight. The freezing process was repeated for the stabilized gel under the same conditions at −10 °C for 8 h (2nd freezing step). The frozen gel was then thawed at 4 °C. The ice crystals in the sample structure were automatically removed. Additionally, real meat samples were included as a comparison in the study. The chicken breast sample was prepared by individually placing it in a plastic bag and boiling at 100 °C for 5 min. All samples were stored at 4 °C until further testing.

In the present study, formulations were developed in accordance with the methodology outlined in our previous study (Chantanuson et al., 2022), specifically to generate a food gel containing 6.0% soy protein powder and approximately 2.5 g of protein. In order to attain an equivalent protein content, the compositions containing cricket powder, rice flour, and sticky rice flour were meticulously adjusted. The specific formulations are listed for reference in Table 2.

2.3. Proximate analysis of rice and sticky rice flours

In accordance with the (AOAC, 2005), the quantification of moisture (925.10), ash (900.02), protein (920.176), fat (920.177) and fiber (985.29), and constituents was conducted. The carbohydrate content was subsequently determined using following formula;

$$\text{Carbohydrate content } (\%) = 100 - (\%\text{ash} - \%\text{protein} + \%\text{fat} + \%\text{fiber}) \quad (1)$$

2.4. Protein solubility measurement

A mixture was prepared by combining 0.4 g of flour with 40 mL of a 0.15 mol/L NaCl solution. This mixture was vigorously stirred using a magnetic stirrer for a duration of 1 h. Subsequently, the mixture underwent centrifugation at 20,000 *g* for 30 min using a high-speed centrifuge (AS ONE AS165, Japan). Following this, the supernatant obtained was filtered using Whatman No.1 filter paper. The biuret method was utilized to determine the concentration of soluble proteins in the filtered supernatant. For discoloration, the biuret method utilized a protein testing reagent (TaKaRa BCA Protein Assay reagent, Japan), and for the measurement of color absorbance, a spectrophotometer (HITACHI UH5300, Japan). Subsequent to the protein content analysis of the supernatant, the soluble protein content of the flour was ascertained using the methodology described by (Morr et al., 1985).

$$\text{Soluble protein content } (\%) = 100 \times \frac{\text{Protein conc.in supernatant } \left(\frac{\text{mg}}{\text{mL}}\right) \times \text{Supernatant volume (mL)}}{\text{Weight of original powder (mg)}} \quad (2)$$

2.5. Analysis of pasting properties

The pasting properties of rice flour and sticky rice flour were determined using a Rapid Visco Analyzer (RVA-4, Newport Scientific, Australia) following the method described by (Rachman et al., 2021). In brief, 2.5 g of dried flour was dispersed in 25 mL of distilled water, and the resulting slurry was heated to 50 °C for 1 min, then heated to 95 °C at a rate of 15 °C/min and held at 95 °C for 4 min, followed by cooling to 50 °C at a rate of 15 °C/min and holding at 50 °C for 2 min.

2.6. Analysis of rheological properties

Rheological measurements were carried out with a rheometer

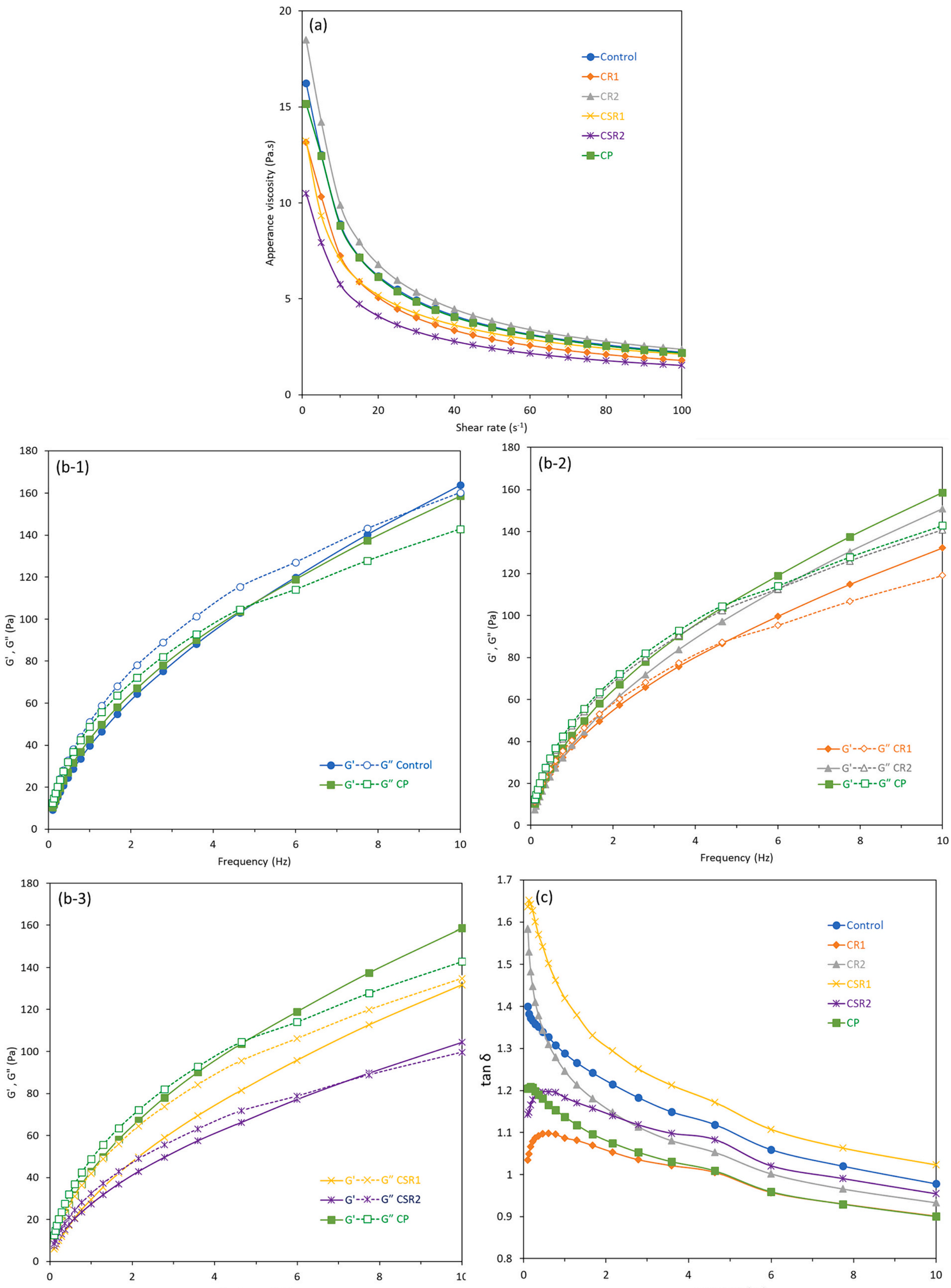

Fig. 1. Rheological properties of the prepared meat analogues: apparent viscosity (a), G′, G″ (b), and tan δ (c).

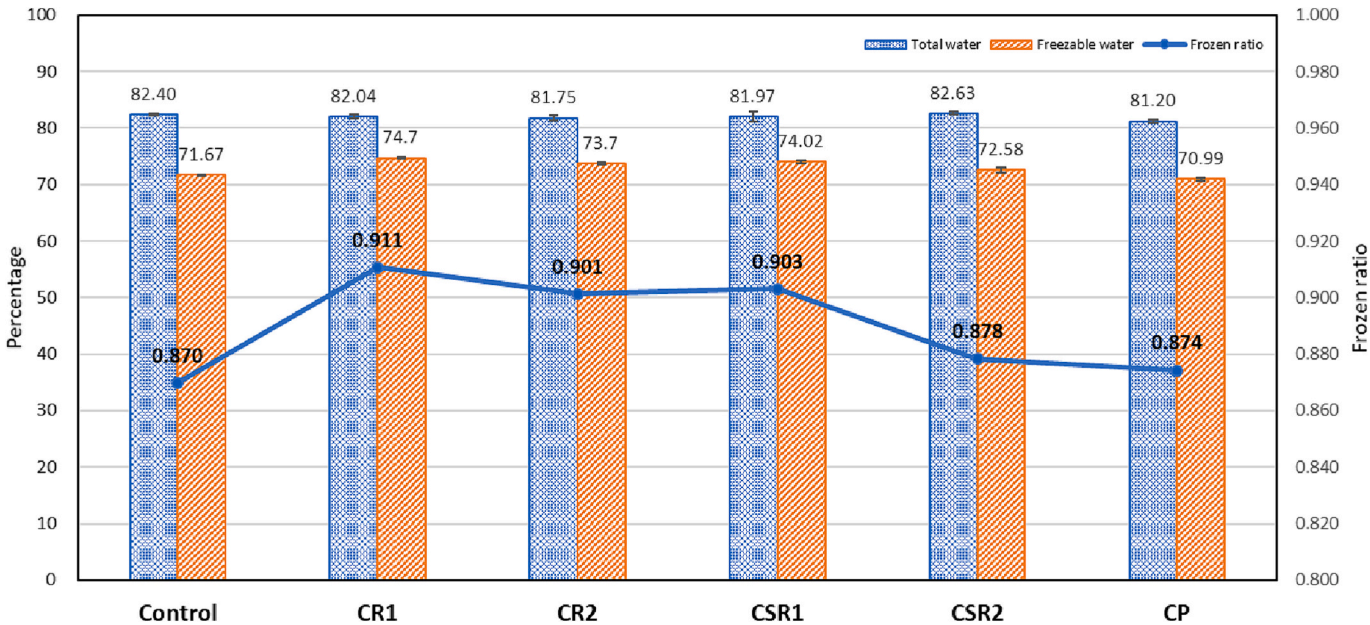

Fig. 2. Total moisture contents, freezable water contents, and frozen ratio of the prepared meat analogues.

(Haake mars II Thermo Electron Corporation, Germany) that included a cone-plate sensor (C35/1 T polished). The flow behavior of the laying sample was studied at shear rates ranging from 0.1 to 100 s^{-1}, flow behavior was depicted by the Herschel-Bulkley model according to eq. (2). An oscillatory amplitude sweep of frequency sweep (0.1 to 10 Hz) was measured at 25 ± 0.1 °C (Xia, Xue, Xue, Jiang, & Li, 2022; Yuliarti et al., 2021).

$$\sigma = \sigma_0 + K(\gamma')^n \quad (3)$$

where σ was the shear stress (Pa), σ_0 was the yield stress (Pa), K was the consistency index (Pa·s), γ' was the shear rate (s^{-1}), and n was the flow behavior index.

2.7. Frozen ratio measurement

The determination of total water content in each sample slurry was carried out using a moisture balance (SHIMADZU MOC—120H, Japan), which was measured by infrared heat drying and mass measurement. Approximately 1 g of the sample slurry was subjected to a heating process at 110 °C by the infrared drying heater, during which its weight was recorded at one-minute intervals until the weight change became <0.05% of the initial sample weight. The disparity in weight between the initial and final samples was utilized to calculate the water content. To measure the freezable water content, we employed a method involving a differential scanning calorimetry (DSC) apparatus equipped with a liquid nitrogen cooling module (NETZSCH DSC 3500 Sirius, Germany). Each sample, weighing approximately 10–20 mg, was enclosed in a 25 μL aluminum-sealed container and subjected to freezing and heating cycles. The DSC profiles were generated by initially cooling the samples to −30 °C at a rate of −2 K/min, holding them isothermally for 5 min, and then heating them to 30 °C at a rate of 2 K/min. The mass of freezable water, M_f (kg), was determined as described as follow;

$$M_f = Q/_{\Delta H} \quad (4)$$

where ΔH is the melting enthalpy of water (J/kg), and Q is the endothermic heat detected by DSC during the thawing process (J). The freezable water content (kg/kg) was calculated by dividing M_f by the weight of the sample. The frozen ratio was determined using the ratio of freezable water content to total water content. To ensure the reliability and consistency of the results, a minimum of three tests were conducted for each sample.

2.8. Color analysis

The color of the samples was measured in terms of CIE-L^*, a^*, and b^* using a CR-400/CR-410 colorimeter (Chroma Meter; Konica Minolta Sensing Inc. Osaka, Japan), following the method described by (Ming-Min & Ismail-Fitry, 2023). Specifically, L^* represented lightness-darkness, a^* represented redness-greenness, and b^* represented yellowness-blueness.

2.9. Water holding capacity measurement

The assessment of water holding capacity (WHC) was conducted using a modified version of the method outlined by (Van Oeckel, Warnants, & Boucqué, 1999). In this investigation, 5 g of samples were positioned on a filter paper (Schleicher & Schuell No. 2040B, Dassel, West Germany) and sandwiched between two cover glasses. A weight of 5 kg was applied for a duration of 5 min to give 49 N to thee sample. WHC was calculated by;

$$\%WHC = \{(W_i - W_f)/W_i\} \times 100 \quad (5)$$

where W_i denotes the initial weight of the sample (g) and W_f represents the final weight of the sample (g).

2.10. Texture analysis

Texture analysis was conducted using a TA-XT plus instrument (Stable Micro Systems, UK) with modifications to the method described by (Barbanti & Pasquini, 2005). Cubic test pieces (3 × 3 × 3 cm) were used to measure firmness with a knife blade (HDP/BS) at room temperature (20 °C). Test speeds of 5.0 mm/s, 10 mm/s, and 10 mm/s were chosen for the test speed, test speed, and post-test speed, respectively. The hardness (N), cohesiveness, adhesiveness (N·*sec*), springiness (%), and chewiness (N) were measured using a cylinder probe (P/5) at test speeds of 0.5 mm/s, 1.0 mm/s, and 10 mm/s, respectively.

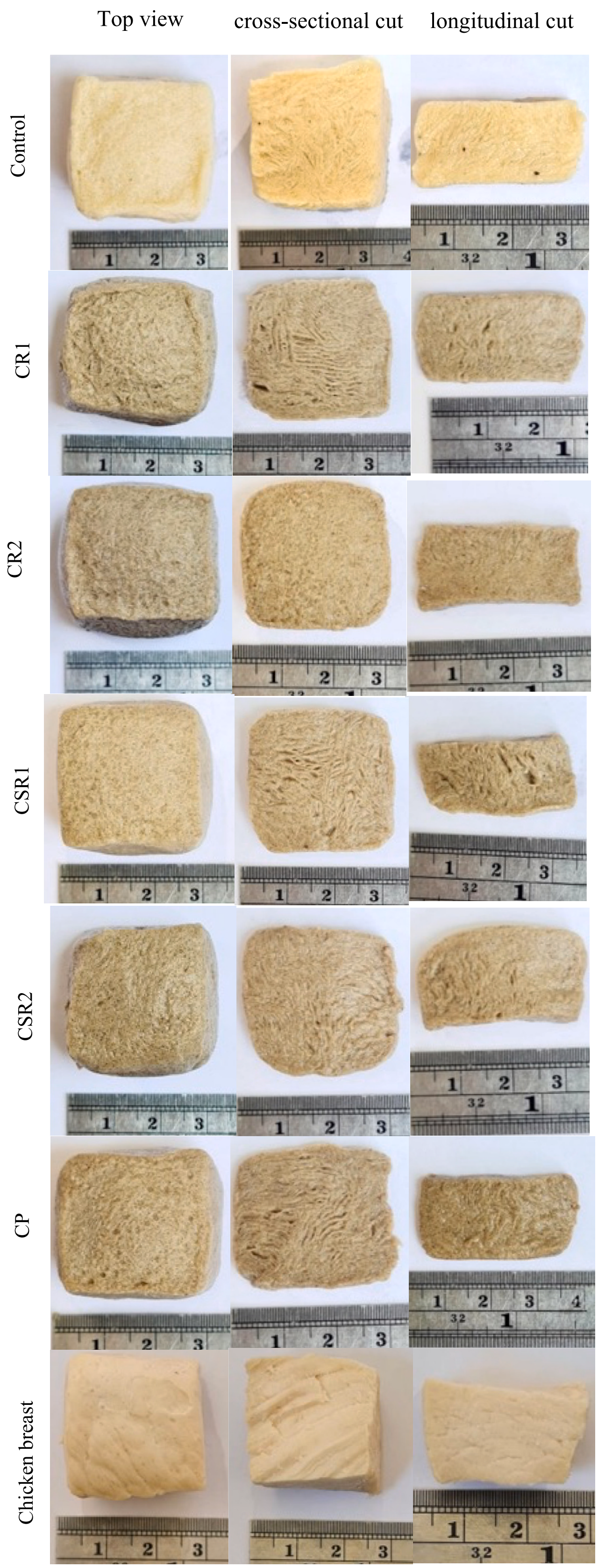

Fig. 3. Aligned structures of the prepared meat analogues.

2.11. Statistical analyses

The results were presented as the mean ± standard deviation (SD) from three independent replicates. Data analysis was performed using one-way ANOVA followed by the Duncan test. Distinct letters used to label various samples indicate statistically significant differences ($p < 0.05$).

3. Results and discussion

3.1. Materials properties

Table 1 presents the compositional analysis results, including moisture, ash, protein, fat, fiber, carbohydrate, and soluble protein content for soy protein powder, rice flour (RF), sticky rice flour (SR), and cricket powder (CP). Both rice flour and sticky rice flour constitute approximately 77.6–78.3% of their composition as carbohydrates. Cricket powders have the highest protein content at 59.8%, with an approximate 20% fat content. Subsequently, soy protein follows, with a protein content of 42.3% and a fat content of 22.9%. Our results were similar to those reported by Magara et al., who found that cricket had a protein content of 55 to 73% (Magara et al., 2021) and soy protein had a higher than that reported in previous studies (~35–40% protein) (Qin, Wang, & Luo, 2022). The present cricket powders demonstrate a substantial fiber content of 5.7%. Furthermore, the analysis reveals that the cricket powders exhibit a superior soluble protein content of 5.9% compared to soy protein powder at 4.7%. Conversely, both normal rice flour and sticky rice flour exhibit minimal soluble protein content, with values of 0.7% and 0.5%, respectively. The investigated parameters play a significant role in determining the final characteristics of the product. Cricket powder (CP) emerges for its elevated protein content, while rice flour (RF) and sticky rice flour (SR) exhibit relatively lower crude protein contents. These ingredients are commonly employed as binding agents in meat analogues owing to their capacity to enhance cohesion among protein molecules (Asgar, Fazilah, Huda, Bhat, & Karim, 2010; Cho & Ryu, 2021).

In the realm of meat analogue production, the utilization of plant polysaccharides such as sugars, fibers, and starch are important. These polysaccharides serve to augment interactions among proteins, water, and lipids during extrusion, thereby imparting a fibrous texture akin to meat, alongside improved appearance and structural integrity (Dinali et al., 2024; Schmid, Farahnaky, Adhikari, & Torley, 2022). The incorporation of polysaccharides into meat analogues offers a promising avenue for enhancing overall quality, primarily attributed to their enhanced gelling, thickening, and water-binding capacities (Fu et al., 2023; Schmid et al., 2022).

3.2. Pasting property

The pasting properties of RF and SR flour can be seen from the RVA measurement results listed in Table 3. Amylose and amylopectin in starch form hydrogen bonds within its granules, which break upon heating. This causes the expansion of granules, and viscosity rises. The well-dispersed amylose and its ability to bind water, which also depends on the rice cultivars, increase the viscosity of rice flour (Varavinit, Shobsngob, Varanyanond, Chinachoti, & Naivikul, 2003). It can be seen that RF (non-glutinous rice), which has a higher amylose content, showed higher final viscosity and trough viscosity and resulted in more than three times higher setbacks than those of SR. On the other hand, SR had higher peak viscosity and breakdown values, but a lower pasting temperature. These flour characteristics could be associated with the resulting meat analogue product developed in the next steps of our experiments.

3.3. Rheological properties

The rheological measurement demonstrates the relationship between apparent viscosity and shear rate, as shown in Fig. 1a. The viscosity of the samples decreases as the shear rate increases, indicating that all samples have a non-Newtonian pseudoplastic flow behavior. Fig. 1b-c shows the deformation properties of the meat analogues. The storage modulus (G') shows the elastic behavior of solid-like characteristics, whereas the loss modulus (G") represents the network's viscous nature (Rao, 2014). The trends of G' and G" suggested that the elastic character dominates the meat analogues, and the increase in cricket powder resulted in a rise in the strength of the protein gel. It was likely that the cricket powder contributed to boosting the resultant gel strength, whereas the rice flour contributed to softening the gels. The employment of sticky rice flour clearly demonstrated this trend. The cross-over points of G' and G" for CR2 were positioned at a higher frequency than that of CR1, suggesting that the increasing amount of rice leads to soft and weaker gel formation (Mariotti, Sinelli, Catenacci, Pagani, & Lucisano, 2009). The cross-over points for CSR1 and CSR2 were positioned in a significantly higher frequency region than the CR series of samples. Fig. 1c shows the tan δ as a function of meat analogue frequency. As discussed above, the concentration of cricket powder in the implant increases the protein network. CSR1 has the highest tan δ value, which indicates weak and liquid-like gel formation. This could be due to an interaction between polysaccharides and proteins, which results in a decrease in viscoelastic behavior (Jiménez-Colmenero et al., 2012).

3.4. Frozen ratio

Water is essential for food to retain its intended properties and has a substantial effect on freezing. Water in food can be separated into two categories during freezing: freezable water and non-freezable water. In food processing, freezable water exhibits comparable characteristics to purified water, as it can be easily extracted following the drying process, reduced to ice, and utilized as a solvent. However, it remains immobile in tissue foods, irrespective of their slicing or mincing. The definition of non-freezable water is water whose motion is constrained by substances in its surroundings. The concept of "water binding ability" pertains to the inherent tendency of water to form bonds with hydrophilic substances, which includes macroscopic biological components. The nature of the nonaqueous substance, the concentration of salt, the temperature, and the pH all influence the degree and intensity of its interaction with the plasticizer, water (Fennema, 1996; Slade, Levine, & Reid, 1991). Water is characterized as non-freezable if it remains liquid at temperatures below the freezing limit. The binding strength was of the utmost importance in decreasing the freezing point of non-freezable water. The DSC test might detect the presence of freezable water. In a DSC test, setting a threshold temperature was equal to defining a threshold binding strength by separating freezable water and non-freezable water content (Lee & Lee, 1995; Lee, Su, & Mujumdar, 2013; Xu, Tao, & Shivkumar, 2016; Yoshida, Hatakeyama, & Hatakeyama, 1993).

By directly dehydrating and freezing, respectively, the total water content and freezable water content were determined using DSC. The frozen ratio, freezable water content, and total water content as determined for each formulation are illustrated in Fig. 2. In representing the water added to the slurry, the total moisture content of each sample varied between 81% and 83% wt. It might utilize evaporation to determine the total amount of water in the slurry. Freezable water and frozen ratios varied based on the materials powder utilized in creating each formulation. The frozen ratio of the control formulation, which used soy protein flour, was 0.870. The frozen ratio of CP is 0.874, which is similar to the control formulation. Because of the high soluble protein content of the cricket powder and soy flour powder, the frozen ratio of the slurry was low due to its high water binding strength. The formulations that added rice flour and sticky rice flour had a higher frozen ratio. Increasing the ratio of rice flour or sticky rice flour in the formulation resulted in higher frozen ratio values. This could be due to a combination of rice and cricket powders, which reduced the water binding strength. This is consistent with the rheological measurement results discussed in the previous section. The combination of rice and cricket powders leads to the formation of a weak, liquid-like gel because it suggests the presence of water that has a weak affinity for the gel network.

3.5. Color and water holding capacity

The color of the artificial meat was examined and presented in Table 4, which shows that the inclusion of crickets led to a significant rise in brightness (L*) and yellowness (b*) ($p < 0.05$). However, it also resulted in elevated redness (a*) when compared to both the control and chicken breast meat. In a study conducted by Mafu, Ketnawa, Phongthai, Schönlechner, and Rawdkuen (2022) that examined the addition of cricket powder to bread, it was observed that the quantity of cricket powder used in the formulation had a significant impact. The observed increment led to a notable reduction in the L* and b* values.

The investigation into the water holding capacity (WHC), as shown in Table 4, revealed that the control formula showed the highest WHC at 52.20%. On the other hand, the formulas combining rice flour and cricket powders, as well as the cricket-only formula (CP), showed reduced efficacy values that ranged from roughly 44.38% to 47.47%. Rice flour and sticky rice flour in the formulations results in an increased frozen ratio. This can be attributed to the poor water binding strength of the rice flour components, according to (Gu, Xiao, Sun, Shi, & Yang, 2018), the addition of rice to the gel resulted in a decrease in the gel's internal structure and a drop-in water holding capacity (WHC).

3.6. Texture and alignment structure formation

Texture characteristics are shown in Table 5., including firmness, hardness, cohesiveness, adhesiveness, springiness, and chewiness. The study revealed that the use of CP-SR led to the attainment of the highest levels of cohesiveness and adhesiveness, which is similar to the control. In a similar manner, the addition of cricket powder can establish chemical connections and contribute to a modest improvement in the structural integrity of the food product. The study conducted by Somjid, Panpipat, Cheong, and Chaijan (2022) is of academic significance. Proteins serve as gelling agents during the cavity filling process. The adhesiveness was seen to increase during the gelling process, as reported by (Nilsson et al., 2023).

Fig. 3 illustrates the fiber-like alignment structures observed in all analyzed samples. In order to compare the macroscopic structures most likely to be associated with the visual appeal of the product, the prepared samples were observed with a digital camera. Remarkably, the introduction of CP, R, and SR within the formulations has led to the formation of distinct aligned structures. Particularly, the CSR2 formulation prominently exhibits well-defined fiber-like appearances, followed by CR1, CSR1, CP, and CR2. According to the former studies (Chantanuson et al., 2022; Chantanuson, Nagamine, Kobayashi, & Nakagawa, 2023), the water binding strength (*i.e.*, freezable water content) of the formulation had a significant influence on the aligned ice crystal formation during freezing. Highly binding strength could bind water in the matrices and prevent structure formation. To achieve an anisotropic meat-like structure by freezing, the gels must maintain the proper binding strength. This paper explained how the combination of cricket powder and rice flour changed the resultant gel properties, especially water binding strength and rheological properties. It is still unclear what mechanism determines ice crystal formation during the freezing process. This study supports the use of cricket powder as a practical resource for meat analogue production and provides evidence that a combination of cricket powder and rice flour can tune the rheological properties as well as the alignment structures.

4. Conclusion

Our study highlights the potential of cricket powder and rice flour as innovative ingredients for meat analogues produced through freeze alignment techniques. We observed a remarkable synergy between these components, leading to the formation of fiber-like alignments that not only enhanced rigidity and stickiness but also mitigated toughness. Moreover, the application of freezing resulted in increased water-holding capacity, attributable to changes in viscoelastic properties. Importantly, the formation of multilayered structures upon freezing was notably influenced by the specific combination of cricket powder and rice flour. This study underscores the significance of formulation optimization, suggesting a promising avenue for fine-tuning textural and appearance properties in meat analogues. By strategically adjusting ingredient ratios, manufacturers can potentially achieve desired sensory attributes, offering consumers high-quality meat substitutes. Our findings contribute to the expanding field of alternative protein sources, endorsing cricket powder and rice flour as viable candidates for sustainable and nutritious food innovation.

CRediT authorship contribution statement

Kyuya Nakagawa: Writing – review & editing, Writing – original draft, Supervision, Investigation, Conceptualization. **Ratchanon Chantanuson:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Parinya Boonarsa:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Nidthaya Seephua:** Investigation, Formal analysis, Data curation. **Sirithon Siriamornpun:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used QuillBot in order to grammar-check and paraphrase. After using this tool or service, the author(s) reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sirithon Siriamornpun reports was provided by Mahasarakham University. If there are other authors, they 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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