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Physicochemical and Antioxidant Properties of Cookies from Coix Seed Incorporated with *Clinacanthus nutans*

Izzah Hayati Yahya[1], Hazrulrizawati Abdul Hamid[1]*, Wan Fatimah Wan Mohd Nowalid[1,3], Ade Chandra Iwansyah[2], Chan Sin Ying[1] and Jianbo Xiao[4]

[1]Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang Al-Sultan Abdullah, Lbh Persiaran Tun Khalil Yaakob, Gambang, 26300 Kuantan, Malaysia
[2]Research Center for Food Technology and Processing, National Research and Innovation Agency, Indonesia Jl. Jogja - Wonosari km 31,5 Kab. Gunung kidul, Yogyakarta 55861, Indonesia
[3]Jabatan Pendidikan Politeknik dan Kolej Komuniti JPPKK, Aras 4-7, Galeria PJH, Jalan P4W, Persiaran Perdana, 62100 Putrajaya
[4]Nutrition and Bromatology Group, Department of Analytical and Food Chemistry, Faculty of Science, University of Vigo, 32004 Ourense, Spain
*Corresponding author (e-mail: hazrulrizawati@umpsa.edu.my)

Coix seed and *Clinacanthus nutans* are recognized for their significant nutritional and medicinal benefits. The objective of this study was to develop and analyze cookies made from Coix seed incorporated with *C. nutans*, assessing their physicochemical properties and antioxidant properties. Six different formulations (T0-T5) were created with varying proportions of Coix seed flour and *C. nutans* powder. The analysis included the evaluation of texture-forming properties, functional characteristics, color measurements, and antioxidant activities. The results showed that the addition of Coix seed flour and *C. nutans* powder significantly affected the physical characteristics of the cookies, such as the spread ratio, bulk density, and water absorption. The T2 cookies comprising 75 g Coix seed flour and 3 g *C. nutans* powder demonstrated the most favorable properties, including a well-balanced texture, high swelling capacity, and optimal antioxidant activity with TPC increased from 0.69 to 1.28 mg GAE/g; IC_{50} decreased from 3291.44 to 1761.72 µg/mL, respectively. Replacing wheat flour with Coix seed flour improved the nutritional value of the cookies, while *C. nutans* further enhanced their functional attributes. These findings highlight the potential of Coix seed and *C. nutans* cookies as antioxidant-enriched, functional snacks that can contribute to healthier dietary choices and the development of functional foods.

Keywords: Physicochemical, antioxidant, Coix seed, *Clinacanthus nutans*, cookies

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Coix seed, derived from the perennial herbaceous plant *Coix lachryma-jobi* L., exists in different varieties, primarily classified into wild and domesticated types [1, 2]. *Coix lachryma-jobi var. ma-yuen* is the most cultivated for food applications, while *Coix lachryma-jobi var. lachryma-jobi* remains largely underutilized despite its potential nutritional and functional benefits. Coix seed is a nutrient-rich grain that contains about 65% carbohydrates, 14% protein, 5% fat, 3% crude fibre, and a mineral composition of 0.24% phosphorus, 0.07% calcium, 0.001% iron [3]. The bioactive compounds present in Coix seed, such as polyphenols, coixenolide, and polysaccharides, contribute to its health-promoting properties. Additionally, studies have shown that it may possess prebiotic properties, enhancing the synthesis of bioactive metabolites such as vitamins, phenyl lactate, and biotin [4]. Moreover, Coix seed has been found to possess anti-inflammatory, antioxidant, and anti-tumor properties, making it a promising option for the development of functional foods [5]. Additionally, it has shown promise in improving intestinal microbiota and metabolic functions, positioning it as a promising ingredient for the development of functional foods.

In parallel, *Clinacanthus nutans is* another valuable plant widely used in Southeast Asian traditional medicine and shares many of the functional and therapeutic traits of Coix seed. It has long been used to treat a wide range of illnesses, such as skin conditions, viral infections, inflammation, insect and snake bites, and even diabetes mellitus. [6, 7].*C. nutans* has a high nutritional content of fat (0.5-2%), protein (5-20%), ash (5-10%), crude fiber (20-40%), and carbs (60-80%) [8]. Additionally, the plant has a wealth of bioactive substances that support its medicinal benefits, including flavonoids, glycosides, cerebrosides, and glycoglycerolipids [9]. Like Coix seed, *C. nutans* exhibit potent antioxidant and anti-inflammatory effects, suggesting complementary functionality when combined. The synergistic potential of these two natural ingredients offers a novel opportunity to design functional bakery products that integrate traditional medicinal plants into modern food applications.

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Growing health and well-being awareness has led to a rise in demand for food products improved with functional components that offer both nutritional and therapeutic benefits [10]. This trend aligns with the global vision of SDG 2, which aims to achieve food security and improved nutrition by developing functional foods with added health benefits. Instead of relying solely on staple grains, incorporating underutilized nutrient-rich sources such as Coix seed and *Clinacanthus nutans* can diversify food systems and enhance dietary antioxidant intake. Hence, scientific exploration of these ingredients in bakery products provides an evidence-based pathway to create health-promoting, functional foods suitable for modern consumers.

The functional properties of Coix seed extend beyond basic nutrition, as it has been recognized for its medicinal potential in traditional and modern pharmacology [11]. It has shown promise in improving intestinal microbiota, reducing inflammation, and improving liver and spleen function. Despite its advantages, Coix seed is still underutilized, and little is known about its pharmacological and chemical composition [12]. The nutritional value of Coix seed is similar to that of staple grains like wheat and rice, making it a promising substitute for creating functional foods [13]. Similarly, *C. nutans*, despite its well-documented medicinal properties, remains underdeveloped in food applications. Traditional knowledge supports its use in treating various illnesses; however, its potential for disease prevention and health promotion, particularly in cancer treatment, has not been fully explored. Given its high levels of phytochemical constituents and antioxidant activity, incorporating C. nutans into functional food products presents a promising avenue for further exploration [14, 15]. The increasing demand for healthier food options among consumers necessitates the discovery and utilization of alternative food sources that provide enhanced nutritional and functional benefits. No previous study has examined the combined incorporation of Coix and *C. nutans* into bakery products.

Therefore, this study aims to develop and characterize Coix seed cookies incorporated with C. nutans by evaluating their physicochemical properties and antioxidant potential. By bridging the gap between traditional medicinal knowledge and modern food science, this research aims to enhance the value of Coix seed and *C. nutans*, thereby fostering their application in functional food development and contributing to improved public health and nutrition.

EXPERIMENTAL

Materials

Materials for formulated cookies were Coix seed (*C. lacryma-jobi var.lacryma-jobi*) *C. nutans* powder, wheat flour, pure butter, stevia sugar, castor sugar, milk powder, egg yolk, cornstarch, butter oil, vanilla essence, baking powder, and salt. For the physicochemical analysis, Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), gallic acid, DPPH (2,2-diphenyl-1-picrylhydrazyl), methanol (analytical grade), and quercetin were utilised.*Coix lacryma-jobi var. lacryma-jobi* were sourced from Murni Herbs in Perak, Malaysia. *C. nutans* was obtained from TKC Herbal Nursery Sdn. Bhd in Nilai, Negeri Sembilan.

Preparation of Cookies

The concentration ranges of Coix seed flour (25-100 g) and *C. nutans* powder (3-15 g) were selected based on preliminary trials and findings from previous studies, which demonstrated the optimal texture and sensory acceptability of composite cookies enriched with non-wheat functional flours [16]. All the ingredients were mixed thoroughly to make the cookies. The cookie dough was made by mixing wheat flour, cornstarch, milk powder, baking powder, and salt together and then left aside. Stevia sugar, castor sugar, and pure butter were blended for 5 minutes until well mixed. Egg yolk, butter oil, and vanilla essence are added to the wet mixture to be mixed. The entire wet mixture was poured into and combined with the dry ingredients until a homogeneous mixture was formed. The dough was refrigerated at 4 °C for 30 minutes. It is taken out and stretched using a rolling pin until its thickness becomes 0.8 cm. Stainless steel moulds with a round shape, 45 mm in diameter, are used to cut the dough into 6 pieces. The doughs are sent into a forced convection oven and baked at 180 °C for 20 minutes. After baking is done, the newly baked cookies are left to cool until they reach room temperature and are stored properly in a container. A total of 6 treatments, designated as T0 to T5, were proposed, featuring different variations of Coix seed flour and *C. nutans* powder. These treatments consisted of varying amounts of Coix seed flour, ranging from 25 g to 100 g, combined with 3 g to 15 g of *C. nutans* powder, as shown in Table 1.

Table 1. Formulation of cookies.

Ingredient (g)	Treatment					
	T0	T1	T2	T3	T4	T5
Wheat flour	125	25	47	70	90	110
Coix seed flour	-	100	75	50	25	0
C. nutans powder	-	0	3	5	10	15

Physicochemical and Characterization of Cookies

The Texture-forming Properties

The bulk density (BD) of formulated and formulated flour was determined according to the method by Adegunwa, Ogungbesan [17] with some modification. A 50 mL graduated cylinder, which had been tared before use, was filled with 10 g of each of the flours. The cylinder base was tapped lightly on any laboratory bench numerous times until the volume became constant. The measurements were performed in triplicate, and equation (1) to be used for the calculation of BD was expressed as follows equation (1).

The swelling capacity (SC) was measured as the method described by Heo, Kim [18], with slight modification, by filling 3 g of flour into a 50 mL measuring cylinder, and the initial volume was read. After that, the measuring cylinder was topped up to 30 mL using distilled water, shaken, turned upside down several times, and left for 24 hours at a room temperature of 25°C. The final volume of the wetted flour was measured, and the measurements were carried out twice. SC was calculated using equation (2).

For the water solubility index (WSI), the method described by Zhang, Song [19] With a few modifications, it was used. In a 50 mL Falcon tube, 0.5 g (W_1) was mixed with 25 mL of distilled water, then the mixture was warmed in a water bath at 80 °C for 30 min. The step was followed by centrifugation of the mixture at 4000 rpm for 40 minutes. Upon finishing the centrifugation, the tube was taken out, and the supernatant was decanted carefully into a pre-weighed vessel (W_2) and allowed to dry at 105 °C for 24 hours. The final weight of the residue (W_3) were weighed and the WSI was expressed as the equation (3).

The water holding capacity (WHC) was evaluated using a method done by Sławińska, Jabłońska-Ryś [20], with slight modification. WHC was determined by first mixing 100 mg of flour (W_1) with 1.5 mL of distilled water in a pre-weighed 2 mL Eppendorf tube (W_2). The suspension mixture was then swirled for 1 min and incubated for 30 min at a room temperature of 25°C. The mixture was centrifuged at 4000 rpm for 40 minutes. After this, the tube was removed, and the supernatant was carefully decanted to be removed, leaving the sediment, which was the sample residue. The tube, along with the residue, was inverted and set aside for 30 minutes to allow any remaining water to evaporate. Finally, the residue was weighed (W_3) and the WSI of each flour was calculated using equation (4) as follows.

Colour Measurement

A colorimeter (HP-200 Precise Color Reader) was used for colour analysis. Parameters including L^*, the brightness, a^*, the red to green value, and b^*, the blue to yellow value, were detected. The browning index, BI, was determined, and the total colour difference, ΔE, between substituted cookies and control cookies was determined using equations mentioned in [21] and [20].

Texture Profile Analysis

The average weight (W), diameter (D), and thickness (T) were measured and recorded for each version of the cookies using an electronic calliper. Measurements were done in triplicate. After that, the spread ratio (D/T) expressed as the ratio of diameter to thickness, was calculated based on collected data.

$$\text{Bulk density (BD)}=\frac{\text{Sample weight (g)}}{\text{Volume of sample after tapping (mL)}} \quad (1)$$

$$\text{Swelling capacity (SC)}=\frac{\text{Final volume (mL)-Initial volume (mL)}}{\text{Sample weight (g)}} \quad (2)$$

$$\text{Water solubility index (\%)}=\frac{W_3(g)-W_2(g)}{W_1(g)}\times 100\% \quad (3)$$

$$\text{Water holding capacity (WHC)}=\frac{W_3(g)-W_2(g)-W_1(g)}{W_1(g)} \quad (4)$$

$$BI=100-L^* \quad (5)$$

$$\Delta E=\left[\left(L^*_{sample}-L^*_{control}\right)^2+\left(a^*_{sample}-a^*_{control}\right)^2+\left(b^*_{sample}-b^*_{control}\right)^2\right]^{1/2} \quad (6)$$

Antioxidant Properties

The determination of TPC and DPPH scavenging free radical activity is performed as the method described and modified by [22], with the extraction of cookie samples using 50% (v/v) ethanol for TPC analysis and 80% (v/v) methanol for DPPH analysis. The TPC of all extracts was determined following the Folin-Ciocalteu method in a 96-well microplate with some modifications. The exact volume of 20 µL extract, followed by 100 µL Folin-Ciocalteu reagent, was pipetted into each well to be incubated for 5 minutes. After that, 80 µL of 0.75% Na_2CO_3 was pipetted into each well and incubated for an additional 20 minutes in a dark condition. The absorbance at a wavelength of 760 nm was measured using a Tecan Infinite F200 Pro plate reader (Tecan Group Ltd., Männedorf, Switzerland), and the measurement was performed three times. With gallic acid as the standard for the calibration curve, the same steps were repeated using gallic acid at a series of concentrations: 0, 0.625, 1.25, 2.5, 5.0, and 10.0 µg/mL, in each well. The results were expressed in mg GAE/g of crude extract.

The antioxidant activity was assessed based on DPPH radical scavenging, with some modification. A stock sample extract with a volume of 2000 µL/mL was prepared prior to analysis. The analysis was conducted in a 96-well microplate, with 200 µL of sample extracts pipetted from a stock solution into the wells of the plate and prepared in 7 serial dilutions, starting from 2000, 1000, 500, 250, 125, 62.5, and 31.25 µg/mL. A volume of 100 µL of 0.2 mM DPPH was then added to the wells, and 100 µL of methanol was added for the blank sample. The microplate was incubated for 30 minutes and then analyzed using a Tecan Infinite F200 Pro plate reader (Tecan Group Ltd., Männedorf, Switzerland) for absorbance measurement at a wavelength of 517 nm against a reagent blank. The measurement was conducted for thrice. With quercetin as the positive control, the same steps were repeated. The radical scavenging activity (RSA) of sample extracts was calculated with equation (5):

$$\text{RSA}\ (\%) = \left[\frac{(A_o - A_s)}{A_o}\right] \times 100 \tag{5}$$

Where A_o represents the absorbance value of the reagent blank, and A_s represents the absorbance value of the sample extract. The results were then expressed as an IC_{50} value in units of µg/mL by interpolation from the linear regression analysis obtained using the RSA value.

Statistical Analysis

All measurements were conducted in triplicate, and data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using IBM SPSS Statistics 26. One-way analysis of variance (ANOVA) was applied to determine significant differences among treatments. When significant differences ($p < 0.05$) were detected, Tukey's Honest Significant Difference (HSD) post-hoc test was used for mean separation.

RESULTS AND DISCUSSION

Physicochemical and Characterization of Cookies

The Texture-forming Properties

Table 2 presents the texture-forming properties, including BD, SC, WSI, and WHC, of wheat flour, Coix seed flour, and *C. nutans* powder. The BD of Coix seed flour and *C. nutans* powder (0.43–0.49 g/mL) were significantly lower than that of wheat flour (0.71 g/mL). Therefore, it is noticeable that the replacement of wheat flour with Coix seed flour and *C. nutans* powder in formulating cookies resulted in a decrease of BD. This is clearly stated in the data recorded in Table 3, whereby the BD of formulated flours, T1 to T5 (0.66–0.69 g/mL) were lower than that of the control flour, T0 (0.71 g/mL). T2 showed the lowest BD. The lower BD is more favorable for obtaining higher-quality, crunchier cookies. BD is impacted by the particle size of flour, where flour with a coarse particle size reduces the BD [23]. In contrast to cookies made with whole wheat flour, the results showed that cookies made with Coix seed flour and *C. nutans* powder had coarser particle sizes, which resulted in greater crunchiness and better quality. In this study, the substitution with Coix seed flour and *C. nutans* powder, particularly in T2, reduced BD values, leading to cookies that were less dense and more desirable in texture. Such cookies are often preferred by consumers due to their crispness and ease of bite, key attributes that contribute to sensory acceptability.

Table 2. Texture-forming properties of different flours.

Sample	Parameter			
	BD (g/mL)	SC (mL/g)	WSI (%)	WHC (g^{water}/g)
Wheat flour	0.71	1.00	10.62	0.89
Coix seed flour	0.49	0.83	18.76	2.21
C. nutans powder	0.43	0.67	16.20	2.02
Overall significance (p)	< 0.001	0.004	0.010	0.015

Table 3. Texture-forming properties of formulated flours.

Sample	Parameter			
	BD (g/mL)	SC (mL/g)	WSI (%)	WHC (g^{water}/g)
T0 (control)	0.71	1.00	10.62	0.89
T1	0.69	1.51	11.54	1.43
T2	0.66	1.25	14.77	1.38
T3	0.68	1.20	12.41	1.33
T4	0.67	1.00	10.38	1.58
T5	0.69	1.97	16.93	1.29
Overall significance (p)	0.008	< 0.001	0.012	0.023

The largest swelling capacity (1.00 mL/g) was obtained by wheat flour, followed by C. Coix seed flour (0.83 mL/g) and *C. nutans* powder (0.67 mL/g). The high protein content of Coix seed flour strengthens its interaction with starch, limiting water hydration and ultimately resulting in lower SC [16]. Substituting Coix seed flour and C. nutans powder with different ratios of wheat flour will increase the SC of cookies. Higher SC is associated with enhanced expansion during baking, which contributes to a softer internal structure and pleasant mouthfeel. From a consumer standpoint, moderate swelling ensures cookies remain tender without being overly dry or hard, balancing texture appeal. Therefore, the improved SC observed in the composite flours supports the development of cookies with both functional and sensory benefits, linking physicochemical improvements to consumer satisfaction and marketability.

The highest WSI was Coix seed flour (18.76%) compared to *C. nutans* powder (16.20%) and wheat flour (10.62%). This is due to higher protein and soluble dietary fibre content present in Coix seed flour, which are responsible for the hydrating properties. The higher the value, the higher the water solubility index [24]. The substitution of Coix seed flour and *C. nutans* powder in cookies had increased the WSI.

The highest WHC was found in Coix seed flour (2.21 g water/g), followed by C. nutans powder (2.02 g water/g), and lastly, wheat flour (0.89 g water/g). Coix seed flour has a strong ability to retain water within its structure [25]. This water-binding capability is primarily due to its high fiber and protein content, indicating that Coix seed flour is rich in these essential compounds.

Colour Measurement

Tables 4 and 5 present the parameters for colour analysis, including L*, a*, b*, ΔE, and BI, of different flours and formulated cookies. The L^* was the highest for wheat flour (L^* = 95.69), second highest for Coix seed flour (L^* = 70.77), and the lowest for *C. nutans* powder (L^* = 54.60). Then, regarding the parameter a^* and parameter for b^*, both the Coix seed flour and *C. nutans* powder showed a higher degree of redness (a^* = 4.42, a^* = 3.54) and yellowness (b^* = 8.29, b^* = 11.69) compared with wheat flour (a^* = 0.39, b^* = 6.59). The total colour difference (ΔE) was higher in *C. nutans* powder (ΔE = 41.52) than in Coix seed flour (ΔE = 25.30). The same trend was also observed in the browning index (BI), with C. nutans powder, Coix seed flour, and wheat flour obtaining values of 45.40, 29.23, and 4.31, respectively.

Table 4. Colour measurement of different flours.

Sample	Parameter				
	L^*	a^*	b^*	ΔE	BI
Wheat flour	95.69	0.39	6.59	-	4.31
Coix seed flour	70.77	4.42	8.29	25.30	29.23
C. nutans powder	54.60	3.54	11.69	41.52	45.40
Overall significance (p)	<0.001	0.004	0.002	-	<0.001

Table 5. Colour measurement of pre-formulated cookies.

Sample	Parameter				
	L^*	a^*	b^*	ΔE	BI
T0 (control)	45.93	13.33	22.05	-	54.07
T1	42.82	7.19	19.92	7.21	57.18
T2	29.57	7.86	12.53	19.70	70.43
T3	33.13	9.47	13.44	15.89	66.87
T4	42.84	9.26	26.83	7.00	57.16
T5	44.21	7.63	22.34	5.97	55.79
Overall significance (p)	< 0.001	0.006	0.002	-	0.010

The colour of cookies is affected by the type of flour utilised. The control cookies (T0) exhibited the highest value L^*, measured at 45.93. T2 showed the lowest degree of brightness ($L^* = 29.57$) and the trend gradually increased from 33.13 to 44.21 for T3 to T5 cookies. This could be due to the increase in the addition of C. nutans powder and wheat flour, but at the same time, a decrease in Coix seed flour is observed starting from T2. For T1 cookies, the substitution of only the Coix seed flour decreased the brightness slightly to 42.82. The results indicate that the addition of *C. nutans* powder has a significant effect on the brightness of cookies, but this effect is smaller when it comes to Coix seed flour.

For parameter a^*, T0 showed the highest degree of redness ($a^* = 13.33$) while T1 showed the lowest value ($a^* = 7.19$). The T3 and T4, which contained a greater portion of C. nutans powder, showed the two highest values of redness, at 9.47 and 9.26, respectively. The T5, which completely excluded Coix seed flour from the formulation, obtained a lower value at 7.63. The results indicate that the redness of cookies is higher when a larger portion of C. nutans and Coix seed flour is substituted for wheat flour. With the decrease of Coix seed flour and increase of *C. nutans* powder, the values of parameter b^* obtained were higher. Cookies appeared to be more yellowish when there is a smaller portion of Coix seed flour and a higher amount of *C. nutans* powder.

The variation in colour parameters (L*, a*, b*) among the formulated cookies reflects the influence of Coix seed flour and *Clinacanthus nutans* on Maillard and caramelization reactions during baking. The decrease in brightness (L*) from 45.93 in the control (T0) to 29.57 in T2 corresponds to the darker hue contributed by polyphenols and chlorophyll-related pigments in *C. nutans*. In addition to pigment contribution, the Maillard reaction between reducing sugars and amino acids likely intensified browning and generated melanoidin compounds, which are known to possess antioxidant properties**.** The decrease in brightness (L*) from 45.93 in the control (T0) to 29.57 in T2 corresponds to the darker hue contributed by polyphenols and chlorophyll-related pigments in *C. nutans*. Similar trends were reported by Sławińska et al. (2024), who found that mushroom-enriched cookies exhibited reduced L* and increased browning index (BI) due to elevated phenolic content. In addition, Kutschera and Krasaekoopt [16] observed that Job's-tear (Coix) flour imparted a yellow-brown tone associated with higher a* and b* values, consistent with our data where both redness and yellowness increased with higher Coix and *C. nutans* proportions. These results indicate that the incorporation of functional plant flours rich in pigments and phenolics not only modifies colour but may also serve as a visual indicator of antioxidant enrichment. The observed ΔE (5.97–19.70) suggests perceptible differences from the control, which could influence consumer appeal depending on preference for darker, "healthier"-looking baked products.

Texture Analysis Profile

Table 6 demonstrates the effects of Coix seed flour and *C. nutans* powder on the weight, thickness, diameter, and spread ratio of formulated cookies. The addition of Coix seed flour and *C. nutans* powder significantly decreased the weight of the formulated cookies (8.20–8.95 g) compared to the control cookies. There were no significant effects on the diameter of the cookies with the addition of Coix seed flour and *C. nutans* powder (3.25–3.80 cm) compared to control cookies. For the thickness, the addition of Coix seed flour and *C. nutans* powder had slightly decreased the values (1.05–1.30 cm) when comparing with that of control cookies.

Table 6. Texture analysis of formulated cookies.

Sample	Parameter			
	Weight (g)	**Diameter (D) (cm)**	**Thickness (T) (cm)**	**Spread Ratio (D/T)**
T0 (control)	10.10	3.65	1.55	2.35
T1	8.44	3.80	1.05	3.62
T2	8.20	3.25	1.15	2.83
T3	8.72	3.50	1.30	2.69
T4	8.95	3.70	1.30	2.85
T5	8.48	3.75	1.25	3.00
Overall significance (p)	< 0.001	0.003	0.010	0.005

Table 7. Antioxidant properties of formulated cookies.

Sample	Parameter	
	TPC (mg GAE/g)	DPPH (IC50) (µg/mL)
T0 (control)	0.69	3291.44
T1	0.98	2840.56
T2	1.28	1761.72
T3	1.21	1982.09
T4	1.18	2339.40
T5	1.06	2711.58
Overall significance (p)	< 0.001	< 0.001

There were slight variations in the values of the weight, diameter, and thickness. The decrease in Coix seed flour and the increase in *C. nutans* powder caused an increasing trend in the weight and diameter of T2, T3, and T4 cookies. The exclusion of *C. nutans* powder or Coix seed flour resulted in similar data on weight and diameter of the cookies, which were 8.44 g and 3.80 cm for T1, and 8.48 g and 3.75 cm for T5. The thickness of the cookies showed a decreasing tendency without the addition of *C. nutans* powder or Coix seed flour, compared to others. This is demonstrated by the 1.05 cm and 1.25 cm readings for T1 and T5, respectively. The spread ratios of cookies ranged from 2.35 to 3.62. The control cookies resulted in the lowest value, and the addition of Coix seed flour and *C. nutans* powder increased the spread ratio of the cookies. The T1 cookies, which were formulated without *C. nutans* powder showed the two highest values of spread ratio among all the formulated cookies, with 3.62 and 3.00. The spread ratio reflects the ability of cookies to rise [26]. A lower value is desired. T2 to T4 cookies, which achieved the values (2.69–2.85) close to that of control cookies (2.35), demonstrated better rising ability.

Antioxidant Properties

The TPC and DPPH scavenging activity of formulated cookies are recorded in Table 7. The control cookies T0 were characterised as having the lowest TPC value at only 0.69 mg GAE/g and the highest IC_{50} value at 3291.44 µg/mL among all the cookies.

A Pearson correlation analysis was performed to determine the relationship between the total phenolic content (TPC) and the antioxidant activity (IC_{50}) of the formulated cookies. The results showed a strong and significant negative correlation ($r = -0.94$, $p < 0.01$), confirming that higher TPC values were associated with lower IC_{50} values, indicating stronger antioxidant activity. This inverse relationship aligns with findings from previous studies that demonstrate phenolic compounds as major contributors to radical scavenging capacity in plant-based functional foods.

Compared with control cookies, all the formulated cookies showed an increase in TPC value, ranging from 0.98 mg GAE/g to 1.61 mg GAE/g, and a decrease in the value of IC_{50}, ranging from 1761.72 µg/mL to 2840.56 µg/mL. The addition of Coix seed flour and *C. nutans* powder had resulted in improved antioxidant activity of the cookies. Among the formulated cookies, T2 had achieved the highest TPC value at 1.28 mg GAE/g and the lowest IC_{50} at 1761.72 µg/mL. Similarly, cookies enriched with fruit or mushroom powders commonly show control TPCs in the 0.2–0.5 mg GAE/g range and enriched products approaching or exceeding ~0.9–1.6 mg GAE/g, consistent with T2 [27]. Both the TPC and DPPH values of the cookies showed the same trend, indicating a positive correlation between the two properties. It is observed that the cookies made without Coix seed flour or *C. nutans* powder significantly decreased the antioxidant properties. According to numerous studies, Coix seed and *C. nutans* are expected to exhibit high antioxidant properties.

The antioxidant trend observed among the formulated cookies may be attributed to synergistic or antagonistic interactions between bioactive compounds present in Coix seed and *C. nutans*. Coix seed is rich in γ-oryzanol and tocols (vitamin E derivatives), which act as potent lipid-phase antioxidants capable of donating hydrogen atoms to stabilize free radicals [28]. Conversely, *C. nutans* contains abundant hydrophilic phenolics and flavonoids, such as schaftoside and vitexin, that primarily scavenge radicals in aqueous systems. The coexistence of these lipophilic and hydrophilic antioxidants can produce synergistic effects by extending radical-scavenging capacity across both phases, thereby enhancing overall antioxidant efficiency. However, at higher *C. nutans* concentrations, the relative dilution of Coix seed bioactives or potential competition for reactive oxygen species could create mild antagonistic effects, explaining the non-linear trend in TPC and IC_{50} values beyond the T2 formulation. Similar biphasic interactions between phenolics and tocopherols have been reported in mixed antioxidant systems, where the efficacy depends on concentration ratio, matrix polarity, and compound partitioning [29]. Hence, the antioxidant behavior in these composite cookies likely reflects a complex interplay between Coix seed's lipid-soluble antioxidants and *C. nutans*'s polyphenolic constituents.

CONCLUSION

The formulation studies on cookie preparation showed that replacing wheat flour with Coix seed flour, incorporating *C. nutans*, significantly impacted the physicochemical properties of the cookies. In the formulation study, T2 cookies, which were made by blending 75 g of Coix seed flour, 3 g of C. nutans, and 47 g of wheat flour, exhibited the most favorable characteristics across all properties except for color appearance. It had achieved a low spread ratio, low bulk density, high swelling capacity, water solubility index, water holding capacity, and the highest antioxidant properties, which had led to its selection for further optimization. The results demonstrated the potential of Coix seed to be made into a healthy alternative option to wheat flour. By incorporating *C. nutans* in the preparation of cookies, the properties and quality of cookies are further enhanced and elevated.

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