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## The effects of feed moisture and dried coconut meal content on the physicochemical, functional, and sensory properties of gluten-free Riceberry rice flour-based extruded snacks

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The objective of this study was to develop an expanded snack from Riceberry rice flour (RBF) and dried coconut meal (DCM), a by-product of coconut milk production, using a twin-screw extruder. A 3 x 3 factorial design was employed to investigate the effects of feed moisture contents (18, 21, and 24%) and DCM levels (0, 5, and 10%) on the physicochemical, functional, and sensory properties of the products. The results showed that increasing feed moisture content significantly reduced the cold peak viscosity but had no significant effect on the hot peak viscosity. DCM was the dominant factor affecting the hot peak viscosity. The substitution of DCM led to a decrease in the expansion of the extrudates while increasing the L\* and b\* values. The texture parameters were affected by both feed moisture and DCM, where increased feed moisture resulted in more rigid structures with higher density and hardness but lower crispiness. Antioxidant levels in the final product decreased by approximately 64 to 70% and 10 to 25%, as determined by DPPH and FRAP assays, respectively, compared to the initial RBF. However, the antioxidant levels remained high in the final product. Feed moisture content had no effect on the antioxidant activity as measured by the DPPH and FRAP assays. The total phenolic content (TPC) of the extrudates decreased as feed moisture and DCM content increased. Furthermore, the anthocyanin content (ATC) decreased when RBF was substituted with DCM. The sensory evaluation revealed that the product with 18% feed moisture content and 10% DCM had the highest overall liking score. These findings offer valuable insights into the potential use of a twin-screw extruder for developing expanded snacks from RBF and DCM and could be useful for further research in this area.

KEYWORDS

extrusion, coconut meal, Riceberry rice, anthocyanin, pasting properties, functional properties, gluten-free, snack

## 1. Introduction

Extrusion is one of the most widely used methods for producing snacks. The physicochemical and sensory properties of extruded products can vary significantly depending on operating conditions and raw material properties. Most snacks available on the market are made from cereals that are high in fat and low in nutrition. Today, consumers

are increasingly interested in foods that not only taste good but also provide high levels of nutrients or other functional components. The healthy snack market is expanding, and new products must be developed to meet consumer demand. To provide more nutrition that partially or completely replaces flour, several nutritious ingredients such as protein (Philipp et al., 2017; Saldanha do Carmo et al., 2019; Zhong et al., 2021), fiber (Alam et al., 2016; Han et al., 2018; Zhong et al., 2021), and phytochemicals (Hirth et al., 2014; Neder-Suárez et al., 2021) have been added to the extruded snack.

Anthocyanins are polyphenol compounds that are found in red, purple, and blue plants such as blackberries, blueberries, bilberries, grapes, strawberries, and black rice. Riceberry rice (*Oryza Sativa* L.), a dark purple rice variety from Thailand, was developed through crossbreeding between Hom Nin rice, a nonglutinous purple rice, and Khao Dawk Mali 105. Riceberry rice has been found to contain high levels of antioxidants, including vitamin E (tocotrienols), anthocyanins (cyanidin-3-O-glucoside, peonidin-3-O-glucoside), and phytosterols (oryzanol, sitosterol, and triterpene alcohol) (Poosri et al., 2019). Therefore, Riceberry rice-based food products may be an excellent choice for gluten-free and health-conscious consumers.

Coconut (Cocos nucifera L.) is a significant economic crop that is mainly grown in tropical regions. Indonesia (16.82 million tons), India (14.70 million tons), and the Philippines (14.49 million tons) are the top three coconut-producing countries globally, with Thailand ranking ninth (0.8 million tons) (FAO, 2020). Coconuts are versatile and can be used in various applications, including food, beverages, wood, and handicrafts. Coconut meat or kernel can be consumed fresh, processed into other foods, or crushed to extract coconut milk, which is a common ingredient in many dishes. The production of coconut milk typically uses screw press or hydraulic extraction methods, which produce between 53 and 62% of coconut milk, resulting in 38 to 47% of coconut meal waste. Although commercial coconut milk manufacturing is growing in Thailand, generating 5,000 metric tons of coconut meal residue (Sangkharak et al., 2020), most of which is primarily used as a lowcost animal feed ingredient and fertilizer, only a small part is used in food products as a source of dietary fiber. Therefore, there is a need to utilize waste products to achieve sustainable development and reduce environmental pollution. Several by-products, such as pomace (Alam et al., 2016) and bagasse from fruits and vegetables (Navarro Cortez et al., 2016), bran, coat, and wasted grains from cereals (Fleischman et al., 2016; Unsaeng et al., 2019; Zhong et al., 2021), have been used in extruded snack applications. However, there have been only a few studies examining the use of coconut meal in extruded snacks (Khan et al., 2015; Dumchaiyapoom et al., 2019).

Thus, this research aimed to investigate the effects of different levels of feed moisture and dried coconut meal on the physicochemical properties (nutritional compositions, pasting properties, expansion index, bulk density, water absorption index, color, and texture), functional properties (DPPH, FRAP, total phenolic content, and anthocyanin content), and sensory evaluation of extrudates. The primary focus of this study was to examine the potential application of dried coconut meal and Riceberry rice flour in the production of healthy extruded snacks and to investigate the effect of the extrusion process on product properties. The findings of this study will provide valuable insights into the feasibility of utilizing these ingredients to create nutritious snacks while also contributing to the reduction of food waste and promoting sustainable development.

## 2. Materials and methods

## 2.1. Raw materials and dried coconut meal preparation

Riceberry rice flour (RBF) and dried coconut meal (DCM) were used to formulate the expanded snacks. Commercial RBF was purchased from a local market (Fancy Carp brand, Charoenworrakit Part., Ltd., Thailand). The mature coconuts were procured from a local market, the shells removed, and the white kernels mechanically scraped. Thick milk was extracted from the kernels using a hydraulic press, followed by thin milk extraction using water (1:1 w/v, 25°C) left for 15 min before pressing. Coconut meal, a by-product of coconut milk extraction, was dried at 70°C for 4 h in a hot-air tray dryer, milled, passed through a 60 mesh sieve, and packed into polyethylene bags. The RBF was substituted with DCM at three different levels (0, 5, and 10% w/w). The proximate compositions of RBF and DCM were determined using a standard method described by AOAC (2002) and are presented in Table 1.

# 2.2. Experiment design and extrusion process

The experiment was carried out using a 3 x 3 factorial arrangement in a completely randomized design (CRD) to investigate the two factors, including feed moisture content (18, 21, and 24% w/w) and DCM (0, 5, and 10% w/w) substituted for RBF.

Extrusion was performed using a pilot scale co-rotating intermeshing twin-screw extruder (CTE-D22L32 model, Chareun

Parameters	RBF	DCM							
Proximate composition									
Moisture (%)	$9.17\pm0.45$	$6.84 \pm 2.52$							
Ash (%)	$1.16\pm0.33$	$1.15\pm0.33$							
Protein (%)	$5.91 \pm 0.98$	$39.48 \pm 2.03$							
Fat (%)	$7.09\pm0.18$	$7.17\pm0.16$							
Carbohydrate (%)	$76.67\pm0.98$	$48.36\pm2.62$							
Antioxidant properties									
DPPH (mg TE/100 g DW)	$335.21 \pm 16.95$	$45.95 \pm 1.71$							
FRAP (mg FeSO <sub>4</sub> /100 g DW)	$36.14\pm1.11$	$0.89\pm0.02$							
TPC (mg GAE/100 g DW)	7,783.96 ± 39.15	$1,\!280.12\pm83.62$							
ATC (mg Cy-3-G/100 g DW)	$33.20\pm0.55$	$3.42\pm0.59$							

RBF, Riceberry rice flour; DCM, dried coconut meal.

TABLE 1 Proximate composition and antioxidant activity of raw material used for expanded snack production.

Tut Co., Ltd., Thailand). The screw had a diameter of 24.70 mm with a length-to-diameter ratio of 32:1. The barrel consisted of six jacketed zones. The temperature from the first zone to the sixth zone was maintained at 30, 45, 80, 125, 130, and  $140^{\circ}$ C, respectively, throughout the experiment. The extrudates were carried out in the twin-screw extruder using a 1.5-mm die diameter. The extruder was powered by a 3.7 kW motor with a screw speed of 400 rpm at a die temperature of 90°C. The raw material was fed into the extrusion barrel at 20 kg/h. The extrudates, in each experiment, were produced in duplicates. The extrudates were packed and stored at room temperature for further analysis.

### 2.3. Pasting properties

The pasting properties of the RBF and extrudate flour were measured using a Rapid Visco Analyzer (Newport Scientific PTY Ltd, Australia) combined with the Thermocline for Windows<sup>TM</sup> (TCW3) software. In the aluminum RVA canister, 4.5 g of sample was mixed with 25 mL of distilled water and corrected to a moisture basis of 14%. The mixture was rotated with a paddle until no lumps were observed. The canister and paddle were inserted into the RVA instrument, and the analysis was started. After 2 min of equilibration at 25°C, the suspension was heated to 95°C for 5 min and maintained for 3 min at the same temperature (95°C). Then, the temperature was reduced to 25°C for 5 min and held for 1 min.

## 2.4. Physical properties of extruded product

### 2.4.1. Expansion ratio

The diameter of extrudates from each treatment was measured using a digital caliper and divided by the die diameter (1.5 mm) of the extruder (Saeleaw et al., 2012).

### 2.4.2. Bulk density

Bulk density is a measure of weight per unit volume. The sesame seed displacement method was used to determine the volume of extrudates (Promsakha na Sakon Nakhon et al., 2018).

### 2.4.3. Water absorption index

The water absorption index (WAI) was determined using a modified method from Li et al. (2019). A 2.5 g sample of the ground product was sieved at 60 mesh, then suspended in 30 mL of water at 30°C in a 50 mL tarred centrifuge tube, stirred intermittently for 30 min, and then centrifuged at 3,000 x g for 10 min. The liquid from the supernatant was gently poured into an evaporating dish. The water absorption index was derived from the weight of the remaining solid.

### 2.4.4. Color measurement

The color parameters were assessed with a colorimeter (200 M model, Datacolor, NJ, USA) in the CIELAB parameters ( $L^*$ ,  $a^*$ , and  $b^*$  values), which are used to characterize color in three dimensions.

### 2.4.5. Texture analysis

The hardness and crispness of the extrudates were determined using the modified methodology of Nascimento et al. (2017). A texture analyzer (TA. XT Plus, Stable Micro Systems, Surrey, UK) was fitted with a 5 kg load cell and a 2 mm diameter stainless steel cylinder probe. A probe punctured the samples to a depth of 50% of the extrudate diameter of each specimen, and the test speed was 1 mm/s. Ten measurements were performed on each treatment. A force-time curve was recorded and analyzed to calculate the peak force and the number of force peaks.

### 2.5. Proximate composition

The proximate compositions of the RBF, DCM, and extrudates were determined according to AOAC (2002) methods. The carbohydrate content was calculated by difference.

## 2.6. Antioxidant activity, total polyphenolic content, and anthocyanin content

The antioxidant activity (DPPH and FRAP), total phenolic content (TPC), and anthocyanin content (ATC) of RBF, DCM, and extrudates were extracted according to the methods of Shao et al. (2021) with some modifications. Two g of each sample was mixed with 20 mL of 70% methanol (v/v) and then centrifuged at 3,000 x *g* for 10 min. The supernatant was filtered by a 0.45  $\mu$ m nylon syringe filter (Minisart<sup>®</sup> SRP15, Sartorius AG, Germany), and the supernatant was transformed and stored at 4°C for further analysis. All extracts were used for antioxidant activity (DPPH and FRAP), TPC, and ATC.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical capacity was determined using the methods suggested by Xu et al. (2015) with some modifications and using a freshly prepared solution of 0.1 mM solution of DPPH in 70% methanol with the absorbance of approximately 1.0 AU at 517 nm. The absorbance was then measured at a wavelength of 517 nm using a double-beam spectrometer (Libra S70, Biochrom, UK). Methanol was used as a blank control. The antioxidant capacity was expressed as milligram of Trolox equivalent (TE) per 100 g dry weight (DW) of the sample (mg TE/100 g DW).

FRAP assay was determined using the method described by Benzie and Strain (1996) with a slight modification. The extract (200  $\mu$ L) was added to a 1,000  $\mu$ L FRAP reagent and incubated for 1 h in darkness before measuring absorbance at 593 nm using a double-beam spectrometer. Fresh ferrous sulfate solutions (FeSO<sub>4</sub>) were used as the calibration curve and expressed as milligram of ferrous sulfate equivalent (FeSO<sub>4</sub>) per 100 g dry weight (DW) of the sample (mg FeSO<sub>4</sub>/100 g DW).

The TPC was measured using the Folin–Ciocalteu colorimetric method reported by Gong et al. (2017) with some modifications. The sample extracts or standard solutions were alkalinized with  $Na_2CO_3$ , mixed well, and then allowed to stand for 2 min, followed by being added to 100  $\mu$ L of Folin–Ciocalteu reagent (Folin–Ciocalteu: Methanol, 1:1), vortexed, and then allowed to stand for 30 min at room temperature. The absorbance was then measured at

760 nm using a double-beam spectrometer. Gallic acid was used as the standard, and the TPC was expressed as milligram of gallic acid equivalent (GAE) per 100 g dry weight (DW) of the sample (mg GAE/100 g DW).

The ATC was determined using a spectrophotometric pH differential protocol described by Ti et al. (2015) with some modifications. Fifty  $\mu$ L of extracts were mixed thoroughly with 3 mL of 0.025 M potassium chloride (pH 1) buffer. The absorbance of the mixture was then measured at 520 and 700 nm against a distilled water blank. Similarly, the extracts were dissolved with sodium acetate buffer (pH 4.5), and the absorbance of these solutions was measured at the same wavelengths using a double-beam spectrometer. The ATC was calculated using the following equation and expressed as milligram of cyanidin-3-glucoside equivalent (Cy-3-G) per 100 g dry weight (DW) of the sample (mg Cy-3-G/100 g DW).

### 2.7. Sensory evaluation

The extrudate samples were coded with random three-digit numbers and served to consumers in a random order. Thirty panelists, who were regular snack consumers and aged between 20 and 40, assessed the samples using a 9-point hedonic scale (1-extremely dislike to 9-extremely like). The experiment was reviewed through the exemption review mode and approved for its adherence to the basic principles of international research ethics by the Human Research Ethics Committee of the Sakon Nakhon Rajabhat University (COA NO. 074/2565, IEC NO. 65-004). Informed consent was obtained from all participants for the experiments.

### 2.8. Statistical analysis

The physicochemical and functional properties of all treatments were analyzed using analysis of variance (ANOVA) at a significance level of 0.05. Duncan's multiple range test (DMRT) was used to compare the means of all treatments. A  $3 \times 3$  factorial design was used to investigate the effect of two factors and evaluate the interaction of the factors on all response variables.

Sensory properties were subjected to variance analysis for statistical significance using a randomized completely block design (RCBD).

## 3. Result and discussion

### 3.1. Pasting properties

Table 2 presents the pasting properties of the extruded flours as measured using the Rapid Visco Analyzer (RVA). The initial degree of starch modification can be determined by re-cooking the processed food product in the RVA. The results indicated that the feed moisture content had a significant effect on pasting properties, including the cold peak viscosity, hold, final viscosity, and setback. Furthermore, the replacement of DCM affected several pasting properties, including the cold viscosity, hot peak viscosity, hold, breakdown, final viscosity, and setback.

The measurement of cold peak viscosity (initial viscosity at 25°C) is important for the production of cereal breakfast, modified starch, and snack product. This property determines the ability of instant food to dissolve in cold water or absorb water at room temperature and transform into a paste, viscous substance, gel, or snack. The RBF (unprocessed flour, data not shown in the table) exhibited a very low initial or cold viscosity (10 cP), a high hot peak viscosity (3,077 cP) caused by the swelling of raw starch granules, and a higher setback viscosity (2,386 cP) than those of the extruded flours. The cold peak viscosity of all extrudate flour varied from 270.25 to 1,013.25 cP. The minimum cold viscosity value of 270.25 cP was observed for design treatment 7, which had 24% feed moisture content and 0% DCM. Factorial analysis indicated that the feed moisture content had a significant ( $p \le 0.01$ ) effect on the cold peak viscosity, with increased feed moisture leading to a decrease in the cold peak viscosity ( $p \le 0.05$ ).

The hot peak viscosity, defined as the maximum viscosity during the heating step (95°C), was affected by the loss of starch granule structure during extrusion, which decreased its capacity for swelling and led to a low viscosity at 95°C. No significant (p > 0.05) differences were found in the hot peak viscosity among the different feed moisture levels, indicating that the starch had already been fully gelatinized during the extrusion process at a low feed moisture content. The DCM content was the dominant factor affecting the hot peak viscosity, with an increase from 5 to 10% DCM resulting in a significant ( $p \le 0.05$ ) decrease from 839.75 to 700.25 cP. However, a statistically significant interaction was observed between the feed moisture content and DCM. This could be due to water absorption by fiber, which reduces the amount of available water molecules for starch granules, leading to the loss of starch to form a gel-like structure. Moreover, Table 2 shows that the viscosity of the cold peak was slightly different from that of the hot peak. It can be assumed that no hot peak viscosity was detected, indicating that these flours were completely gelatinized during extrusion (Huang et al., 2020). Conversely, a hot peak viscosity greater than the cold peak viscosity indicates the presence of intact starch granules or a partially maintained starchy structure.

When flour is combined with water and heated, the starch granules initially swell, leading to an increase in the viscosity of the paste. Hold viscosity is determined by continuously stirring and heating the paste during the heating cycle, causing the starch granules to break down, resulting in the lowest viscosity. The results of the study showed that the hold viscosities of all extrudates ranged from 55.00 to 194.00 cP. The factorial design revealed that extrudates containing 5% and 10% DCM had significantly lower hold viscosity than those containing 0% DCM. The breakdown is an index of starch stability to withstand heating and shear stress during cooking. The factorial results showed that the substitution of 5% DCM resulted in a higher breakdown compared to 0 and 10% DCM, while feed moisture content had no significant effect on breakdown.

The final viscosity of flour is an indicator of its ability to form a viscous paste after cooling. Setback is defined as the difference between the hold and final viscosity. Both feed moisture and DCM were significant parameters affecting final viscosities and setback values. Masatcioglu et al. (2014) reported that samples with higher

TABLE 2 Pasting pro	perties of extrudates	made from			
Treatment	Factors				
	Feed moisture (%)	DCM			
1	18	0			
2	18	5			
3	18	10			
4	21	0			
5	21	5			
6	21	10			
7	24	0			
8	24	5			

#### TA m RBF as affected by feed moisture and DCM content.

Treatment	Facto	ors	Pasting properties							
	Feed moisture (%)	DCM (%)	Cold peak viscosity, 25°C (cP)	Hot peak viscosity, 95°C (cP)	Hold (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)		
1	18	0	$980.75 \pm 79.25  a$	$994.00\pm53.31a$	$122.50\pm34.49b$	$871.50 \pm 51.16 \text{ ab}$	$290.50\pm47.64bcd$	$168.00\pm13.54~cd$		
2	18	5	779.75 $\pm$ 38.99 b	$787.50\pm55.66b$	$65.50\pm31.89~bc$	$722.00\pm36.24bc$	$267.25 \pm 41.80 \text{ bcd}$	$201.75 \pm 29.38 \text{ bc}$		
3	18	10	$545.75\pm 87.75c$	$583.50 \pm 156.27  cd$	$68.50\pm20.22~bc$	$515.00 \pm 146.07 \ d$	$220.75 \pm 86.91 \text{ d}$	$152.25\pm67~d$		
4	21	0	$648.50. \pm 108.38 \ { m bc}$	$800.50\pm89.66~b$	$116.50 \pm 29.13 \text{ b}$	$684.00 \pm 60.71  cd$	$348.00 \pm 38.23 \text{ b}$	$231.50 \pm 18.55 \ b$		
5	21	5	$710.50 \pm 172.62 \text{ bc}$	$702.00\pm199.76c$	$55.00\pm30.02c$	$647.00\pm229.78cd$	$221.50\pm5.19~d$	$166.50 \pm 35.21 \text{ d}$		
6	21	10	$795.25 \pm 38.87 \text{ b}$	$801.50 \pm 73.43 \text{ b}$	$90.50\pm47.27~bc$	$711.00 \pm 31.39 \text{ bc}$	$260.00\pm62.71cd$	$169.50\pm17.33~\text{cd}$		
7	24	0	$270.25 \pm 29.63 \text{ d}$	$473.75 \pm 32.25 \text{ d}$	$194.00\pm18.86\mathrm{a}$	$279.75 \pm 29.90 \text{ e}$	$548.75 \pm 31.64  a$	$354.75 \pm 15.86$ a		
8	24	5	$1{,}013.25\pm73.71a$	1,029.75 $\pm$ 76.57 a	$118.50\pm70.49b$	$911.25 \pm 34.82  a$	$308.50 \pm 72.45 \text{ bc}$	$190.00\pm10.16~cd$		
9	24	10	$664.50 \pm 174.28 \text{ bc}$	$715.75 \pm 188.03 \text{ bc}$	$80.25\pm25.14~bc$	$635.50 \pm 124.42  cd$	$251.50 \pm 24.51 \text{ d}$	$171.25\pm3.20~cd$		
ANOVA test (p-va	alue)									
Feed moisture			**	ns	**	ns	***	***		
DCM			***	**	***	*	***	***		
Feed moisture x DCM			***	***	ns	***	***	***		
DUNCAN multipl	e range test									
Feed moisture				ns		ns				
18			$768.75\pm 201.29a$	$788.33 \pm 197.28$	$85.50\pm38.23~\mathrm{b}$	$702.83 \pm 173.78$	$259.90\pm63.82~b$	$174.00 \pm 44.51 \text{ b}$		
21			$718.08 \pm 125.26 \text{ ab}$	$768.00 \pm 130.08$	$87.33\pm42.19~\text{b}$	$680.66 \pm 128.16$	$276.50 \pm 67.35 \text{ b}$	$189.16\pm38.64~b$		
24			649.33 ± 332.56 b	$739.75 \pm 260.85$	$130.91 \pm 63.73  a$	$608.83 \pm 285.17$	369.58 ± 141.29 a	$238.66 \pm 86.68$ a		
DCM										
0			633.16 ± 311.57 b	$756.08 \pm 231.38 \text{ ab}$	$144.33\pm44.77\mathrm{a}$	$611.75 \pm 261.68 \text{ b}$	$395.75 \pm 121.07$ a	$251.41 \pm 78.86$ a		
5			$834.50 \pm 168.29  \text{a}$	$839.75 \pm 185.33  a$	$79.66\pm52.16~\mathrm{b}$	$760.08 \pm 169.04  a$	$265.75\pm5.35\mathrm{b}$	$186.08 \pm 28.91 \text{ b}$		
10			$668.50 \pm 155.05 \text{ b}$	$700.25 \pm 162.92 \ b$	$79.75\pm31.33~b$	$620.50 \pm 145.08 \text{ b}$	$244.08\pm60.05~b$	$164.33 \pm 37.35 \text{ b}$		

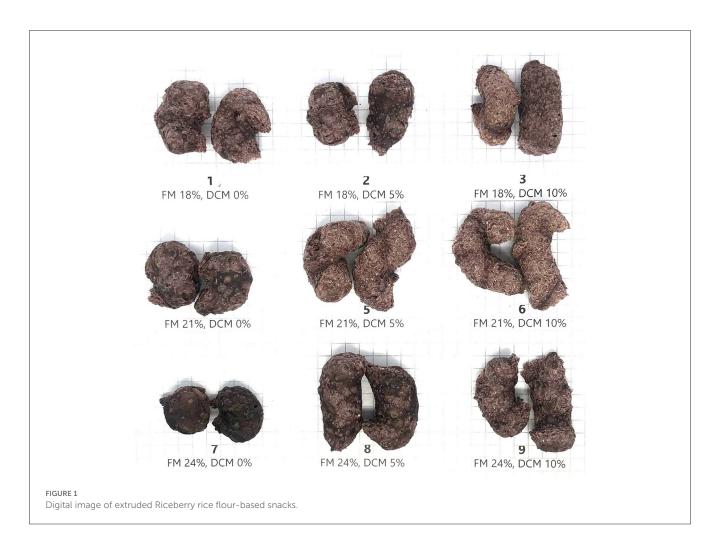
ns, not significant, \*p ≤ 0.05, \*\*p ≤ 0.01, \*\*\*p ≤ 0.001. Different letters after the mean value ± standard deviation in the same column indicate significant differences (p ≤ 0.05) using a DMRT test, where a was the highest value. RBF, Riceberry rice flour; DCM, dried

coconut meal.

TABLE 3 Physical properties of extrudates made from RBF as affected by feed moisture and DCM content.

Treatment	t Factors		Expansion index	Bulk density (g/cm <sup>3</sup> )	WAI	L*	a*	b*	Hardness (Max. force, kg)	Crispness (No. of peaks)
	Feed moisture (%)	DCM (%)								
1	18	0	$2.96\pm0.20a$	$0.38\pm0.06~\text{d}$	$19.52\pm0.33~bcd$	$35.26\pm1.31a$	$8.31\pm0.33~ab$	$4.45\pm0.34bc$	$2.29\pm0.37~d$	$75.00\pm9.51~a$
2	18	5	$2.69\pm0.14c$	$0.41\pm0.05cd$	$19.36\pm0.29bcde$	$30.21\pm2.86~b$	$8.21\pm0.39~\text{abc}$	$4.27\pm0.20~bcd$	$3.43\pm0.69bc$	$39.80 \pm 11.33 \text{ d}$
3	18	10	$2.46\pm0.25~d$	$0.47\pm0.08~bcd$	$17.51\pm0.58\mathrm{f}$	$37.62\pm3.79a$	$7.96\pm0.27bc$	$5.80\pm0.74a$	$3.92\pm0.68~ab$	$48.15\pm8.25~\mathrm{c}$
4	21	0	$2.67\pm0.32c$	$0.46\pm0.08~bcd$	$20.58\pm0.57~b$	$26.40\pm1.93cd$	$8.46\pm0.21a$	$3.77\pm0.25cd$	$4.26\pm1.27\mathrm{a}$	$48.60\pm5.83~\mathrm{c}$
5	21	5	$2.67\pm0.18c$	$0.52\pm0.08~ab$	$18.13\pm0.93~\text{ef}$	$35.15\pm2.18\mathrm{a}$	$7.77\pm0.15c$	$4.82\pm0.92\mathrm{b}$	$2.91\pm0.65c$	$62.25 \pm 11.71 \text{ b}$
6	21	10	$2.91\pm0.22c$	$0.49\pm0.07~bc$	$18.49\pm0.30~\text{cdef}$	$34.37\pm2.12\mathrm{a}$	$8.00\pm0.32bc$	$4.68\pm0.42~b$	$3.73\pm0.54~ab$	$53.25 \pm 6.49 \text{ c}$
7	24	0	$2.80\pm0.18~ab$	$0.60\pm0.01\mathrm{a}$	$19.79\pm0.49bc$	$23.75\pm0.68~\text{d}$	$7.87\pm0.20bc$	$3.61\pm0.17~d$	$3.74\pm1.15~ab$	$17.55\pm5.78~\mathrm{f}$
8	24	5	$2.85\pm0.17bc$	$0.48\pm0.09bc$	$21.87\pm2.10a$	$27.71\pm3.13~\mathrm{bc}$	$7.95\pm0.42bc$	$4.38\pm0.64bcd$	$3.36\pm0.71bc$	$28.20 \pm 12.83$ e
9	24	10	$2.74\pm0.25~ab$	$0.52\pm0.07~ab$	$18.18\pm0.38~\text{def}$	$30.15\pm0.85~\text{b}$	$8.07\pm0.15~abc$	$4.28\pm0.14bcd$	$4.25\pm0.86a$	$41.65 \pm 6.41 \text{ d}$
ANOVA test (	o-value)									
Feed moisture			***	***	**	***	ns	**	**	*
DCM			***	ns	***	***	ns	***	***	***
Feed moisture x 0	Coconut		***	*	***	***	ns	*	***	***
DUNCAN mu	ltiple range test									
Feed moisture							ns			
18			$2.70\pm0.29\mathrm{b}$	$0.42\pm0.07b$	$18.79\pm1.02\mathrm{b}$	$34.63\pm4.13\mathrm{a}$	$8.16\pm0.34$	$4.84\pm0.84a$	$3.21\pm0.94b$	54.31 ± 17.93 a
21			$2.67\pm0.22\mathrm{b}$	$0.49\pm0.07\mathrm{a}$	$19.07\pm1.27~\mathrm{b}$	$31.97\pm4.54\mathrm{b}$	$8.07\pm0.37$	$4.42\pm0.73$ ab	$3.63\pm1.03a$	$54.70 \pm 10.07$ a
24			$2.86\pm0.19a$	$0.53\pm0.09a$	$19.95\pm1.95a$	$27.20\pm3.25\mathrm{c}$	$7.96\pm0.26$	$4.09\pm0.05~b$	$3.78\pm0.98a$	29.13 ± 13.26 b
DCM				ns			ns			
0			$2.85\pm0.28a$	$0.48\pm0.12$	$19.96\pm0.64a$	$28.46\pm5.29\mathrm{c}$	$8.21\pm0.35$	$3.94\pm0.45c$	$3.43\pm1.30~\text{b}$	$47.05 \pm 24.72$ a
5			$2.72\pm0.17b$	$0.47\pm0.09$	$19.78\pm2.02a$	$31.02\pm4.07~\mathrm{b}$	$7.98 \pm 0.36$	$4.49\pm0.64b$	$3.23\pm0.71\text{b}$	$43.42 \pm 18.48 \text{ b}$
10			$2.66\pm0.24b$	$0.49\pm0.07$	$18.06\pm0.58b$	$34.05\pm3.94a$	$8.20\pm0.39$	$4.92\pm0.81a$	$3.97\pm0.72a$	$47.68\pm8.46~\mathrm{a}$

ns, not significant, \* $p \le 0.05$ , \*\* $p \le 0.01$ , \*\*\* $p \le 0.001$ . Different letters after the mean value  $\pm$  standard deviation in the same column indicate significant differences ( $p \le 0.05$ ) using a DMRT test, where a was the highest value. RBF, Riceberry rice flour; DCM, dried coconut meal.



levels of feed moisture content exhibited higher setback values. The substitution of DCM resulted in a decrease in both final viscosity and setback, indicating that DCM may limit the ability to form a paste after cooling. In a previous study, Muñoz-Pabon et al. (2022) reported that a high fiber content from adding quinoa flour results in a lower retrogradation value compared to the cereal extrudate without quinoa. Moreover, Khan et al. (2015) reported that increasing the amount of virgin coconut meal resulted in a significant decrease in breakdown, final viscosity, and setback. This may be due to the formation of a complex during extrusion that combines lipids, proteins, fiber, starch, and high molecular weight compounds. However, the observed changes in viscosity may vary depending on the type of material used and extrusion parameters.

## 3.2. Physical properties of extrudate product

### 3.2.1. Expansion ratio

Based on the ANOVA results of the full factorial experiment, Table 3 presents the results, including the expansion ratio, bulk density, WAI, color measurement (L\*, a\*, and b\* values), and textural properties (hardness and crispiness). In the industrial production of extruded products, expansion is a critical factor as product containers are usually filled by weight rather than volume. Expanded products are expected to have a light, puffed structure (Nascimento et al., 2017).

The results indicated that both feed moisture and DCM content significantly ( $p \le 0.001$ ) affected the expansion of the extruded products. The extrudates produced with a feed moisture content of 24% showed a higher expansion than those with a feed moisture content of 18 and 21%. Additionally, the extrudates containing 5 and 10% DCM exhibited lower radial expansion ratios than the control (0%). It was also observed that the interaction between feed moisture and DCM content was statistically significant ( $p \le 0.001$ ). Figure 1 illustrates examples of extrudates made from RBF under different extrusion process conditions.

As reported by Raghavendra et al. (2006), defatted coconut residue had 63.24% dietary fiber, including 4.53% soluble fiber and 58.71% insoluble fiber. The presence of fiber particles can reduce the elastic properties of starch, leading to lower radial expansion. Similar effects have been observed in other fiber sources such as tomato skin (Dehghan-Shoar et al., 2010) and wheat bran (Hernández-Díaz et al., 2007). However, some studies have found that product expansion increased with the addition of fibers such as barley and mushrooms (Brennan et al., 2012, 2013). On the other hand, other researchers have reported that the expansion ratio of products was not significantly affected by the inclusion of dietary fibers in the flour-based extruded snack

#### TABLE 4 Proximate composition and antioxidant activity of extrudates made from RBF as affected by feed moisture and DCM content.

Treatment	Facto	Factors Proximate composition						Antioxidant activity		TPC (mg GAE/100 g DW)	ATC (mg Cy- 3-G/100 g DW)
	Feed moisture (%)	DCM (%)	Moisture (%)	Ash (%)	Lipids (%)	Protein (%)	Carbohydrate (%)	DPPH (mg TE/100 g DW)	FRAP (mg FeSO <sub>4</sub> /100 g DW)		
1	18	0	$8.03\pm0.68~bc$	$0.90\pm0.31~\text{ab}$	$3.30\pm0.26~\text{de}$	$7.17\pm0.11~\text{b}$	$80.59\pm0.89a$	$112.75\pm2.51~\text{ab}$	$30.21\pm0.96~bc$	$4{,}913.98\pm206.37a$	$15.45\pm1.66~\mathrm{ab}$
2	18	5	$9.23\pm0.71~b$	$0.83\pm0.19~ab$	$4.20\pm0.94cd$	$7.11\pm0.23~b$	$78.63\pm1.86~ab$	$105.89\pm5.06~ab$	$27.23\pm1.71~\text{d}$	$4{,}736.36\pm91.21a$	$15.37\pm0.42~ab$
3	18	10	$6.45\pm0.38a$	$1.16\pm0.20~ab$	$5.88\pm1.24a$	$7.36\pm0.36~b$	$79.15\pm0.82a$	$108.59\pm3.35~ab$	$30.62\pm0.22~abc$	4,281.25 ± 89.53 b	$10.65\pm0.78~\mathrm{c}$
4	21	0	$9.89\pm0.15~ab$	$0.91\pm0.73~ab$	$2.94\pm0.61~\text{e}$	$7.10\pm0.24b$	$79.16\pm1.33a$	$108.26\pm12.31~\text{ab}$	$27.62\pm1.11~\text{d}$	$3,808.58\pm76.29\mathrm{c}$	$16.23\pm0.63~a$
5	21	5	$8.05\pm1.86~bc$	$0.74\pm0.32~b$	$4.62\pm0.62~bc$	$7.21\pm0.13~b$	$79.38\pm2.15a$	$119.30 \pm 11.07  a$	$32.41\pm1.74a$	2,678.24 ± 357.31 d	$13.45\pm2.26~\mathrm{b}$
6	21	10	$9.92\pm4.27~ab$	$1.25\pm0.16~\text{ab}$	$5.60\pm0.37~ab$	$7.15\pm0.13~b$	$76.09\pm4.12~bc$	$107.07\pm9.68~ab$	$29.88\pm2.06~bc$	$2,723.70 \pm 251.62 \text{ d}$	$16.30\pm0.50~a$
7	24	0	$12.31\pm0.25a$	$1.32\pm0.27a$	$2.82\pm0.33~\text{e}$	$7.93\pm0.57a$	$75.62\pm0.63c$	$99.26\pm2.41~b$	$29.02\pm0.41cd$	3,982.25 ± 76.70 bc	$16.39 \pm 2.81$ a
8	24	5	$10.42\pm0.72~ab$	$1.24\pm0.16~\text{ab}$	$5.14\pm0.37~\mathrm{abc}$	$7.07\pm0.21~b$	$76.13\pm0.22~bc$	$108.52\pm14.25~\text{ab}$	$31.16\pm1.56~\text{ab}$	$2,280.30 \pm 347.94$ e	$16.22 \pm 1.61$ a
9	24	10	$8.08\pm0.67~bc$	$1.23\pm0.16~ab$	$4.24\pm1.22cd$	$7.14\pm0.06~\mathrm{b}$	$79.31\pm1.75a$	$109.81\pm3.35~ab$	$30.12\pm0.88~bc$	$1{,}613.16\pm337.07\mathrm{f}$	$14.40\pm2.37~\mathrm{ab}$
ANOVA test (	(p-value)										
Feed moisture			**	ns	ns	ns	*	ns	ns	***	*
DCM	DCM		**	ns	***	ns	ns	ns	*	***	*
Feed moisture x	DCM		*	ns	*	ns	**	ns	**	***	**
DUNCAN mu	ultiple range te	st									
Feed moisture			ns	ns	ns		ns	ns			
18			$7.90\pm1.31~b$	$0.96\pm0.26$	$4.46 \pm 11.39$	$7.21\pm0.26$	$79.45\pm1.44a$	$109.07\pm4.66$	$29.35 \pm 1.88$	4,643.86 ± 305.83 a	$13.83\pm2.53~\text{b}$
21			$9.29\pm2.59a$	$0.97\pm0.48$	$4.38 \pm 1.25$	$7.15\pm0.16$	$78.20\pm2.98~ab$	$111.55\pm11.55$	$29.97\pm2.55$	3,070.17 ± 592.53 b	$15.33\pm1.86$ a
24			$10.27\pm1.88a$	$1.26\pm0.18$	$4.07\pm1.21$	$7.38\pm0.52$	$77.02\pm1.97~\mathrm{b}$	$105.87\pm9.17$	$30.10\pm1.24$	2,625.24 ± 1,072.83 c	$15.67 \pm 2.30$ a
DCM				ns		ns	ns	ns			
0		$10.08\pm1.87a$	$1.04\pm0.48$	$3.02\pm0.44c$	$\begin{array}{c} 7.21 \pm 0.23 \\ ab \end{array}$	$78.45\pm2.36$	$106.76\pm8.88$	$28.95\pm1.36~\mathrm{b}$	4,234.93 ± 521.34 a	$16.03 \pm 1.78$ a	
5			$9.23\pm1.49$ ab	$0.94\pm0.31$	$4.65\pm0.74~b$	$7.13\pm0.19\mathrm{b}$	$78.05\pm2.08$	$111.24 \pm 11.51$	$30.26\pm2.76a$	3,231.63 ± 1,154.94 b	$15.02\pm1.90~\text{ab}$
10			$8.14\pm2.70~b$	$1.21\pm0.16$	$5.23\pm1.19a$	$7.40\pm0.51a$	$78.18\pm2.85$	$108.49\pm5.86$	$30.21\pm1.13a$	2,872.70 ± 1,164.84 c	$13.78\pm2.78~\mathrm{b}$

ns, not significant, \* $p \le 0.05$ , \*\* $p \le 0.01$ , \*\*\* $p \le 0.001$ . Different letters after the mean value  $\pm$  standard deviation in the same column indicate significant differences ( $p \le 0.05$ ) using a DMRT test, where a was the highest value. RBF, Riceberry rice flour; DCM, dried

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coconut meal.

Treatment	Facto	rs	Liking score							
	Feed moisture (%)	DCM (%)	Appearance	Color	Taste	Flavor	Texture	Overall liking		
1	18	0	$6.30\pm1.49a$	$6.40\pm1.72~\mathrm{ab}$	$6.30\pm1.65~\mathrm{b}$	$6.30\pm1.86~\text{b}$	$6.80\pm1.70~\mathrm{ab}$	$6.80\pm1.70~ab$		
2	18	5	$5.00 \pm 2.07$ bc	$5.90 \pm 1.37 \text{ ab}$	$6.30\pm1.34~\text{b}$	$5.90\pm1.77~\mathrm{bc}$	$5.90 \pm 1.86$ bc	$5.70\pm1.71~\mathrm{cde}$		
3	18	10	$5.80\pm1.90~\text{ab}$	$5.10\pm1.91c$	$7.60\pm1.27~\mathrm{a}$	$7.30\pm1.83a$	$7.75\pm1.77$ a	$7.60\pm1.60~\mathrm{a}$		
4	21	0	$5.65\pm1.42~\text{ab}$	$6.30\pm1.94~\text{ab}$	$6.10\pm1.41~\mathrm{b}$	$4.90 \pm 1.41$	$4.75\pm1.71~\text{d}$	$4.90\pm1.44~\mathrm{e}$		
5	21	5	$6.20\pm1.10a$	$5.90\pm1.29~\text{ab}$	$5.85\pm1.63~\text{b}$	$5.80\pm1.54bc$	$6.90\pm1.94~\text{ab}$	$6.10\pm1.97~\mathrm{bcd}$		
6	21	10	$6.40\pm1.56a$	$6.00\pm1.48~\text{ab}$	$5.90\pm1.37~\mathrm{b}$	$4.80\pm1.70\mathrm{c}$	$5.70\pm1.71\mathrm{cd}$	$5.30 \pm 1.55$ de		
7	24	0	$4.60\pm1.75\mathrm{c}$	$5.45\pm1.95\mathrm{b}$	$4.80\pm1.67\mathrm{c}$	$3.70\pm1.68~\mathrm{e}$	$3.20\pm1.57~\mathrm{e}$	$3.80\pm1.79~\mathrm{f}$		
8	24	5	$6.15\pm1.59a$	$6.70\pm1.17\mathrm{a}$	$6.35\pm1.31~\text{b}$	$5.70\pm1.62~{ m bc}$	$5.40\pm1.60\mathrm{cd}$	$6.35\pm1.49bc$		
9	24	10	$6.10\pm1.71\mathrm{a}$	$6.65\pm1.34a$	$6.25\pm1.33~\text{b}$	$5.45\pm1.73~{ m bc}$	6.35 ± 1.63 bc	$6.35\pm1.56~{ m bc}$		

TABLE 5 Sensory liking scores of extrudates made from RBF as affected by feed moisture and DCM content.

Different letters after the mean value  $\pm$  standard deviation in the same column indicate significant differences ( $p \le 0.05$ ) using a DMRT test, where a was the highest value. RBF, Riceberry rice flour; DCM, dried coconut meal.

(Brennan et al., 2008). Therefore, the effect of fiber content on expansion may also depend on other process conditions such as barrel temperature, screw speed, and residence time, which are considered the governing factors.

#### 3.2.2. Bulk density

The texture of the final product of extruded starch-based snacks is directly related to its density. The bulk density of all extrudates, which ranged from 0.38 to 0.60 g/cm<sup>3</sup>, was significantly influenced by feed moisture content ( $p \le 0.001$ ) but showed no significant effect from DCM. Increasing feed moisture content tends to increase bulk density. Furthermore, the interaction between feed moisture content and DCM exhibited a significant effect ( $p \le 0.05$ ). In starch-based extruded snacks, water acts as a plasticizer, which reduces the viscosity of the dough and gelatinization in the extruder, thereby increasing product density (Pardhi et al., 2019). This result is in agreement with those of Khan et al. (2015), who observed that an increase in the amount of virgin coconut meal in snack products led to a decrease in the expansion ratio while bulk density increased.

### 3.2.3. Water absorption index

The results of this study demonstrated that the WAI of extruded flour was slightly increased with increased feed moisture content. Similar findings have been reported by Sahu et al. (2022) for maizebased extruded snacks. Additionally, the results also show that the addition of DCM decreased the WAI. This finding is in agreement with those of Wang et al. (2017), who explained that adding cherry pomace significantly decreased the WAI of snacks due to competition for water absorption between the pomace and starch. Earlier, Dumchaiyapoom et al. (2019) found that WAI decreased with increased coconut meal in mixed rice and glutinous rice-based extrudates. Furthermore, it can be observed that the interaction had a significant ( $p \le 0.001$ ) effect on the WAI.

#### 3.2.4. Color

Increasing the feed moisture content resulted in a significant decrease in L<sup>\*</sup> and b<sup>\*</sup> values, as shown in Table 4. The color of the final product can be influenced by various reactions that occur during the extrusion process, such as pigment degradation, the Maillard reaction, caramelization, and hydrolysis. The substitution of DCM increased both the L<sup>\*</sup> and b<sup>\*</sup> values. This could be explained by the fact that the anthocyanin in RBF is responsible for the purple color of the extrudates. Furthermore, the a<sup>\*</sup> value indicated that the substitution of DCM did not significantly (p > 0.05) affect the red color.

### 3.2.5. Texture analysis

The hardness and crispness were measured by puncture force, which represents cell wall rupture resistance and structural development during extrusion. The force and time plot for each treatment during the fracturing exhibits as many peaks as the cell wall ruptures, which is correlated with the crispiness (Saeleaw et al., 2012). The values of maximum force (hardness) and the number of force peaks (crispiness) were within the range of 2.29 to 4.26 kg and 17.55 to 75.00 peaks, respectively.

The hardness and crispiness of extrudates were affected by both feed moisture and DCM content. An increase in feed moisture content results in more rigid structures with higher density and hardness but lower crispiness. The extrudates had the hardest texture when RBF was substituted with DCM at 10%. The previous study by Khan et al. (2015) also showed that increasing the amount of virgin coconut meal increased the amount of protein and fiber, resulting in an increase in hardness. A significant interaction ( $p \le 0.001$ ) occurred between feed moisture and DCM on the texture parameters. At moisture contents of 24%, crispiness increased with increasing amounts of DCM.

### 3.3. Proximate composition

Table 4 presents the chemical composition of the products. Moisture content ranged from 8.03 to 12.31%, ash content ranged from 0.74 to 1.32%, lipid content ranged from 2.82 to 5.88%, protein content ranged from 7.07 to 7.93%, and carbohydrate content ranged from 75.62 to 80.59%. Interestingly, the feed moisture content had no impact on the ash, lipid, or protein content. However, the substitution of DCM for RBF resulted in a slightly higher fat content.

# 3.4. Antioxidant activity, total polyphenolic content, and anthocyanin content

The results presented in Table 4 indicate that extrusion significantly decreased the antioxidant activity, TPC, and TAC of all extrudates compared to the unextruded RBF samples (as shown in Table 1). The DPPH and FRAP assays were used to measure antioxidant activity, and their values ranged from 99.26 to 119.30 mg TE/100 g DW and 27.23 to 32.41 mg FeSO<sub>4</sub>/100 g DW, respectively. The antioxidant levels decreased by nearly 64.41 to 70.39% using the DPPH assay and by 10.32 to 24.65% using the FRAP when compared to the initial RBF flour, but the levels were still high in the final product. Feed moisture changes had no impact on antioxidant activity (p > 0.05). By DPPH assay, there was no significant interaction (p > 0.05) between feed moisture and DCM content on the antioxidant activity of extrudate products. However, the FRAP assay revealed that the amount of DCM had a small effect on the antioxidant activity, with FRAP values of 28.95, 30.26, and 30.21 mg FeSO<sub>4</sub>/100 g DW at DCM levels of 0, 5, and 10%, respectively, as shown in Table 3. These findings are similar to those of previous studies on colored ricebased extrudates by Sompong et al. (2011), which showed that the different feed moisture levels (12 and 16%) had no statistically significant effect on the FRAP and DPPH values. Due to the complexity of the extrusion process, which involves numerous variables that can influence the antioxidant properties of extruded snacks, it is possible that other factors such as temperature and residence time may play a significant role in the final antioxidant properties of the snacks.

The TPC in the extrudates ranged from 1,613.16 to 4,913.98 mg GAE/100 g DW, which reduced in the range of 36.87 to 79.28% compared to the initial RBF (7,783.96 mg GAE/100 g DW). The factorial design was evaluated, and both the feed moisture content and DCM variables had a significant ( $p \le 0.001$ ) effect on the TPC. As the feed moisture and DCM increased, the TPC of extrudates decreased. This observation is in agreement with the previous study by Sompong et al. (2011), which reported that the TPC of red rice extrudates decreased as the moisture content increased from 12 to 16%.

The ATC of extrudates varied from 10.65 to 16.39 mg Cy-3-G/100 g DW. The losses in ATC caused by extrusion were 50.63 to 67.92% when compared with raw RBF (33.20 mg Cy-3-G/100 g DW). The higher feed moisture content may have prevented anthocyanin loss. Khanal et al. (2009) found that the extrusion

process reduced anthocyanin by 33 to 42% in blueberry pomacesorghum flour-based extruded snacks, whereas increasing feed moisture reduced loss. In addition, the ATC decreased when DCM was substituted.

### 3.5. Sensory evaluation

The results of the hedonic scores showed significant differences  $(p \leq 0.05)$  among the sensory characteristics, as presented in Table 5. The appearance and color attributes of all extrudates received low scores because some panelists did not enjoy the dark purple hue of the product, possibly due to the prevalence of yellow snacks in the market. The extrudates with 18% feed moisture content and 10% DCM had the highest score for overall liking (7.60), indicating that the panelists moderately to very much preferred this sample. The authors suggest that incorporating flavoring powder as a coating for snacks could potentially enhance their sensory acceptance scores in terms of taste and flavor. Conversely, the samples with 24% feed moisture content and 0% DCM had the lowest scores. This could be explained by the fact that the density, hardness, and crispiness may have related to the sensory score.

## 4. Conclusion

The results of this study revealed that increasing feed moisture content significantly decreased the cold peak but had no significant effect on the hot peak and breakdown. DCM was the dominant factor affecting the hot peak. In addition, extrudates containing DCM had significantly lower hold, final viscosity, and setback than those without DCM. The substitution of DCM for RBF also led to an increase in L\* and b\* values. Increasing feed moisture content resulted in harder structures with decreased crispiness. Feed moisture content had no effect on antioxidant activity. TPC decreased as feed moisture and DCM levels increased. The product with 18% feed moisture content and 10% DCM had the highest overall liking score and contained DPPH, FRAP, TPC, and ATC at 108.59 mg TE/100 g DW, 30.62 mg FeSO<sub>4</sub>/100 g DW, 4,281.25 mg GAE/100 g DW, and 10.65 mg Cy-3-G/100 g DW, respectively. Overall, this study successfully developed a new product using RBF and DCM as raw materials with the potential for commercialization and the ability to reduce waste from the coconut milk industry. However, this study is limited as it only investigated the effects of two variables, namely feed moisture and DCM content. Therefore, future research could use response surface methodology (RSM) to optimize and examine the combined effects of DCM with other extrusion parameters, such as barrel temperature, feed rate, and screw speed. A comprehensive understanding of the reactions that occur during extrusion is essential for achieving the desired snack quality and advancing the development of novel snack products.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## **Ethics statement**

The studies involving human participants were reviewed and approved by Human Research Ethics Committee of the Sakon Nakhon Rajbhat University (COA NO. 074/2565, IEC NO. 65-004). The patients/participants provided their written informed consent to participate in this study.

## Author contributions

All authors listed have significantly contributed to the development and writing of this article and approved the submitted version.

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## **Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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