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## Development of stabilized coconut-waste peptides as bioactive ingredients to improve yogurt texture and functionality

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In this study, enzymatic production and encapsulation of bioactive peptide fractions from coconut cake were carried out with the aim of fortifying yogurt. Then, the properties of peptides (nutritional indices, biological activities, etc.), powders (physicochemical, functional, structural, and morphological), and fortified yogurts (texture, syneresis, viscosity, and sensory indices) were investigated. The 10 KDa peptide fraction (10 PF) demonstrated superior antioxidant effects across multiple assays, including DPPH radical scavenging (77.6%), OH radical scavenging (78.4%), ABTS radical scavenging (79.6%), TEAC (2.14), reducing power (0.98), total antioxidant activity (1.86), and Fe<sup>2</sup> (71.4%) and Cu<sup>2</sup> (33.3%) ion-chelating activity, outperforming crude proteins and larger peptide fractions. Subsequently, 10 PF was encapsulated *via* spray drying with maltodextrin (MD) alone and in combination with pectin (P/MD) to enhance the stability and usability. Characterization by FTIR and scanning electron microscopy confirmed successful peptide incorporation into the carrier matrix, yielding spherical particles with smooth surfaces and improved physicochemical properties such as flowability and physical stability. Notably, antioxidant activity retention post-encapsulation was high, ranging from approximately 87% to 94%. Fortification of yogurt with 3% peptide resulted in improved yogurt properties (such as firmness, cohesiveness, WHC, viscosity, and reduced syneresis). However, high peptide concentrations resulted in the appearance of bitterness. Enzymolysis and spray-drying create antioxidant-rich peptides from coconut cake for use in supplements or pharmaceuticals.

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### Sustainability spotlight

This manuscript presents a well-integrated and timely contribution to food science and functional ingredient development by transforming an underutilized agro-industrial by-product—coconut cake—into value-added, bioactive peptide ingredients and demonstrating their practical application in yogurt fortification. The work is significant for several reasons. First, it identifies and isolates a 10 KDa peptide fraction with robust and multi-assay antioxidant capacity (DPPH radical scavenging, OH radical scavenging, ABTS radical scavenging, TEAC, reducing power, total antioxidant activity, and metal-chelating activity), showing clear superiority over crude protein and larger fractions. This provides a strong biochemical rationale for targeting specific peptide sizes in functional ingredient development. Second, the study couples enzymatic hydrolysis with spray-drying microencapsulation (maltodextrin and pectin/maltodextrin matrices) to address common stability and handling challenges for peptide bioactives; the high post-encapsulation activity retention (~87–94%) and favorable powder physicochemical properties (flowability, stability, morphology, etc.) demonstrate practical feasibility for industrial supply chains. Beyond analytical rigor, the manuscript advances application-level knowledge by directly testing the encapsulated peptides in a real food matrix. Yogurt fortified at 3% peptide concentration exhibited meaningful improvements in texture-related quality metrics (firmness, cohesiveness, water-holding capacity, viscosity, etc.) and reduced syneresis—attributes of clear commercial and consumer relevance—while also highlighting the sensory trade-off of bitterness at higher loadings. This balance of functional benefit, technological solution (encapsulation), and sensory evaluation strengthens the translational value of the research for food formulators, nutraceutical developers, and sustainability-focused industries, aiming to valorize food-processing residues. Overall, the study offers a convincing, practically oriented pathway from byproducts to market-ready bioactive ingredients with measurable benefits for product quality and potential health-related functionality.

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# 1 Introduction

The increase in population and industrial life has been associated with an increase in food-related risks and diseases in recent years, which has, therefore, doubled the importance of using natural and health-promoting bioactive compounds (such as probiotics, phytochemicals, vitamins, and peptides) in the enrichment and production of functional food products.<sup>1</sup> Among the diverse bioactive compounds, peptides or “small digestibles” and hydrolysates can play a key role in improving the health level and preventing various diseases.<sup>2,3</sup> Bioactive peptides (fractions consisting of 2–20 amino acids and inactive in the amino acid sequence of the primary protein) are mainly produced and released after enzymatic hydrolysis with different proteases.<sup>4,5</sup> The positive physiological effects and health-promoting properties of these compounds include scavenging all kinds of destructive free radicals (antioxidant activity), chelating pro-oxidant metal ions (*e.g.*, Fe<sup>2+</sup> and Cu<sup>2+</sup>), growth inhibition of spoilage and pathogenic bacteria, and anticancer, antithrombotic, antihypertensive, immunomodulatory, and hypocholesterolemic activities.<sup>6–8</sup> These advantages have led to investigations on the production of bioactive peptides from a variety of plant, animal, and marine protein sources, as well as waste and by-products of the food industry.<sup>9,10</sup>

Despite the aforementioned advantages, factors such as high bitterness (mainly resulting from the release of hydrophobic amino acids such as tryptophan, isoleucine, leucine, valine, phenylalanine, and lysine), physicochemical instability (under the influence of temperature, ionic strength, light, pH, metal ions, and oxidizing/reducing agents), and high hygroscopic nature, have seriously challenged the physical stability, preservation of bioactivity, and direct usability of peptides in food formulations.<sup>11</sup> Encapsulation is the main solution to overcome the bitterness and stabilize and maintain the antioxidant activity of bioactive proteins/peptides. Therefore, several methods have been investigated for encapsulation of these compounds using micro- and nanocarriers.<sup>12</sup> Among a variety of methods, spray-drying is the most widely used process to stabilize wide-ranging bioactive compounds through their solidification. Flexibility, high speed of the process, cost-effectiveness, and application on an industrial scale are some of the features that have encouraged the microencapsulation of various bioactive and health-promoting compounds (such as phenolic compounds, vitamins, minerals, carotenoids, fatty acids, essential oils, anthocyanins, probiotics, flavor compounds, and all kinds of drugs) using this process.<sup>13</sup>

However, the more complex structures of proteins/peptides cause aggregation, conformational changes, denaturation, and ultimately loss of biological activity of these compounds during atomization/dehydration.<sup>14</sup> Therefore, various types of protein carriers, polysaccharides, and surfactants have been used to maintain the stability of bioactive proteins/peptides in various studies, including peptides from oleaster stone,<sup>11</sup> bee pollen,<sup>15</sup> stripped weakfish,<sup>16</sup> chicken breast,<sup>17</sup> whey protein,<sup>18</sup> Arthrospira,<sup>19</sup> spent-hen meat,<sup>20</sup> *Perinereis aibuhitensis*,<sup>21</sup> and sesame protein.<sup>22</sup> One of the important sources of polysaccharide

carriers is pectin. Pectin is a D-galacturonic acid polysaccharide with  $\alpha$  (1→4) chain. This polysaccharide is used as a stabilizer and emulsifier in many food formulations.<sup>23</sup> Its technological advantages cause its annual need to be estimated at \$1.5 billion in 2025. Therefore, many research studies have not been conducted regarding the extraction and use of pectin from waste or by-products of agricultural industries (such as citrus peel, banana, mango, *etc.*).<sup>24</sup>

Coconut cake is one of the by-products of coconut milk production and oil extraction.<sup>25</sup> This product accounts for a suitable option for the production of functional peptides, given the significant amount of coconut production globally (~60 million tons) and the high protein content (up to 25%).<sup>26</sup> To our knowledge, no research has been conducted on the enrichment of yogurt with microencapsulated peptide fractions derived from coconut waste. (1) Protein extraction and hydrolysis (extract protein from coconut cake and perform enzymatic hydrolysis to break it down into smaller peptides); (2) hydrolysate fractionation (separate the hydrolysate into distinct fractions based on molecular weight differences for further analysis); (3) nutritional and amino acid evaluation (analyze the amino acid composition and assess the nutritional value of the extracted proteins and peptides); (4) antioxidant activity investigation (study the antioxidant properties of the protein and peptide fractions to determine their bioactive potential); (5) microencapsulation and functional properties (evaluate the impact of microencapsulation (using maltodextrin and maltodextrin–pectin blends) on the production efficiency, physical properties (hygroscopicity, flowability, particle size), and functional characteristics); and (6) yogurt fortification and quality assessment (fortify yogurt with the peptide fractions and examine its physical properties (texture, viscosity, *etc.*), sensory attributes, and retention of antioxidant activity under different carrier conditions).

## 2 Materials and methods

### 2.1 Materials

The chemicals used in the analyses included ABTS, Alcalase 2.4 L (Novo Nordisk, Bagsvaerd, Denmark), Coomassie Brilliant Blue (G250), DPPH, ferrozine, Trolox, and pyrocatechol violet, all obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Alpha-deoxyribose was sourced from Fluka (Stockholm, Sweden). TCA (trichloroacetic acid), potassium persulfate, ferrous chloride, and other chemicals were procured from Merck (Darmstadt, Germany). Maltodextrin (DE18-20) was acquired as pooran powder (Isfahan, Iran).

### 2.2 Protein extraction from coconut meal (CM)

The study utilized a by-product obtained from cold-pressed coconut oil extraction, which was ground into a powder (~0.2–1.0 mm). The protein concentrate was prepared using the method described by Akbarbaglu *et al.*<sup>27</sup> The process involved: (1) oil removal: hexane was used in a 1 : 5 (w/v) ratio for 4 hours to defat the coconut meal powder. (2) Protein extraction: the defatted flour was treated with a 0.1% saline solution at pH 9.5.



(3) Protein precipitation: the pH was adjusted to 4.2 using 0.5 M HCl, followed by neutralization to pH 7 with 0.5 M NaOH. (4) Freeze-drying: the protein concentrate was lyophilized using a freeze dryer (Christ, Germany).

### 2.3 Preparation of protein hydrolysates

The dissolution of coconut meal protein (CMP) at a concentration of 5% w/v was carried out in 0.01 M PBS for 30 minutes at 50 °C. Enzymatic hydrolysis was then performed using Alcalase under controlled conditions (pH 8 and 50 °C) with an enzyme-to-substrate ratio of 2% for 120 minutes. Enzyme activity was terminated by transferring the reaction mixture to a water bath at 95 °C for 15 minutes. The resulting dispersion was centrifuged at 7000×g for 10 minutes, and the supernatant containing the hydrolysates was collected. The hydrolysates were freeze-dried using a freeze dryer (Christ, Germany) at −20 °C under a pressure of 0.1 mbar and stored at −18 °C.<sup>28</sup>

### 2.4 Fractionation of bioactive peptides

The coconut meal protein hydrolysate (CMPH) was fractionated using ultrafiltration membranes (Amicon Ultra, Millipore, UK) with molecular weight cut-offs of 10, 30, and 100 kDa. The hydrolysate was sequentially filtered through: (1) 100 kDa membrane (PF-100): retained peptides with MW 30–100 kDa; (2) 30 kDa membrane (PF-30): retained peptides with MW 10–30 kDa; (3) 10 kDa membrane (PF-10): retained peptides with MW <10 kDa.

The resulting fractions were freeze-dried and stored at −18 °C for further analysis.<sup>29</sup>

### 2.5 Amino acid composition

Complete hydrolysis and acid digestion of each sample was performed with HCl (22 g/100 mL; 110 °C) for 24 h. The sample was diluted to 250 mL, and an aliquot was filtered through a 0.22 μm MCE membrane syringe filter (Guangzhou Jet Bio-Filtration, China). The filtrate was derivatized using the AccQ-Fluor Reagent Kit (Waters, Milford, MA, USA). Finally, amino acids were quantified with an HPLC (Nova-Pak C18, 4 μm, Waters, Milford, MA) equipped with an AccQ-Tag amino acid C18 column (Waters, Milford, MA, USA) and coupled to a UV detector (Waters, Milford, MA, USA). The absorbance was recorded at a wavelength of 248 nm. Mobile phase A was 100% AccQ-Tag Eluent, mobile phase B was 100% acetonitrile (HPLC grade), and mobile phase C was 100% water (HPLC grade). The gradient employed was: 0–0.5 min 100% A; 0.5–18 min 99% A and 1% B; 18–19 min 95% A and 5% B; 19–28 min 91% A and 9% B; 28–35 min 83% A and 17% B; 35–38 min 60% B and 40% C; and 38–40 min 100% A, at a constant flow rate of 1 mL min<sup>−1</sup>. Quantification was done by comparing the retention time and area of the Amino Acid Standard Hydrolysate (Thermo Scientific Pierce, Rockford, IL, USA) and Empower software (Waters, Milford, MA, USA). The concentration and identification of tryptophan were determined after alkaline digestion. The results were reported for each sample as the amount of AA (mg) per gram of dry matter.<sup>30</sup>

Essential amino acids (EAAs) and protein efficiency ratios (PER) were evaluated as follows:

$$E/T (\%) = (\text{Thr} + \text{Cys} + \text{Val} + \text{Met} + \text{Ile} + \text{Leu} + \text{Tyr} + \text{Phe} + \text{Lys} + \text{His} + \text{Trp}) \times 100\% / \text{TAA} \quad (1)$$

$$\text{PER} = -0.468 + 0.454 \times \text{Leu} - 0.105 \times \text{Tyr} \quad (2)$$

### 2.6 Characterization of antioxidant activity

**2.6.1 DPPH-RSA.** A 1.5 mL aliquot of each CMP/CMPH solution (concentration range: 10–50 mg mL<sup>−1</sup>) was combined with 1.5 mL of 0.2 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution. The mixture was incubated under dark conditions for 30 minutes and subsequently centrifuged at 4000×g for 10 minutes. The absorbance of the supernatant was recorded at 517 nm.<sup>31</sup> The radical scavenging activity (RSA) was quantified using a standard equation.

$$\text{Inhibition} (\%) = [1 - (\text{sample}_{\text{Abs}} / \text{blank}_{\text{Abs}})] \times 100 \quad (3)$$

**2.6.2 ABTS free radical scavenging activity.** A mixture of ABTS and potassium persulfate was prepared at final concentrations of 7 mM and 2.45 mM, respectively. The mixture was kept in the dark for 12 hours and then diluted with 0.2 M PBS (pH 7.4) until the final absorbance reached 0.70 at 734 nm. Next, 30 μL of CMP/CMPH solution (1–20 mg mL<sup>−1</sup>) was added to 3 mL of ABTS solution, vortexed for 10 seconds, and incubated in the dark for 6 minutes. The absorbance was then measured at 734 nm. To determine the Trolox equivalent antioxidant capacity (TEAC), a standard curve was generated by reacting various concentrations of Trolox (50–1000 μM) with ABTS.<sup>29</sup>

**2.6.3 Hydroxyl-RSA.** The reaction system comprised 0.2 mL of CMP/CMPH solution (10–50 mg mL<sup>−1</sup>), 0.5 mL of α-deoxyribose (10 mM), 0.2 mL of FeSO<sub>4</sub>-EDTA (10 mM), 0.9 mL of phosphate-buffered saline (PBS; 0.2 M, pH 7.4), and 0.2 mL of hydrogen peroxide (10 mM). The mixture was incubated at 37 °C for 1 hour. Post-incubation, 1.0 mL of thiobarbituric acid (TBA; 1.0%) and 1.0 mL of trichloroacetic acid (TCA; 3%) were introduced. The solution was heated in a water bath for 15 minutes, cooled in an ice bath, and the absorbance was measured at 532 nm.<sup>28</sup>

**2.6.4 Reducing power assay.** A reaction mixture containing 0.5 mL of CMP/CMPH solution (10–50 mg mL<sup>−1</sup>), 0.5 mL of phosphate buffer (0.2 M, pH 6.6), and 0.5 mL of 1% potassium ferricyanide was incubated at 50 °C for 20 minutes. Following this, 0.5 mL of TCA (10%) was added, and the mixture was centrifuged at 3000×g for 10 minutes. The supernatant (1.0 mL) was mixed with 1.0 mL of distilled water and 0.2 mL of 0.1% ferric chloride. The absorbance was recorded at 700 nm.<sup>32</sup>

**2.6.5 Total antioxidant activity (TAA) assay.** A mixture of 0.2 mL CMP/CMPH solution (2.5–20 mg mL<sup>−1</sup>) and 2 mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was incubated at 90 °C for 90 minutes. The absorbance of the cooled samples was measured at 695 nm, with higher values indicating greater TAA.<sup>33</sup>



**2.6.6 Fe<sup>2+</sup> chelating activity assay.** The reaction mixture consisted of 1 mL of CMP/CMPH solution (10–50 mg mL<sup>-1</sup>), 50 μL of iron(II) chloride (2 mM), 1.8 mL of double-distilled water, and 0.1 mL of ferrozine solution (5 mM). The mixture was vortexed, incubated at 30 °C for 10 minutes, and the absorbance was measured at 562 nm.<sup>31</sup>

**2.6.7 Cu<sup>2+</sup> chelating activity assay.** 1 mL of 0.2 mM CuSO<sub>4</sub> solution was mixed with 1 mL of CMP/CMPH solution (10–50 mg mL<sup>-1</sup>) and kept at 30 °C for 5 minutes. 1 mL of 10% TCA was then added, and the mixture was centrifuged at 2000×g for 10 minutes. To 2 mL of the supernatant, 1 mL of 10% pyridine and 20 μL of 0.1% pyrocatechol violet were added. The mixture was vortexed, incubated at 30 °C for 5 minutes, and the absorbance was measured at 632 nm.<sup>34</sup>

## 2.7 Spray-drying microencapsulation

A feed solution was prepared by dissolving 2 g of pure CMPH in 20 mL of phosphate buffer (pH 7.4) and mixing it with 20 mL of maltodextrin (20% w/v) at a 1:3 core-to-carrier ratio. For comparison, 2 g of pectin replaced maltodextrin in another sample. Operation was conducted using a Büchi B-290 mini spray dryer with the following parameters: inlet temperature of 140 °C, outlet temperature of 80 °C, feed rate of 5 mL min<sup>-1</sup>, drying air speed of 0.54 m<sup>3</sup> h<sup>-1</sup>, nozzle diameter of 0.7 mm, and air pressure of 5.6 bar. The resulting powders were stored in airtight bags under refrigeration.<sup>35</sup>

**2.7.1 Physicochemical and functional properties.** The spray-dried powders were characterized for production yield (MC), water activity (Aw), bulk density (BD), hygroscopicity, flowability (angle of repose), solubility, and particle size following established methodologies.<sup>19</sup>

**2.7.2 Morphological properties.** Particle morphology was analyzed using scanning electron microscopy (SEM; HITACHI PS-230, Japan) at an accelerating voltage of 25 kV, after coating the samples with a gold layer.

**2.7.3 FTIR analysis.** The chemical structure of the peptide and spray-dried powders was examined *via* Fourier transform infrared (FTIR) spectroscopy. Samples were mixed with potassium bromide (KBr; 1:100 ratio), pressed into disks, and analyzed over a range of 4000–400 cm<sup>-1</sup> using a Shimadzu 8400 spectrophotometer (Japan).

**2.7.4 Retention of antioxidant activity.** The retention of antioxidant activity (AA) post-microencapsulation was calculated for DPPH, ABTS, and hydroxyl radical scavenging; TAA; reducing power; and Fe<sup>2+</sup>/Cu<sup>2+</sup> chelating activity using the formula:

$$\text{Antioxidant retention (\%)} = \frac{\text{AA in SD-peptides/}}{\text{AA in feed solution}} \times 100 \quad (4)$$

## 2.8 Yogurt fortification

To prepare yogurt, first milk (~3.4% fat, 3.5% protein and 4.8% lactose) was purchased from a dairy factory (Pegah, Mashhad). Peptide powder was added to the milk in different proportions (1, 2 and 3% w/v) and dissolved. Then, a heating process (95 °C,

5 min) was performed in a container on a heater. Then, the milk temperature was reduced to 42 °C, and YO-MIX 883 yogurt starter (~0.01% w/v) was added. After that, the mixture was incubated for 5 hours at 42 °C and finally transferred to the refrigerator (4 °C).<sup>36</sup>

### 2.8.1 Fortified yogurt analysis

**2.8.1.1 Physicochemical, and textural properties.** Adding coconut peptide as a fortifying agent can change the chemical-physical and organoleptic properties of yogurt to some extent. For this purpose, factors such as pH, hardness (g), cohesiveness (g), water holding capacity (WHC), syneresis (%) and apparent viscosity (Pa s) were investigated according to the methods described by Gantumur *et al.*<sup>37</sup>

**2.8.1.2 Sensory property evaluation.** A 5-point hedonic test was used to evaluate the sensory properties of the samples. For this purpose, the samples were coded. Then, evaluation forms were provided to semi-trained panelists (aged between 20 and 35 years). The items included in the evaluation sheets included taste, color, odor, consistency, and overall acceptability of the product. The evaluators rated the control yogurt and the fortified samples on a scale of 1 (very bad) to 5 (very good).<sup>38</sup>

## 2.9 Statistical analysis

Data were analyzed using SPSS software (version 21.0) with one-way ANOVA. All experiments were conducted in triplicate, and Duncan's test ( $p < 0.05$ ) was employed for mean comparisons.

# 3 Results and discussion

## 3.1 Amino acid and nutritional quality

Coconut cake (CC) is recognized as a valuable source of protein (>20%). As a plant-based protein, coconut proteins are particularly beneficial in the food industry due to their lower cost compared to animal-based proteins.<sup>39</sup> To assess the nutritional quality and bioactivity of these proteins, their amino acid sequence needs to be analyzed. Therefore, the protein extracted from coconut meal (crude protein), its hydrolysate (CMPH) and peptide fraction filtered through a 10 kDa molecular weight membrane (PF-10) were evaluated. Fig. 1 shows the chromatogram image of the peptides produced. Since the highest antioxidant activity is associated with the peptide fraction <10 kDa (Fig. 2 and 3), it can be concluded that the type of amino acid in the fraction is an impacting factor. Indeed, peptides have been reported to show higher antioxidant activity than native proteins.<sup>20</sup>

Essential amino acids (EAAs) (%) in most plant-based sources (26 ± 2% of total protein) are low when compared with animal-based proteins (37 ± 2% of total protein); on the other hand, EAAs of some plant-based proteins like soy (27%), brown rice (28%), pea (30%), corn (32%), and potato (37%) can meet the WHO/FAO amino acid requirements.<sup>40</sup> EAAs of CC-samples were 32.5% for crude protein, 32.8% for CPH and 35.7% for the PF-10 sample (Table 1).

All amino acids, mainly the essential ones (histidine, threonine, methionine, valine, phenylalanine, isoleucine, leucine, lysine, and tryptophan) are needed for human nutrition. In all samples, all these EAAs are available; moreover, hydrophobic



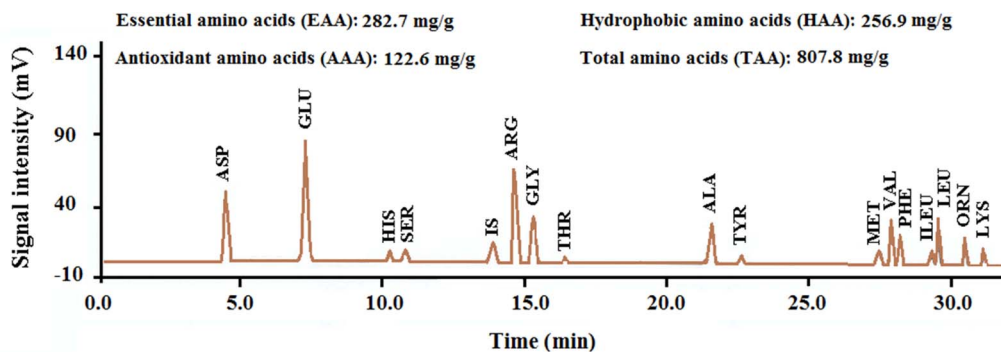


Fig. 1 HPLC chromatogram showing the amino acid profile of coconut-meal peptide.

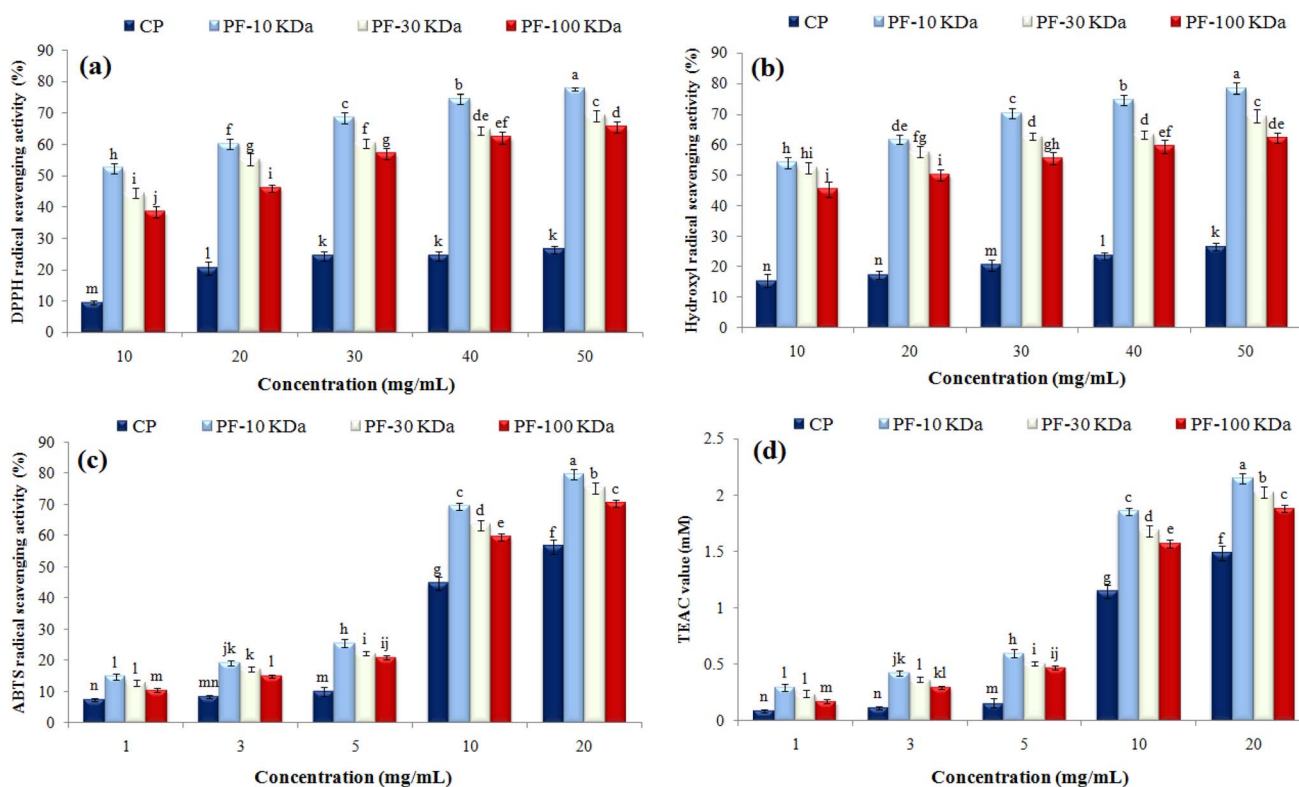


Fig. 2 Effect of molecular weight (MW) and peptide concentration on (a) DPPH radical scavenging, (b) hydroxyl radical scavenging, (c) ABTS radical scavenging, and (d) Trolox equivalent antioxidant capacity of coconut-meal protein and its peptide fractions.

amino acids (HAA) like alanine, valine, and leucine were found at 46.7, 39.3, and 51.4 mg g<sup>-1</sup> dry sample in the PF-10 KDa sample, respectively (Table 1). Tryptophan (4.7–6.9 mg g<sup>-1</sup> dry sample), methionine (13.3–17.5 mg g<sup>-1</sup> dry sample), histidine (7.5–40.1 mg g<sup>-1</sup> dry sample), tyrosine (21.1–27.3 mg g<sup>-1</sup> dry sample) and lysine (32.1–39.8 mg g<sup>-1</sup> dry sample) are antioxidant amino acids (AAAs) detected in all three samples (Table 1). Also, the high value of PER index (1.91) in the peptide fraction indicates its high nutritional value and digestibility.<sup>30</sup>

Other studies have also examined the amino acid composition and some nutritional indicators of some grains and seeds. For example, chia seeds contain all essential amino acids (EAAs), with glutamine being the most abundant and histidine

the least prevalent.<sup>41</sup> In contrast, cereals and pulses are deficient in certain EAAs, notably lysine, methionine, and threonine. Quinoa is, however, rich in lysine, with concentrations typically ranging from 2.4 to 7.8 g per 100 g of protein. Its methionine content varies between 0.3 and 9.1 g per 100 g of protein, while threonine levels range from 2.1 to 8.9 g per 100 g of protein.<sup>42</sup> Amaranth is another notable pseudocereal, containing significant quantities of lysine, tryptophan, arginine, and sulfur-containing amino acids. Specifically, amaranth's lysine content is twice that of wheat and three times higher than that of maize.<sup>43</sup> Buckwheat also stands out for its high lysine content (624 mg per 100 g of dry matter), addressing a common deficiency in cereals.<sup>41</sup>



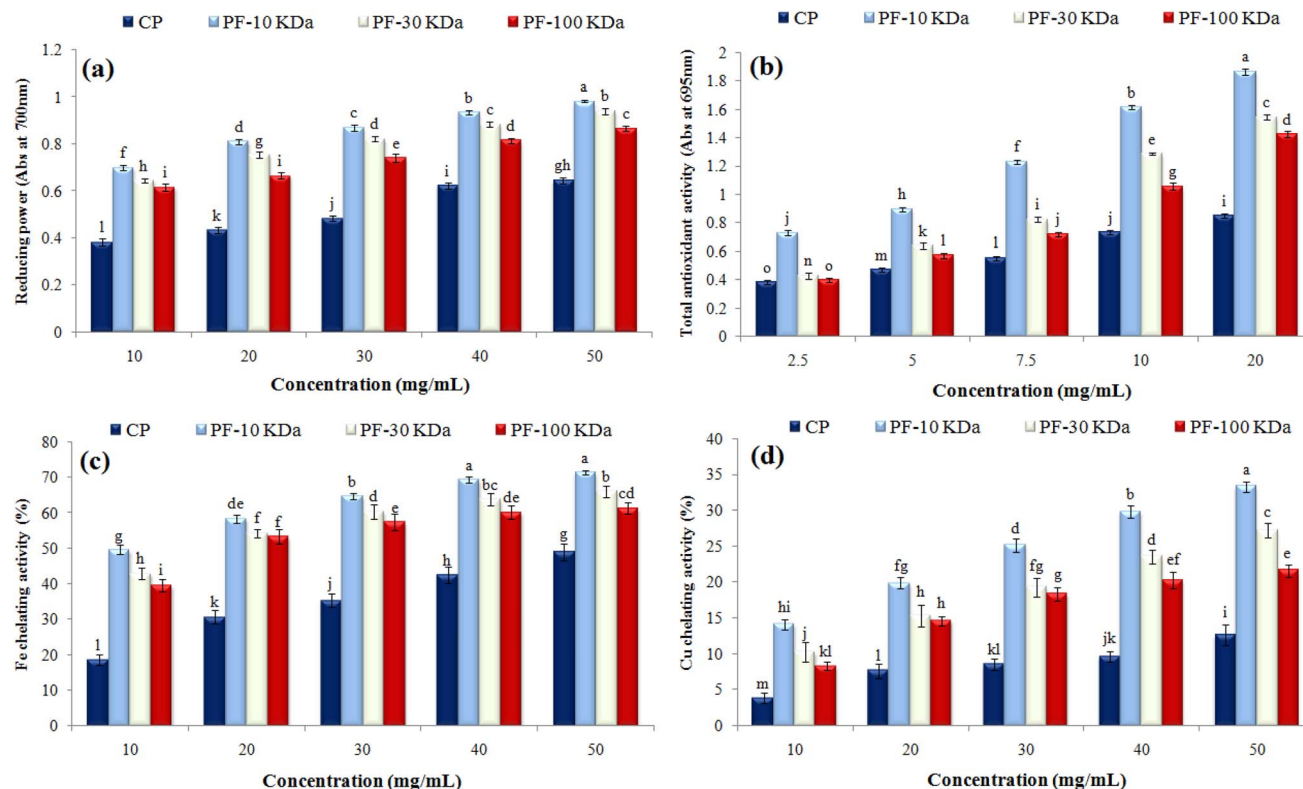


Fig. 3 Effect of molecular weight (MW) and peptide concentration on (a) reducing power activity, (b) total antioxidant activity, (c)  $\text{Fe}^{2+}$  chelating activity, and (d)  $\text{Cu}^{2+}$  chelating activity of coconut-meal protein and its peptide fractions.

Table 1 Amino acid composition of coconut cake protein (CMP), its hydrolysate (CPH) and peptide fraction (CMPF) (mg amino acid per g dry sample)<sup>a</sup>

Amino acids	Crude protein	CPH	PF-10 KDa
Aspartic acid	82.7	87.5	72.4
Glutamic acid	161.8	166.2	155.2
Histidine	20.2	31.9	39.6
Serine	40.1	39.7	34.5
Arginine	112.6	110.8	102.3
Glycine	41.4	45.2	40.9
Threonine <sup>b</sup>	24.8	27.3	22.6
Alanine	38.2	45.4	52.7
Tyrosine	21.1	27.3	25.1
Methionine <sup>b</sup>	13.3	17.3	27.5
Valine <sup>b</sup>	36.9	40.2	44.3
Phenylalanine	36.2	39.5	40.1
Isoleucine <sup>b</sup>	24.6	27.3	42.5
Leucine <sup>b</sup>	47.1	53.1	58.2
Lysine <sup>b</sup>	32.1	39.3	45.8
Tryptophan <sup>b</sup>	4.7	6.8	6.9
HAA	222.1	256.9	297.3
AAA	91.4	122.6	144.9
PER	1.45	1.66	1.91
TAA	737.8	807.8	810.6

<sup>a</sup> CPH: coconut protein hydrolysate; PF-10: peptidic fraction (<10 KDa).

<sup>b</sup> Essential amino acids; hydrophobic amino acids (HAA) = alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan, proline and methionine; antioxidant amino acids (AAA) = tryptophan, methionine, histidine, tyrosine and lysine; total amino acids (TAA).

The nutritional potential of haustorium protein, derived from coconut, was evaluated by comparing its amino acid profile to the FAO reference standards. Aspartic acid dominated the profile at 29.9%, followed by alanine (11.4%), proline (9.32%), and glutamic acid (6.35%). Essential amino acid analysis revealed elevated levels of histidine, valine, and lysine relative to FAO/WHO benchmarks, with threonine matching the reference value. However, haustorium exhibited significant deficiencies in aromatic amino acids (32.5% chemical score), leucine (45.7%), sulfur-containing amino acids (57.6%), and isoleucine (68%). These findings highlight the need for complementary formulations incorporating pseudo-cereals and millets to achieve a balanced nutritional profile. Additionally, the high aspartate and glutamate content (36%) may enhance the flavor, suggesting potential culinary applications.<sup>44</sup>

### 3.2 Characterisation of antioxidant activity (AA)

**3.2.1 DPPH and OH radical scavenging.** To evaluate the antioxidant activity of various fractions of CMP with different molecular weights (MW), DPPH and hydroxyl (OH) radical scavenging activity (%) at 10, 20, 30, 40 and 50 mg mL<sup>-1</sup> concentrations were analyzed (Fig. 2a and b). The PF-10 KDa CP fraction at 50 mg mL<sup>-1</sup> concentration has the highest DPPH and OH radical scavenging activity, which was near 80%. The lowest results were obtained for the CP sample at all concentrations for both DPPH (10%) and OH radical scavenging (15%) activity. Comparing different peptide fraction results for DPPH



and OH radical scavenging, it can be concluded that the activity trend is PF-10 > PF-30 > PF-100 KDa.

Mundi and Aluko<sup>45</sup> reported that peptide fractions with greater content of hydrophobic aliphatic amino acids (valine, isoleucine, and leucine) and hydrophobic aromatic amino acids (phenylalanine and tyrosine) exhibited higher DPPH inhibitory activity. Each amino acid has its own mechanism to show antioxidant activity, for instance, the antioxidant activity of peptides with aromatic amino acids tryptophan and phenylalanine is associated with their ability to function as radical scavengers, while the antioxidant activity of tyrosine is linked to the unique ability of its phenolic groups to donate hydrogen.<sup>46</sup>

**3.2.2 ABTS radical scavenging activity and TEAC index.** The antioxidant activity of CP and its three fractions characterized as ABTS radical scavenging activity (%) and Trolox equivalent antioxidant capacity (TEAC) (mM) at different concentrations (1, 3, 5, 10 and 20 mg mL<sup>-1</sup>) are presented in Fig. 2c and d. Similar to previous antioxidant activity experiments for ABTS and TEAC, the highest results were found for the smallest fraction (PF-10 KDa) at a higher concentration 50 mg mL<sup>-1</sup>. The MW of the peptide was found to be significant for antioxidant activity. These findings correlate with peptide fractions with lower molecular weights exhibiting higher antioxidant activities compared to those with higher molecular weights.<sup>46</sup> The reason for this can be basically related to the main structure characteristics of peptides, like (a) amino acid composition: lower molecular weight peptides tend to have higher concentrations of antioxidant amino acids such as tryptophan, methionine, histidine, lysine, and tyrosine. These amino acids have strong electron- or proton-donating abilities, which enhance the antioxidant activity, (b) free amino acids: the fractionation process increases the number of free amino acids in lower molecular weight peptides, which contributes to their higher antioxidant activity, (c) hydrophobic amino acids: lower molecular weight peptides contain more hydrophobic amino acids (*e.g.*, valine, leucine, tyrosine, *etc.*), which are more effective in scavenging lipophilic radicals like DPPH and (d) structural properties: smaller peptides are more likely to interact with and neutralize free radicals due to their increased solubility and accessibility.<sup>28,31,33</sup>

**3.2.3 Reducing capacity and total antioxidant activity.** Reducing capacity and total antioxidant activity results of the

samples are presented in Fig. 3a and b. The results showed that the 10 KDa-PF samples have a strong reducing power and high total antioxidant activity. The total antioxidant activity of the PF-10 peptide fraction varies between 0.72 to 1.87 at the lowest and highest concentrations, and for reducing capacity it was from 0.7 to 0.98, respectively. It was found that the trend of reducing capacity and total antioxidant activity of each different concentration is CP < PF-100 KDa < PF-30 KDa < PF-10 KDa. The MW of the peptides and concentration are two main factors, the highest result being related to samples with 20 mg mL<sup>-1</sup> concentration. These results suggest that the ability to donate electrons and reduce ferric ions to ferrous ions is enhanced significantly ( $p < 0.05$ ) by enzymatic hydrolyzation of CP. Indeed, this can be related to the release of free antioxidant amino acids (like tyrosine, tryptophan, histidine, methionine and lysine) after enzymatic hydrolyzation and their higher concentration in the fractions with a relatively low MW (10 KDa).<sup>47,48</sup>

**3.2.4 Metal ion chelating activity.** The chelating activity of CP and peptide fractions is presented in Fig. 3c for Fe<sup>2+</sup> ions and in Fig. 3d for Cu<sup>2+</sup> ions. The trend is very similar to reducing capacity and TAA: the highest chelating ability was found for the PF-10 KDa sample at 50 and 40 mg mL<sup>-1</sup> concentrations with 70% for chelating Fe<sup>2+</sup> and at 50 mg mL<sup>-1</sup> concentration, with around 34% for Cu<sup>2+</sup> ions. PF-10 and PF-30 KDa samples at concentrations of 30, 40 and 50 mg mL<sup>-1</sup> had the highest Fe<sup>2+</sup> and Cu<sup>2+</sup> chelating activity ( $p < 0.05$ ). The most important health-promoting properties of bioactive peptides include the inhibition of free radicals and chelation of metal ions to reduce the lipid-oxidation rate.<sup>33</sup> Like antioxidant activity, significant differences ( $p < 0.05$ ) in the chelating activity of Fe<sup>2+</sup> and Cu<sup>2+</sup> can be related to the function of enzymes in the production of peptides with more acidic and basic amino acids, histidine with an imidazole ring and the degree of hydrolysis.<sup>49</sup> According to our findings, the smallest peptide fraction has the highest ionic chelating activity and this is in common with Farvin *et al.*<sup>50</sup> who reported the highest iron chelating activity in a cod (*Gadus morhua*) protein hydrolysate with MW < 3 KDa.<sup>50</sup> In another study, enzymatic hydrolysis of rice bran protein (with pepsin and pepsin–trypsin combination) and subsequent fractionation of the hydrolysate using ultrafiltration (<3, 3–5 and 5–10 KDa) were performed. The results showed that the

Table 2 Effect of carrier composition on the physical properties of the microencapsulated coconut peptide fraction<sup>a</sup>

Carrier type	Carrier : core	Production yield (%)	Moisture content (%)	Water activity	Bulk density (g mL <sup>-1</sup> )
—	0 : 1	35.3 ± 2.8 <sup>b</sup>	3.5 ± 0.16 <sup>b</sup>	0.34 ± 0.01 <sup>a</sup>	0.45 ± 0.01 <sup>a</sup>
MD	3 : 1	59.9 ± 3.4 <sup>a</sup>	3.2 ± 0.14 <sup>c</sup>	0.31 ± 0.01 <sup>b</sup>	0.36 ± 0.01 <sup>b</sup>
MD : P	2 : 1 : 1	55.1 ± 2.6 <sup>a</sup>	4.1 ± 0.17 <sup>a</sup>	0.35 ± 0.01 <sup>a</sup>	0.31 ± 0.01 <sup>c</sup>
Carrier type	Carrier : core	Angle of repose (°)	Solubility (%)	Hygroscopicity (%)	Size (µm)
—	0 : 1	32.7 ± 2.2 <sup>a</sup>	93.2 ± 1.6 <sup>b</sup>	38.9 ± 2.2 <sup>a</sup>	9.4 ± 0.8 <sup>b</sup>
MD	3 : 1	25.9 ± 1.1 <sup>b</sup>	97.2 ± 1.2 <sup>a</sup>	23.7 ± 2.1 <sup>b</sup>	10.8 ± 0.8 <sup>a</sup>
MD : P	2 : 1 : 1	27.9 ± 1.6 <sup>b</sup>	90.3 ± 1.8 <sup>b</sup>	26.9 ± 1.4 <sup>b</sup>	12.2 ± 0.5 <sup>a</sup>

<sup>a</sup> MD: maltodextrin; P: pectin. Different letters in the same column indicate statistically significant differences ( $P < 0.05$ ).



antioxidant and metal ion chelating activities were higher in smaller peptides (with a lower molecular weight). They attributed this finding to the higher reactivity of the lower molecular weight fractions compared to the other fractions.<sup>29</sup>

### 3.3 Powder characterization

**3.3.1 Production yield.** In the spray drying encapsulation, the type of carrier and its ratio to the core can influence the production yield (%) and other physical properties.<sup>51</sup> In this study, the carrier-to-core ratio was kept constant at 3 : 1 (Table 2). The production yields for MD (59.9%) and MD/P (55.1%) showed no significant differences ( $p > 0.05$ ), indicating that the encapsulation materials had similar production yields. It has been reported that proteins generally result in low-yield production during spray drying for the encapsulation of bioactive compounds.<sup>52</sup> Therefore, hydrocolloids, either alone or in combination with proteins, appear to be more effective encapsulation materials. However, MD combined with whey protein concentrate exhibited the highest yield at 57.4%. Similarly, Balci-Torun and Ozdemir<sup>51</sup> reported a process yield of 86% when strawberry flavour was encapsulated using MD as the wall material *via* spray drying. In contrast, Pellicer *et al.*<sup>53</sup> applied spray drying to encapsulate the same material with MD and obtained a lower process yield of 66%. Chlorella peptide was spray-dried by MD and MD/P with 54.9% and 48.1% yields, respectively.<sup>4</sup>

**3.3.2 Physical, functional and flowability indices.** Some physical properties of PF-10 KDa before and after encapsulation are presented in Table 1. The encapsulated sample with MD/P exhibited the highest moisture content at 4.1%, while the sample encapsulated with MD had the lowest moisture content at 3.2%. The sample without any carrier showed an intermediate moisture content of 3.5%. According to previous reports, proteins have a higher water holding capacity,<sup>11</sup> which may explain these findings. Notably, moisture content is a crucial factor in evaluating the stability of powders. Encapsulating bioactive peptides with suitable materials enhances their stability.<sup>4</sup> The water activity (aw) of PF-10 KDa (0.34) and the

encapsulated sample with MD/P (0.35) showed no significant difference ( $p > 0.05$ ) from each other, while encapsulation with MD was different for both ( $p < 0.05$ ) with 0.31 aw. Water activity depends on free water in the food matrix as well in encapsulated materials, and it can depend on hydrophobic groups of the hydrolysates and their interactions with the carrier, resulting in conformational changes that exposed more hydrophilic groups to the surface, increasing the moisture, water activity and hygroscopicity of the encapsulated hydrolysate.<sup>54</sup>

The bulk density and angle of repose results are given in Table 2. The bulk density was measured for three different samples, showing the following trend: PF-10 KDa (0.45 g mL<sup>-1</sup>) > PF-10 KDa encapsulated with MD (0.36 g mL<sup>-1</sup>) > PF-10 KDa encapsulated with MD/P (0.31 g mL<sup>-1</sup>) with significant differences. Our findings differ from those of Akbarbaglu *et al.*,<sup>19</sup> who attributed the higher bulk density in MD samples to viscosity changes in the feed material compared to gum Arabic and WPC, which are known to produce powders with smaller particle sizes. Based on the angle of repose (Table 2), a significant decrease from 32.7° to 25.9° and 27.9° was observed after encapsulation with MD and MD/P materials, respectively ( $p > 0.05$ ). Also, another study reported that the addition of stevioside as a carrier significantly reduced the angle of repose from 48.47° to 40.29°, compared to using a soluble soybean polysaccharide carrier.<sup>55</sup> Furthermore, the increased product yield of peptide microcapsules may be attributed to enhanced powder mobility during spray drying, as indicated by the reduced angle of repose. Thus, it can be concluded that encapsulation with MD and MD/P improves particle mobility.

The solubility of samples serves as an indicator of the presence of hydrophilic polymers and hydroxyl groups within the sample.<sup>56</sup> In our experiment, the highest solubility (97.2%) was observed for the PF-10 KDa peptide encapsulated with MD. However, no significant difference ( $p < 0.05$ ) was noted between the PF-10 KDa sample and the MD/P encapsulated sample, which exhibited solubility values of 93.2% and 90.3%, respectively. In other study, peptide microcapsules with higher porosity were found to absorb water more rapidly.<sup>55</sup>

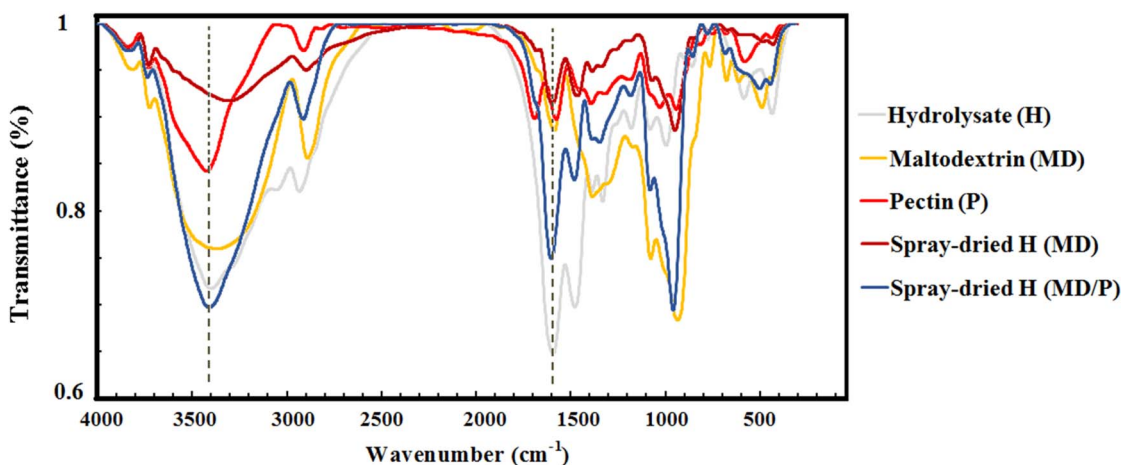


Fig. 4 Effects of spray-drying encapsulation by maltodextrin and combination of maltodextrin with pectin as a carrier on the structural properties (FTIR) of the coconut-meal bioactive peptide.



The hygroscopicity (%) of MD and MD/P samples was found to be 23.7% and 26.9%, respectively, with no significant difference ( $p > 0.05$ ) between them. Both values were lower than those of the sample without a carrier, indicating that encapsulation effectively reduced the hygroscopicity of the bioactive peptide sample. Hygroscopicity, or moisture sensitivity, refers to a solid's ability to absorb and retain water from its surroundings. This property arises from polar functional groups or binding sites that facilitate hydrogen bonding with water, making the material hydrophilic and prone to moisture uptake. Encapsulation using MD has been shown to reduce hygroscopicity due to its inherently low hygroscopicity and ability to increase the glass transition temperature ( $T_g$ ) of spray-dried powders, maintaining them in a stable glassy state. These properties enhance moisture protection and stability.<sup>56</sup>

The particle size of the PF-10 sample without a carrier was 9.4  $\mu\text{m}$ , which significantly increased to 10.8  $\mu\text{m}$  and 12.2  $\mu\text{m}$  after encapsulation with MD and MD/P, respectively. However, the combination of MD with P had no significant effect on particle size ( $p > 0.05$ ). In comparison, spirulina peptides had particle sizes of 4.7  $\mu\text{m}$  with MD and 7.7  $\mu\text{m}$  with MD/P during the spray-drying process.<sup>19</sup> Similarly, spray-dried oleaster seed peptides exhibited particle sizes of 2.5  $\mu\text{m}$  with MD and 4.07  $\mu\text{m}$  with MD/P.<sup>11</sup>

**3.3.3 Structural and conformational features (FTIR).** To evaluate the interaction of spray-drying encapsulation materials pectin (P), maltodextrin (MD), and their combination (MD/P) with the peptide hydrolysate fraction (PF-10 KDa) sample, Fourier Transform Infrared (FTIR) spectroscopy was performed. In general, the spectra of all samples and microcapsules (Fig. 4) showed the same peak patterns. The PF-10 KDa sample (gray line) showed main vibration bands at 3400, 2900, 1600 and 1500  $\text{cm}^{-1}$  and unique bands at 1300, 600, and 400  $\text{cm}^{-1}$ . For P (the red line) the main FTIR spectral bands were near 3400, 1700 and 1500  $\text{cm}^{-1}$  wavenumbers. Previous research reported that the FTIR spectra of pectin showed a band around 3411  $\text{cm}^{-1}$  (O–H stretching), an absorption band around 1750  $\text{cm}^{-1}$  (C=O stretching vibration of ester carbonyl), and an absorption band around 1630  $\text{cm}^{-1}$  (C=O stretching vibration of the carboxyl group).<sup>57</sup> The MD spectrum reported by Kang *et al.*<sup>58</sup> revealed absorption bands at 3392  $\text{cm}^{-1}$  (O–H stretching), 2925  $\text{cm}^{-1}$  (C–H stretching), 1653  $\text{cm}^{-1}$  (C=O stretching), 1457  $\text{cm}^{-1}$  ( $\text{CH}_2$  bending), 1371  $\text{cm}^{-1}$  (O–H bending), 1158, 1081, and 1020  $\text{cm}^{-1}$  (C–O stretching and C–O–H bending), and 928, 848, 761, 708, and 576  $\text{cm}^{-1}$  (vibrations of the pyranoid ring).<sup>58</sup> The MD/P sample (blue line) showed a similar pattern of maximum intensity bands at 3400  $\text{cm}^{-1}$  and 2900  $\text{cm}^{-1}$  for MD (yellow line) and different peaks (smaller) at 1600, 1400, and 1300  $\text{cm}^{-1}$ . Based on the spectra of CMPHs reported by Wang *et al.*,<sup>57</sup> the characteristic bands of coconut peptide hydrolysate appeared at 3455 (O–H stretching), 2927 (C–H stretching in phytol), 2855 (symmetric and asymmetric  $\text{CH}_2$  and  $\text{CH}_3$  stretching), 1745 (C=O stretching), 1694 (C=O stretching), 1607 (C=N stretching of the aromatic system in chlorophyll and C=C), and 1286  $\text{cm}^{-1}$  (C–O stretching). In their experiments, these peaks indicated the presence of CMPHs in all

microcapsules. Consequently, it was confirmed that microencapsulation by spray-drying was successfully performed.<sup>57</sup>

The hydroxyl peak intensities for all microcapsules were significantly lower than those for P and MD. This suggests that the hydroxyl groups in P and MD were involved in chemical reactions, like hydrogen bonding or esterification, during spray-drying. A peak corresponding to the amine or carbonyl group of CMPH appeared at 1613  $\text{cm}^{-1}$  in the spectra of microcapsules coated with P/MD but at a lower wavenumber in microcapsules prepared using MD alone. Comparison of the spectra of carrier materials alone and their combination with the core showed changes in the wavenumbers. For example, this phenomenon was also reported in the alginate blend with cashew gum. As the alginate content is increased, absorption peaks with higher intensities were observed at 1615  $\text{cm}^{-1}$  and 1417  $\text{cm}^{-1}$  due to the presence of carboxylate groups in greater quantity in alginate than in cashew gum.<sup>59</sup>

Abdin *et al.*<sup>60</sup> observed a shift in wave numbers due to strong absorption between the core and encapsulation materials. They claimed that the FTIR spectra of sodium alginate showed principal bands at wavenumbers around 3280, 1595, 1405, and 1025  $\text{cm}^{-1}$ , attributed to O–H,  $\text{COO}^-$  (asymmetric),  $\text{COO}^-$  (symmetric), and C–O–C stretching, respectively. The band at wavenumber 3280  $\text{cm}^{-1}$  shifted to 3235  $\text{cm}^{-1}$  as a strong absorption band due to O–H stretching. Moreover, the symmetrical stretching band of  $-\text{COO}-$  groups shifted from 1415  $\text{cm}^{-1}$  to 1530  $\text{cm}^{-1}$  after interaction with sodium alginate and anthocyanin from the *Syzygium cumini* extract.<sup>60</sup>

By comparing band intensities and new peaks in the FTIR spectra, the 10-PF sample (gray) shows the strongest bands, similar to the MD sample (yellow) and the MD/P-encapsulated sample (blue). The increased band intensities for the MD/P sample indicate stronger internal interactions, which likely explain why MD/P particles encapsulate peptides more effectively than MD alone.

**3.3.4 Morphology (SEM) and particle size.** The morphological characteristics of the PF-10 sample encapsulated with MD and MD/P during spray drying are shown in Fig. 5a and b. According to Table 2, there were no significant differences in particle size between PF-10 samples encapsulated with MD and MD/P. Encapsulation increased the particle size: the PF-10 sample had a size of  $9.4 \pm 0.8 \mu\text{m}$ , while PF-10 encapsulated with MD and MD/P measured  $10.8 \pm 0.8 \mu\text{m}$  and  $12.2 \pm 0.5 \mu\text{m}$ , respectively. The microstructure of powders is critical for their properties, with target sizes typically below 100  $\mu\text{m}$ <sup>11</sup> or even less than 50  $\mu\text{m}$ ,<sup>19</sup> as particle size affects physical properties such as flowability, ingredient blending, and sensory characteristics like flavor, appearance, and texture. Smaller particles, however, tend to attract moisture and clump during drying.<sup>35</sup> Encapsulation with MD as a single wall material (Fig. 5a) resulted in more homogeneous, shriveled (wrinkled) surface morphologies, while encapsulation with MD/P (Fig. 5b) produced particles with diverse morphologies, including both shriveled and smooth spherical surfaces with hollowed regions between particles. These findings indicate that the choice of the shell material significantly influences particle morphology. Furthermore, particles produced with MD alone were reported to



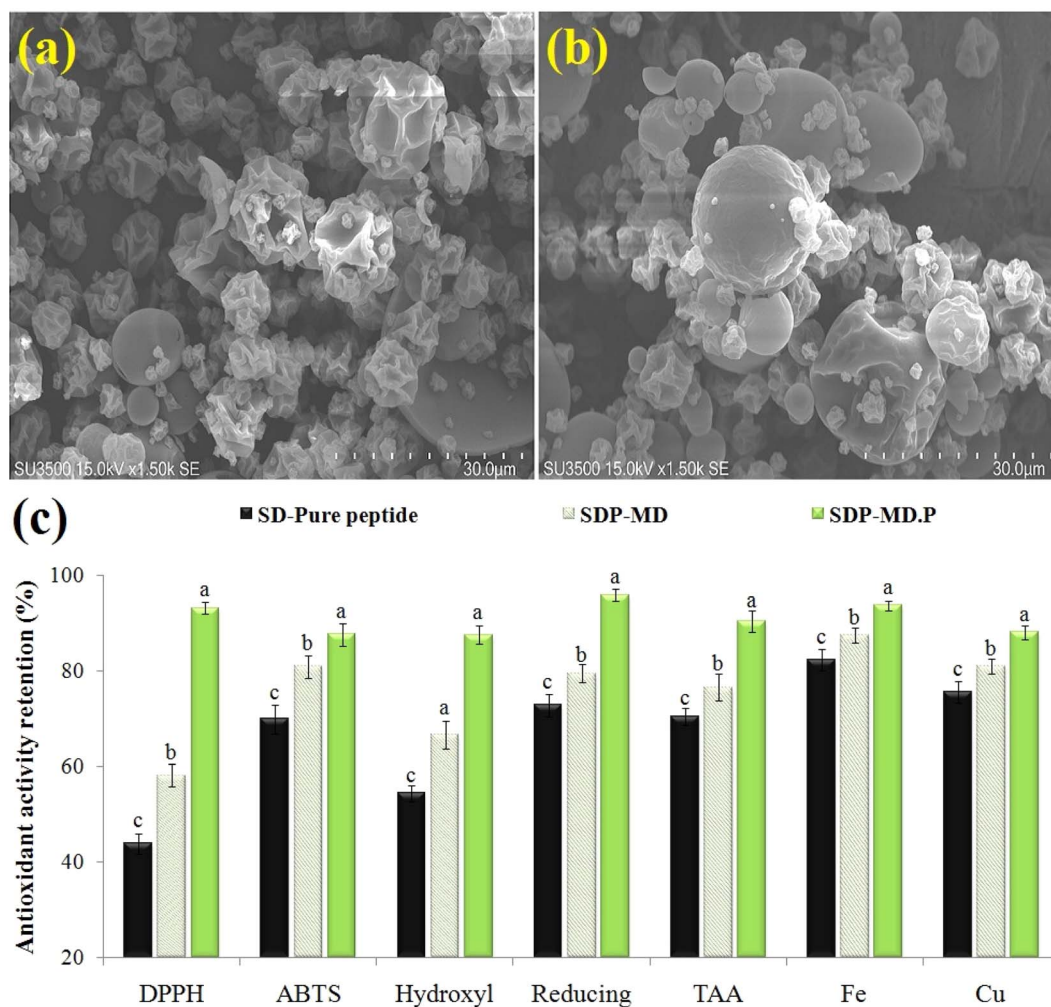


Fig. 5 Effects of spray-drying encapsulation by (a) maltodextrin and (b) combination of maltodextrin with pectin as a carrier on the morphological properties. (c) Retention of antioxidant activity of the coconut-meal bioactive peptide.

exhibit more surface shrinkage compared to those encapsulated with MD/GA combinations.<sup>35</sup> Our results demonstrate that MD and MD/P as encapsulating agents can form smooth, wrinkled structures with no visible cracks, pores, or broken walls. Additionally, optimizing spray drying conditions is critical for achieving desirable particle surface characteristics.<sup>57</sup>

**3.3.5 Retention of AA.** Fig. 5c illustrates the retention of antioxidant activity for the PF-10 sample encapsulated with two carriers during the spray-drying process. Based on various methods, including DPPH radical scavenging, ABTS radical scavenging, hydroxyl radical scavenging, reducing power, total antioxidant activity (TAA), and Fe<sup>2+</sup> and Cu<sup>2+</sup> chelating activity, the MD/P combination was found to be the most effective carrier for encapsulating the PF-10 sample ( $p < 0.05$ ). While the pure peptide exhibited the lowest retention of antioxidant activity (40–80%), the PF-10 sample encapsulated with the P material showed a higher retention (60–90%). The highest retention (90–98%) was observed for the PF-10 sample encapsulated with the MD/P combination ( $p < 0.05$ ). Notably, the greatest difference in retention was recorded for the DPPH

radical scavenging method, where a 50% gap was observed between the pure peptide and the MD/P-encapsulated peptide. Based on our findings and previous research, two primary factors influence the stability of peptide antioxidants during spray-drying: (1) the structure and amino acid sequence of the peptide and (2) the type of encapsulating material used.<sup>14</sup> The stabilization effects of carriers are due to mechanisms such as peptide entrapment within the carrier matrix, water replacement, and glassy immobilization.<sup>61</sup> The efficiency of an encapsulation method and material can be assessed by comparing antioxidant activity retention before and after encapsulation. The most desirable outcomes are achieved when antioxidant activity retention (%) is maximized after encapsulation.<sup>11</sup> Various protein/polysaccharide-based materials have been reported as effective shells for protecting bioactive peptides from processing and environmental stresses, such as heat and oxidative damage, during spray-drying.<sup>19</sup>

Encapsulation of nutraceuticals in WPI or WPIF is an effective means of enhancing their reducing capacity. This may be due to its binding to whey proteins through hydrophobic



**Table 3** Effects of different proportions of SD-CPH on the physico-chemical properties, texture, and viscosity of fortified yogurts<sup>a</sup>

(a)	pH	Hardness (g)	Cohesiveness (g)
CY	4.54 ± 0.1 <sup>a</sup>	25.6 ± 3.2 <sup>d</sup>	0.59 ± 0.02 <sup>c</sup>
FY-1%	4.38 ± 0.1 <sup>ab</sup>	41.9 ± 3.3 <sup>c</sup>	0.65 ± 0.02 <sup>b</sup>
FY-2%	4.40 ± 0.1 <sup>b</sup>	47.5 ± 2.9 <sup>b</sup>	0.74 ± 0.02 <sup>a</sup>
FY-3%	4.33 ± 0.1 <sup>b</sup>	58.3 ± 3.1 <sup>a</sup>	0.72 ± 0.02 <sup>a</sup>
(b)	WHC (%)	Syneresis (%)	Viscosity (Pa s)
CY	64.1 ± 3.6 <sup>c</sup>	18.5 ± 1.2 <sup>a</sup>	1.32 ± 0.1 <sup>c</sup>
FY-1%	73.3 ± 2.7 <sup>b</sup>	16.4 ± 1.1 <sup>b</sup>	3.34 ± 0.3 <sup>b</sup>
FY-2%	79.4 ± 2.8 <sup>a</sup>	13.3 ± 0.7 <sup>c</sup>	4.74 ± 0.4 <sup>a</sup>
FY-3%	80.3 ± 2.5 <sup>a</sup>	11.1 ± 1.2 <sup>d</sup>	5.31 ± 0.2 <sup>a</sup>

<sup>a</sup> CY: control yogurt; FY: fortified yogurt. SD: spray-dried. CPH: coconut protein hydrolysate. Different letters in the same column indicate statistically significant differences ( $P < 0.05$ ).

interactions, thereby facilitating electron transfer to  $Fe^{3+}$ .<sup>62</sup> It is clear that encapsulation is the best way to improve the reducing capacity of peptides. The higher encapsulation efficiency and loading amount can be evaluated from the higher reducing power activity.<sup>63</sup>

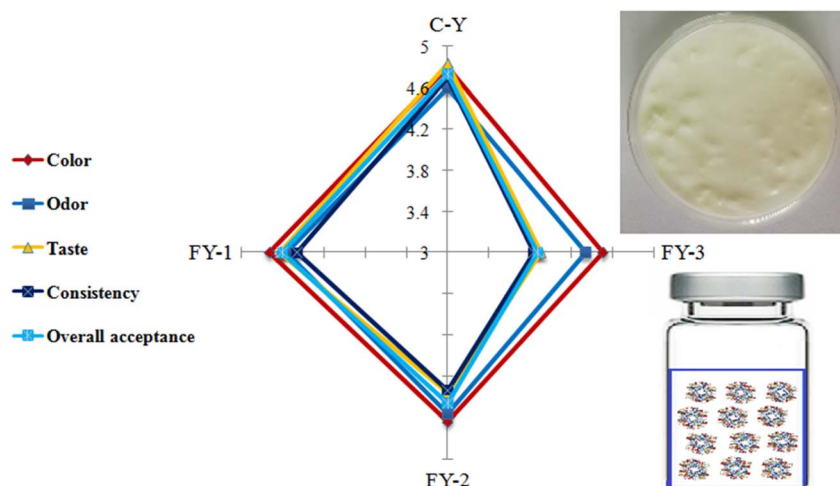
### 3.4 Fortified yogurt analysis

**3.4.1 Physical, chemical, and textural properties.** Table 3a and b shows the characteristics of yogurt enriched with different proportions of encapsulated peptide. Increasing the peptide concentration (especially to 3%) resulted in a decrease in pH from ~4.54 (control sample) to ~4.33. Under the above conditions, the firmness (from 25.6 to 58.3 g) and cohesiveness (from ~0.59 to 0.72 g) of the yogurt increased significantly. Increasing peptide concentration also resulted in an increase in water retention capacity (from ~64 to 80%) and a decrease in syneresis (from ~18.5 to 11.1%). Finally, the viscosity of the samples increased from about 1.32 to 5.31 Pa s with increasing

solids and peptide concentration to 3% (Table 3b). Regarding the above results, the following reasons can be stated: (1) increased reaction and complex formation between peptides and casein micelles as well as improved gel strength; (2) increased uniformity, gel formation strength as well as water retention capacity by peptides leading to changes in syneresis and WHC.<sup>36,64</sup>

In another study, natural yogurt was enriched with different concentrations (0, 0.2, 0.5 or 1% w/v) of low molecular weight peptides derived from casein hydrolysis. The results showed increased acidity, reduced fermentation time and syneresis, improved rheological and textural properties, and antioxidant activity.<sup>36</sup> In another study, the effects of adding fermented whey protein concentrate on yogurt properties were investigated. The results of that study showed that the use of fermented protein increased the antioxidant activity of yogurt compared to samples enriched with whey protein concentrate or isolate. Also, the water holding capacity, viscosity, formation of a larger and stronger protein network, structural and textural properties, as well as the growth of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* bacteria improved after enrichment.<sup>37</sup>

**3.4.2 Sensory property evaluation.** Fig. 6 shows the sensory characteristics of control and enriched yogurt samples. Enrichment of yogurt with coconut peptide up to 2% did not show a significant effect on the color of the samples. However, high peptide concentrations (3%) resulted in reduced flavor characteristics (creating bitterness), changes in texture, and ultimately the overall acceptability. However, samples enriched with 2% peptide had acceptable scores from the evaluators. Given the health benefits of peptides, as well as the positive effects on the physical properties, texture, and syneresis of yogurts, it can be considered acceptable. In a similar study, yogurts enriched with fermented whey protein concentrate exhibited better textural, microstructural, and sensory properties compared to other samples (enriched with commercial WPC and WPI).<sup>37</sup>



**Fig. 6** Sensory parameters of fortified yogurts with spray-dried coconut peptide fractions.



## 5 Conclusion

Food peptides and hydrolysates have significant health benefits, but their use in the food sector is limited by processing challenges. Spray drying addresses these issues by enhancing powder properties, including thermal protection, encapsulation, granulation, taste masking, shelf life, and compatibility with food matrices, enabling wider applications in functional foods. Microparticles containing maltodextrin with pectin and the core coconut meal protein fraction (<10 KDa) showed the best encapsulation, as well as better yield; moreover, all the microparticles showed higher antioxidant activity than the cured coconut protein. The addition of encapsulated coconut peptides resulted in changes in pH, reaction with casein proteins, increased gel strength, viscosity, water holding capacity (WHC), and decreased syneresis in yogurt samples. However, high peptide concentrations (3% w/v) resulted in reduced sensory properties and the appearance of bitterness in fortified yogurts. Future research should prioritize peptide safety, focusing on cytotoxicity and allergenicity, while optimizing wall material concentration and targeted release profiles. Developing specialized assays to evaluate the bioactivity and bioavailability of encapsulated peptides post-release is also essential.

## Author contributions

Reza Mobasserfar: resources, investigation. Atefeh Karimidastjerd: writing – original draft. Yugal Kishore Mohanta: review & editing. Fatemeh Yousefi Kopaei: formal analysis, software. Zahra Akbarbaglu: supervision, data curation, review & editing. Khashayar Sarabandi: supervision, conceptualization, data curation, review & editing.

## Conflicts of interest

The authors declare no conflicts of interest related to the research presented in this study.

## Data availability

Data are available on request from the corresponding author.

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