

Utilization of coffee pulp extract and honey for the development of functional cube candies

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ABSTRACT

Coffee pulp is a major by-product of the coffee industry, which rich in various phytochemicals and showing potential for use as a functional ingredient in food products. This study aims to use coffee pulp extract and honey to develop functional tea cube candy, focusing on the effects of varying honey content and extraction times of coffee pulp. The results showed that longer extraction times increased moisture content (ranging from 1.08% to 1.57%), while water activity (a_w) was not significantly different. Extraction time significantly influenced total soluble solids (TSS) and colour parameters. Higher honey proportions and longer extraction times were positively correlated with increased total phenolic content (TPCs) and enhanced antioxidant activities (both DPPH and FRAP). The candy is safe for consumption based on the results of microbiological testing and 5-HMF concentrations (ranging from 4.92 to 9.05 $\mu\text{g/g}$). 5-Hydroxymethylfurfural (5-HMF) analysis was performed using a precursor mass of m/z 127 and a product mass of m/z 108.9 through the dilute-and-shoot UHPLC-MS/MS method, which effectively quantified method. Sensory evaluations indicated that both honey and extraction time positively affected the appearance, texture, and overall liking of functional candy. In conclusion, incorporating honey and coffee pulp extract resulted in a pleasant product with enhanced phenolic acid content and antioxidant activity. This approach enhances the value of both coffee pulp and honey but also provides consumers with healthier options.

Key words: Cascara; candy; bioingredients; by-product; functional food.

1 INTRODUCTION

Coffee is indeed a globally popular beverage. According to the USDA Foreign Agricultural Service report (United States Department of Agriculture - USDA, 2024), the projected annual global production is approximately 10.1 million metric tons for the 2023/2024 period, with a compound annual growth rate of approximately 1% over the past decade. This report indicates steady demand, stable production levels, and the significance of coffee in the global market. Nevertheless, the commonly consumed part of the coffee is the bean, while the rest is usually unused or used as animal feed. In recent years, coffee by-products have gained attention and have been studied for their potential benefits (Klingel et al., 2020). Coffee pulp or cascara is the one of main by-product of the coffee processing industry, specifically from the outer layers of the coffee cherry that are removed during milling. It is rich in phytochemicals including chlorogenic acid, epicatechin, dicaffeoylquinic acid, catechin, rutin, etc. (Esquivel; Jiménez, 2012). It has potential health benefits, including slowing weight gain, improving hyperleptinemia, enhancing glucose tolerance, and modulating gut microbiota (Braojos et al., 2023; Rungraung; Muangpracha; Trachootham, 2023). Previous studies have shown that coffee pulp extract is safe for consumption. An *in vivo* oral sub-chronic toxicity study demonstrated that aqueous coffee pulp extract was safe in a mouse model at 1,000 mg/kg

body weight/day (equivalent to approximately 57.7-84.9 mg for humans) (Gil-Ramírez et al., 2024). Clinical studies have also reported concentrated coffee pulp juice as safe at 28g per day (Rungraung; Muangpracha; Trachootham, 2023). This has led to coffee gaining more attention and being developed into a variety of products, including candy. However, previous reports have found that its acceptance is only moderate (3.20 - 4.09 on a 6-point scale for overall acceptance) (Umayya et al., 2024).

In recent years, societal interest in consuming functional foods has significantly increased (Roudbari et al., 2024). This heightened interest has driven research and development of new functional ingredients and products to meet consumer expectations. Candy serves as a suitable matrix for incorporating functional ingredients in product development. It is one of the most popular confectionery products worldwide; however, it generally has low nutritional value, with most varieties primarily containing sugar. A study by Goins et al. (2024) found that greater consumption of added sugars and carbohydrate-rich foods is associated with an elevated risk of developing metabolic syndrome. *In vivo* and clinical studies have also demonstrated that sugar (sucrose) can stimulate weight gain, glucose intolerance, insulin resistance, and other characteristics of metabolic syndrome (Heidari-Beni; Kelishadi, 2017). Therefore, sugar may not be suitable for formulations. Substituting sugar with

other sweeteners, such as honey, is a common technique that can lead to healthier formulations. Although many studies focus on the development of healthy candy recipes or the evaluation of the potential health benefits of coffee pulp and honey, as well as the applications of honey and coffee pulp in the food industry, such as the development of edible films to improve mechanical and functional properties (Biratu; Woldemariam; Gonfa, 2024). However, the research that using coffee pulp extract and honey to develop functional candy is lacked, particularly studies that assess their effects on physicochemical properties and consumer acceptance. Therefore, the present study aims to investigate the potential of using coffee pulp extract as a functional ingredient to formulate candy with honey and evaluate the effects of coffee pulp extract and honey content on the physicochemical properties and consumer acceptance of the product. This approach not only enhances the value of the coffee by-product (coffee pulp) but also provides consumers with products that may help reduce the risk of metabolic diseases

2 MATERIAL AND METHODS

2.1 Chemicals and raw materials

The fresh longan (*Dimocarpus longan* Lour.) honey were collected from a local apiarist in Northern Thailand. Coffee pulp was removed from the coffee cherries (*Coffea arabica* L.) by a de-pulper and dried at approximately 60 degrees Celsius for 15 hours. Honey and dried coffee pulp were stored in a dry place at room temperature until they were used in the study. Glucose syrup and Lime powder was purchased from Foodchem Ltd. Ethanol was purchased from Honeywell (Seelze, Germany). 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and gallic acid obtained from Sigma-Aldrich (Sydney, Australia). Folin-Ciocalteu reagent, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), iron (III) chloride (FeCl_3), hydrochloric acid, and ascorbic acid was purchased from Merck (Darmstadt, Germany). 5-Hydroxymethylfurfural (5-HMF; purity $\geq 98.0\%$) were purchased from Supelco, Inc. (Bellefonte, PA, USA). LC/MS-grade formic acid, acetonitrile and hexane were purchased from Fisher Chemical (Fair lawn, NJ, USA). 18.2 M Ω ultrapure water was obtained from ELGA PURELAB Quest UV (High Wycombe, England). All microbiological growth medium was purchased from Himedia (Maharashtra, India).

2.2 Tea cube candy preparation

In this study, dried coffee pulp (from the same lot) was extracted with hot water at three different extraction times (5, 10 and 15 minutes) and mixed with honey (from the same lot) at three contents (300, 350, and 400 g) to evaluate their effects using a 3x3 factorial design. Briefly, the dried coffee pulp extract was mixed with honey and glucose syrup (16%).

The mixture was then heated to 140 degrees Celsius and mixed with lime powder (6.6%). Finally, the mixture was poured into moulds and left to cool to room temperature.

2.3 Physicochemical properties analysis

Moisture content was determined according to AOAC International (Association of Official Analytical Chemists - AOAC, 2002) with method 969.38 Water activity (a_w) was measured using a 4TE Dew Point Water Activity Meter (Aqualab, WA, USA). Total soluble solid content (TSS) was calculated from the solution of each sample (2 g of sample mixed with 8 mL of water), following a previous study with some modifications (Sarkar et al., 2023), and measured using a Brix refractometer, with results expressed as degrees Brix at 20 degrees Celsius. The colour parameters, including L^* , a^* , and b^* , were measured on the surface of sample using a CR-10 Plus Colour reader (MINOLTA KONICA, Japan).

2.4 Total phenolic content (TPCs) analysis

TPCs was determined by the Folin-Ciocalteu colorimetric method with a minor modification (Akther et al., 2023). The solution (0.1 g of sample mixed with 4 mL of water) was mixed Folin-Ciocalteu reagent and Na_2CO_3 solution. The mixed solution was transferred into a 96-well plate and incubated at room temperature for 150 minutes in the darkness. The absorbance of the solution was measured at 765 nm using a BioTek Synergy HTX Microplate Reader (Agilent, CA, USA), with results expressed as mg gallic acid equivalent per kg of dry weight (mg GAE/kg DW).

2.5 Determination of antioxidant activities

Antioxidant capacity was determined according to our previous studies. The DPPH assay mixture consisted of the sample solution (10-1,000 ppm) and DPPH solution (250 $\mu\text{g}/\text{mL}$ of 0.2% DPPH in ethanol). It was placed into a 96 well-plate and incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance was measured at the wavelength of 517 nm by using an RT-2100C microplate reader (Mediatech, Australia). The free radical scavenging capacity was presented as a percent radical scavenging calculated from the absorbance loss of DPPH radicals with reference to a dose-response curve for ascorbic acid. The FRAP reagent solution was first made containing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl_3 in water in the ratio 10:1:1 (v/v). The sample solution was mixed with the FRAP reagent in the ratio 9:1 (v/v) and incubated in the dark at room temperature for 4 minutes. After incubation, the absorbance was measured at the wavelength of 593 nm with UV-Visible 1800 Spectrophotometer (Shimadzu, Japan). The results were presented as μmol of Fe (II)/g sample with reference to a dose-response curve for FeSO_4 .

2.6 5-HMF analysis

5-HMF was analyzed using a UHPLC-MS/MS system, consisting of an Ultimate 3000 connected to a TSQ Quantis Triple Quadrupole Mass Spectrometer (Thermo Scientific, Waltham, MA, USA). In brief, A 10 g sample was extracted with 20 mL of 50% acetonitrile and 20 mL of hexane, then centrifuged for 20 minutes at 2500g. The lower phase was collected and re-extracted for 2 time. Finally, the extract was dilute at 100-fold with water and filtered through a 0.2-micron nylon filter. UHPLC was performed by using Hypersil GOLD C18 column (1.9 μ m particle size, 100 $\times$ 4.6 mm, ThermoScientific, MA, USA) that maintained at 40 degrees Celsius with gradient run at a flow rate of 0.2 mL/minutes with two mobile phase, 0.5% formic acid in 5% acetonitrile (A) and acetonitrile (B). The gradient program included: 0.0–4.2 minutes, 5–5% B; 4.2–4.3 minutes, 5–0% B; 4.3–6.3 minutes, 0–0% B; 6.3–6.4 minutes, 0–5% B; 6.4–15.0 minutes, 5–5% B. The injection volume was 1 μ L. Mass spectrometric was performed in electron spray ionization with positive ion mode (ESI+) at spray voltage of 4.8 kV, ion transfer tube temperature at 325 degrees Celsius, vaporizer temperature at 350 degrees Celsius, sheath gas, auxiliary gas and sweep gas at 50, 10 and 2.5 arbitrary units, respectively.

2.7 Microbiological contamination analysis

All analysis followed the bacteriological analysis manual by the U.S. Food and Drug Administration (2007). In brief, 5 g of each sample in 0.85% NaCl solution was diluted using a 10-fold serial dilution to optimize concentration. The aerobic plate count was determined using Plate Count Agar at 35 $\pm$ 2 degrees Celsius for 24-48 hours. *Escherichia coli* counts were determined through serial selection using lauryl tryptose broth, brilliant green lactose bile broth, and eosin methylene blue agar, respectively, to isolate colonies of *Escherichia coli*. All medium was incubated at 35 $\pm$ 2 degrees Celsius for 24-48 hours. Mold and yeast counts were determined using Potato Dextrose Agar and Yeast Malt Extract Agar, respectively, at 30 $\pm$ 2 degrees Celsius for 24-48 hours.

2.8 Sensory evaluation

The sensory evaluation was evaluated according to recommendations for central location testing, with 100 randomly selected of untrained panellists evaluating the samples (Stone; Sidel, 2004). Each panellist was presented with six samples of the same size, packaged identically with lids and coded with 3-digit random numbers to avoid bias. Panellists were provided with drinking water to cleanse their palate between tastings. A 9-point hedonic scale was used to evaluate the following attributes: appearance, texture, and overall liking.

2.9 Statistical Analysis

All data were reported as mean $\pm$ standard deviation from triplicate experiments. SPSS software version 17 (IBM, IL, USA) or GraphPad prism (GraphPad Software, MA, USA) version 9 was used for statistical analysis. The variances of processes were separated for analysing the sample data significantly different among means tested using ANOVA with post-hoc test ($p < 0.05$). Duncan's or Turkey's B test were chosen to post-hoc test because it imposes no limitations on the size or family-wise error rate of the sample groups being compared. The responses of sensory evaluation were analyzed with response surface methodology (RSM) by Design Expert software version 7.0 (Stat-Ease, MN, USA).

3 RESULT

3.1 Physicochemical properties of functional candy

From the results of the physicochemical properties of functional honey candy with coffee pulp extract (Table 1), the moisture content increased with longer extraction times across all honey contents, suggesting that more water is retained in the extract. While water activity (a_w) did not significantly change (range 0.37-0.41) as the extraction time of coffee pulp increased for all honey contents. The L^* values tend to decrease with longer extraction times, indicating darker extracts, while the increase in a^* and b^* values with longer extraction times. suggests that the extracts become redder and yellower, respectively.

3.2 Total phenolic content (TPCs) and antioxidant activities of functional candy

As shown in Table 2, TPCs, DPPH radical scavenging activity, and FRAP values were influenced by both the honey content and the extraction time of coffee pulp. The highest values for all parameters were observed at 400 g of honey and 15 minutes of extraction, with TPCs at 26.60 mg GAE/g, DPPH at 14.89%, and FRAP at 22.57 μ M Fe(II)/g. Lower values were recorded at 300 g honey and 5 minutes, indicating that both factors significantly affect the antioxidant properties of the product.

3.3 Safety evaluation of functional candy

The safety of the product in this study was evaluated based on microbiological contamination and 5-hydroxymethylfurfural (5-HMF) levels. Microbiological analysis included total aerobic plate count, *E. coli*, yeast, and mold, assessed over a 150-day storage period. Results from both day 0 and day 150 showed no detectable microbial contamination, indicating that the candy was either sterile or had microbial counts below detection limits, in compliance

with safety standards. 5-HMF was analyzed using the UHPLC-MS/MS technique with selected reaction monitoring (SRM) mode. This technique is known for its high sensitivity and selectivity, enabling precise quantification even at very low concentrations. The analysis of 5-HMF was performed with a collision energy of 10 V and an RF lens of 78 V, at a precursor mass of m/z 127.1 (Figure 1A) and a product mass of m/z 108.9 (Figure 1B). It was achieved good linearity with an average coefficient of determination (R^2) exceeding 0.9995 (Figure 1C). Chromatograms of the 5-HMF standard (Figure 1D) and

candy sample (Figure 1E) were obtained using UHPLC-MS/MS with a dilute and shoot clean up technique. Based on the results of linearity (0.9995), repeatability (2.94% RSD) and sensitivity (LOD: 0.17 and LOQ: 0.51 ppm), the dilute and shoot-LC-MS/MS method for 5-HMF analysis demonstrates highly performance and effective method. The 5-HMF levels of functional honey candy with coffee pulp extract ranged from 4.92 to 9.05 $\mu\text{g/g}$ (shown in Figure 2) results in this study indicates that prolonging the extraction time of coffee pulp leads to higher concentrations of 5-HMF.

Table 1: Physicochemical properties of functional honey candy with coffee pulp extract.

Honey (g)	Extraction time (minutes)	Moisture (%)	aw ns	TSS (Degree Brix)	L*	a*	b*
300	5	1.08 $\pm 0.01\text{g}$	0.39 ± 0.00	51.0 $\pm 0.0\text{a}$	26.3 $\pm 0.5\text{ab}$	3.3 $\pm 0.8\text{e}$	5.0 $\pm 0.3\text{d}$
300	10	1.12 $\pm 0.02\text{f}$	0.40 ± 0.00	35.0 $\pm 0.0\text{c}$	27.2 $\pm 0.4\text{a}$	4.6 $\pm 0.3\text{cd}$	6.5 $\pm 0.5\text{c}$
300	15	1.38 $\pm 0.00\text{c}$	0.41 ± 0.00	37.0 $\pm 0.0\text{b}$	26.3 $\pm 1.6\text{ab}$	4.8 $\pm 1.5\text{bc}$	8.0 $\pm 0.6\text{ a}$
350	5	1.24 $\pm 0.01\text{d}$	0.38 ± 0.00	50.0 $\pm 0.0\text{a}$	25.2 $\pm 0.8\text{bc}$	3.9 $\pm 0.7\text{d}$	5.2 $\pm 0.7\text{d}$
350	10	1.35 $\pm 0.00\text{cd}$	0.39 ± 0.00	37.0 $\pm 0.0\text{b}$	25.0 $\pm 0.7\text{c}$	4.8 $\pm 0.7\text{bc}$	6.7 $\pm 1.1\text{bc}$
350	15	1.45 $\pm 0.01\text{b}$	0.40 ± 0.00	31.0 $\pm 0.0\text{e}$	25.0 $\pm 0.7\text{c}$	5.4 $\pm 0.8\text{bc}$	8.1 $\pm 0.9\text{a}$
400	5	1.15 $\pm 0.00\text{ef}$	0.38 ± 0.00	50.0 $\pm 0.0\text{a}$	25.4 $\pm 0.6\text{bc}$	4.2 $\pm 0.7\text{cd}$	5.7 $\pm 0.5\text{cd}$
400	10	1.22 $\pm 0.01\text{d}$	0.37 ± 0.0	32.0 $\pm 0.0\text{d}$	25.4 $\pm 0.4\text{bc}$	6.0 $\pm 0.2\text{ab}$	8.5 $\pm 0.6\text{ a}$
400	15	1.57 $\pm 0.01\text{a}$	0.37 ± 0.0	31.0 $\pm 0.0\text{ e}$	25.0 $\pm 0.9\text{c}$	7.0 $\pm 1.5\text{a}$	8.5 $\pm 1.2\text{a}$

Table shows mean $\pm$ SD of moisture, water activity (a_w), total soluble solid content (TSS), lightness (L^*), redness (a^*), and yellowness (b^*) for triplicate experiments. In each column, different superscript letters ($^{a-g}$) mean significant differences ($p < 0.05$) obtained from ANOVA with Duncan's post hoc test. ^{ns} was meaning to not statistic significantly.

Table 2: Total phenolic content (TPCs) and antioxidant activities of functional honey candy with coffee pulp extract.

Honey (g)	Extraction time (minutes)	TPC (mg GAE/g)	DPPH (%)	FRAP (μM of Fe (II)/g)
300	5	15.10 $\pm$ 0.0027f	11.26 $\pm$ 0.01a	18.59 $\pm$ 0.02a
300	10	23.20 $\pm$ 0.0449c	12.56 $\pm$ 0.02bc	19.58 $\pm$ 0.0 3b
300	15	24.50 $\pm$ 0.0245b	12.59 $\pm$ 0.01bc	20.56 $\pm$ 0.00c
350	5	19.10 $\pm$ 0.0152e	12.26 $\pm$ 0.01b	19.56 $\pm$ 0.01 b
350	10	24.30 $\pm$ 0.0122b	13.26 $\pm$ 0.01cd	20.22 $\pm$ 0.01c
350	15	25.10 $\pm$ 0.0151ab	13.59 $\pm$ 0.02cd	21.59 $\pm$ 0.01d
400	5	22.60 $\pm$ 0.0026d	13.00 $\pm$ 0.00c	20.56 $\pm$ 0.00c
400	10	25.20 $\pm$ 0.0021ab	14.26 $\pm$ 0.00d	21.15 $\pm$ 0.00d
400	15	26.60 $\pm$ 0.0024a	14.89 $\pm$ 0.00e	22.57 $\pm$ 0.00e

Table shows mean $\pm$ SD of total phenolic content (TPCs), antioxidant activity obtained from the DPPH assay (DPPH), and antioxidant activity obtained from the FRAP assay (FRAP) for triplicate experiments. In each column, different superscript letters ($^{a-f}$) mean significant differences ($p < 0.05$) obtained from ANOVA with Duncan's post hoc test.

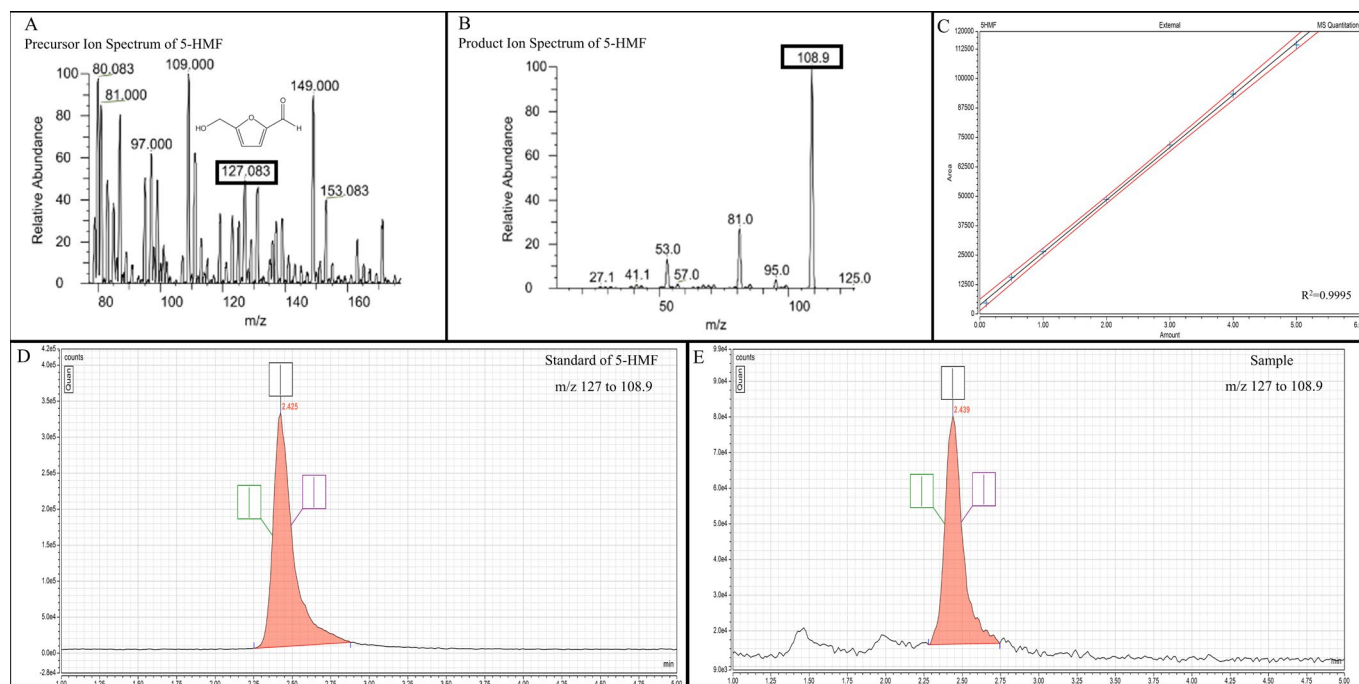


Figure 1: 5-Hydroxymethylfurfural (5-HMF) analyzed by UHPLC-MS/MS. Precursor MS spectra (A) and product ions (B) of 5-HMF. Average calibration curves of 5-HMF (C) generated by the linear plots between areas under the curves of the quantitative product ions and the concentrations of standard solutions. R² values were obtained from linear regression. Chromatograms for quantified product ions of 5-HMF in standard solution (D) and sample (E).

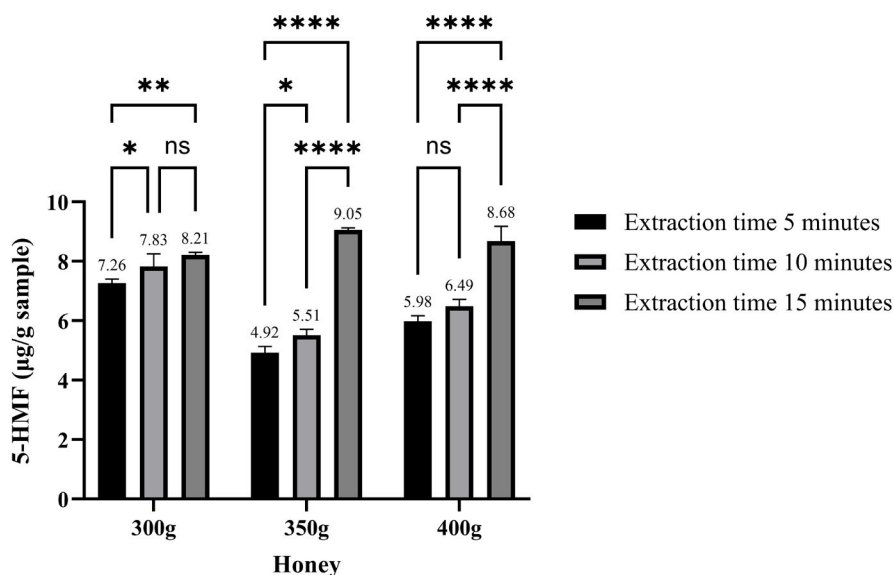


Figure 2: The 5-HMF content of functional honey candy with coffee pulp extract. *, **, *** and **** mean significant differences $p < 0.05$, < 0.01 , < 0.001 and < 0.0001 , respectively, obtained from ANOVA with Turkey's B post hoc test. ^{ns} was meaning to not statistic significantly.

3.4 Sensory evaluation of functional candy

The sensory evaluation of the functional candy is shown in Figure 3. The statistics of regression models were shown in Table 3. The influence of honey content and

extraction time of coffee pulp on sensory evaluation were analyzed with response surface methodology (RSM) by regression models following equations (x: honey content; y extraction time of coffee pulp):

$$\text{Appearance} = 5.228 + 0.117x + 0.145y + 0.033x^2 - 2.667e^{-3}y^2 - 0.025xy \quad (1)$$

$$\text{Texture} = 2.878 + 0.517x + 0.757y - 0.217x^2 - 0.039y^2 - 0.01xy \quad (2)$$

$$\text{Overall Liking} = 4.961 + 0.417x + 0.312y + 0.033x^2 - 0.015y^2 - 0.065e^{-2}xy \quad (3)$$

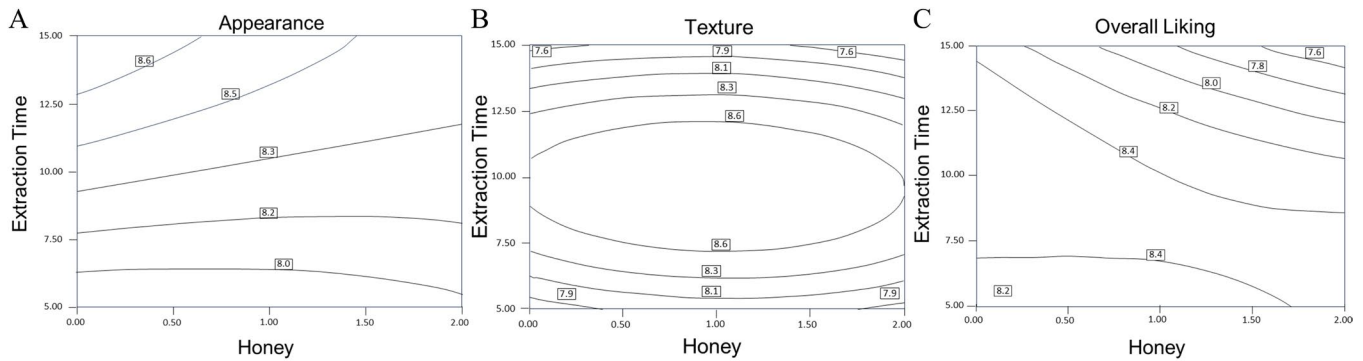


Figure 3: Sensory evaluation of functional honey candy including appearance (A), texture (B), and overall liking (C) were analyzed with response surface methodology (RSM).

Table 3: Response surface methodology (RSM) for sensory evaluation analysis with model statistic.

Source	F-Value	p-value	(Prob>F)
Appearance: R-Squared = 0.8827			
Model	3.56	0.0358	Significant
Lack of fit	6.59	0.0845	Not significant
Texture: R-Squared = 0.9963			
Model	4.89	0.0385	Significant
Lack of Fit	5.57	0.0985	Not significant
Overall Liking: R-Squared = 0.8799			
Model	2.59	0.0215	Significant
Lack of Fit	5.74	0.0875	Not significant

The cube candy shows in Figure 4. For appearance and overall liking, the linear terms for honey content and extraction time were positive. This indicates that an increase in honey content and extraction time of coffee pulp can improve appearance and overall liking. However, the quadratic term for extraction time was negative. It indicates that beyond a certain point, the influence of extraction time on these attributes will diminish. For texture, the linear terms were positive, while the quadratic terms were negative for both honey content and extraction time. This indicates that an increase in honey content and extraction time of coffee pulp can enhance texture. However, the acceptance of texture may diminish beyond a certain point. Across all regression models, we did not find a positive combined effect between honey and extraction time on sensory.



Figure 4: Cube candy from honey and coffee pulp.

4 DISCUSSION

Based on the results of the physicochemical properties, extraction time is suggested to be a key factor influencing the TSS and color parameters of the product. Longer extraction times for coffee pulp may extract more compounds, that can bind and form complexes with sugars or other soluble solids, affecting on their solubility (Jakobek, 2015). Additionally, extended extraction time increases the likelihood that the solvent and heat will break down cellular structures and penetrate the raw material, resulting in a higher yield of various substances, including pigments (Mahfoudhi et al., 2015). Likewise, the longer extraction times allow more phenolic compounds to be extracted from the coffee pulp, which is reflected in the higher TPCs. This finding is consistent with a previous study was reported that after 15- minutes extraction of polyherbal, TPCs was significantly increased compare with 5-minutes extraction (Hamad; Hartanti, 2023). In addition to the effect of extraction time, TPCs also depends on the amount of honey used. This is because honey contains a wide range of bioactive compounds, including phenolic acids and flavonoids (Al-Kafaween et al., 2023; Jiamjariyatam et al., 2024). Similarly, the results for antioxidant activity showed that the value increased with the increase in honey and the time spent extracting the substances. The result indicates a strong positive correlation between TPCs and antioxidant activities (both DPPH and FRAP). Compared with the previous study, the candies in this study showed higher antioxidant activity than pure honey (urban honey; 2-8% DPPH inhibition) and higher TPCs than other candies product that contain with coffee pulp (0.15-0.75 mg GAE/g), but lower than the functional candy containing fruits and herbs (Nicewicz; Nicewicz; Pawłowska, 2021; Yadav et al., 2021). These findings suggest that both honey and coffee pulp extract are sources of phenolic compounds with antioxidant activity, which show potential to enhance the functional properties of candy. Antioxidants can inhibit oxidative damage by scavenging or neutralizing free radicals, enhancing the activity of antioxidant enzymes, which may help against oxidative stress (Zhou et al., 2023). Grosso (2018) reported that oxidative stress is associated with non-communicable diseases (NCDs), and that antioxidant-rich foods are linked to a decreased risk of overall and NCD-related mortality including CVDs, cancers, etc. However, *in vitro* antioxidant assays are still debated due to concerns about the appropriateness of the methods used to determine antioxidant capacity in humans and the challenge of extrapolating potential effects on human health (EFSA, 2018). Therefore, *in vivo* and clinical studies on antioxidant activity and oxidative stress biomarkers are still needed to support and confirm the effects of such products on human health.

The product in this study demonstrated safety based on both microbiological and 5-HMF evaluations. No

microbial growth was detected throughout the storage period, which corresponds to the product's low water activity ($a_w < 0.4$). Additionally, the presence of natural antimicrobial compounds, which obtain from coffee pulp extract and honey (e.g., chlorogenic acid), may resulting in resistance to microbial growth (Lima et al., 2019). 5-HMF was chosen as one of the safety indicators because honey and coffee, which were used as ingredients to formulate the functional candy in this study, are recognized sources of 5-HMF (Lee et al., 2019). 5-HMF is a furanic compound that is formed by the Maillard reaction through the decomposition of 3-deoxyglucosone, or by caramelization through the dehydration of sugars after storage or heat processing (Capuano; Fogliano, 2011). It has reports regarding the potential negative effects of 5-HMF on human health, including mutagenic and genotoxic properties, which could impact consumer safety, especially when it is transformed into 5-sulfooxymethylfurfural (5-SMF) (EFSA, 2011; Monien et al., 2012; Choudhary et al., 2020). In this study, a dilute-and-shoot clean-up technique was used to prepare samples before analysis with LC-MS/MS. This technique can reduce both the cost and time associated with sample preparation. In addition, Dilute and shoot-LC-MS/MS method dementated highly effective in terms of sensitivity and linearity. Compared with other analytical methods in previous studies, it was found to be as efficient as the SPE-LC-HRMS method (Imperiale et al., 2022) and superior to the HPLC-UV and AOAC methods (Niemes et al., 2018; Pasiar; Kiriakou; Proestos, 2017). he 5-HMF levels in functional honey candy are within the normal range typically found in food products, where 5-HMF concentrations can range from 0.04 to 22,500 µg/g depending on several factors (Choudhary et al., 2020). Previous studies have reported that coffee pulp is nutrient-rich, containing high levels of carbohydrates (35%), including arabinose, glucose, sucrose, and xylose, which are precursors for the formation of 5-HMF (Urbaneja et al., 1996; Janissen; Huynh, 2018). Consistent with the findings of Özkaynak Kanmaz (2018), it was observed that the 5-HMF content increased with higher extraction temperatures and longer extraction times due to the decomposition of hemicellulose into various sugars, such as glucose, galactose, mannose, xylose, and arabinose. However, functional honey candy with coffee pulp extract in this study did not have a high 5-HMF content. This may be due to the high lignin content (31.5%) in coffee pulp, which can degrade into phenolic compounds with antioxidant activity (Janissen; Huynh, 2018; Diono; Sasaki; Goto, 2008), combined with honey, which also contains antioxidant compounds (Jiamjariyatam et al., 2024) that may reduce 5-HMF formation. Consistent with Abrantes, Moura-Nunes and Perrone (2022 was reported that phytochemical compounds that have antioxidant activity, such as gallic acid, may mitigated 5-HMF formation by preventing the oxidation of Maillard Reaction intermediate.

Consumer acceptance is the important factors for food product development. Based on RSM of sensory evaluation, texture is the major factor on consumer acceptability. Honey content and extraction time of coffee pulp have a great effect on the texture. A previous study reported that increasing the proportion of honey in candy can decrease hardness by reducing direct compression of the product and increase the coefficient of spill ability, an indirect measure of its viscosity (Yankova-Nikolova et al., 2024). Likewise, the supplementation of natural crude extracts significantly influences the decrease in hardness of food products through the interaction of phenolic compounds and other ingredients (Roudbari et al., 2024). Therefore, higher textural acceptability results from reducing the hardness of the candy to an appropriate level. These findings are consistent with the effect of isomaltulose on gummy jelly properties, which showed that changes in hardness significantly affect consumers' sensory acceptability. The impact will vary depending on the product attributes that consumers expect (Jiamjariyatam, 2018).

5 CONCLUSIONS

This study demonstrated the potential of honey and coffee pulp as bioingredients in developing functional candy with enhanced antioxidant properties. The inclusion improved physicochemical traits, increased phenolic content and antioxidant activity, met safety standards and good consumer acceptability. The optimal formula was 350g honey and 10% coffee pulp extract (10 min extraction). However, this study demonstrated dilute-and-shoot technique proved effective for 5-HMF analysis that reducing both the cost and time associated with sample preparation.

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7 AUTHORS' CONTRIBUTION

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