PROGRESS IN ENGINEERING APPLICATION AND TECHNOLOGY
e-ISSN: 2773-5303

PEAT

Vol. 7 No. 1 (2026) 634-644
https://publisher.uthm.edu.my/periodicals/index.php/peat

Evaluation of Functional Instant Coffee and Chocolate Powders for Targeting Gout-Related Oxidative Stress and Inflammation

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DOI: https://doi.org/10.30880/peat.2026.07.01.071

Article Info

Received: 12 January 2026
Accepted: 20 February 2026
Available online: 25 May 2026

Keywords

Gout, Functional beverage, Antioxidant and anti-inflammatory activity, Herbal enrichment, Instant coffee and chocolate powder

Abstract

Gout is a metabolic disorder associated with hyperuricemia, oxidative stress and inflammatory processes, leading to growing interest in functional food–based management strategies. This study reports investigation of the antioxidant and anti-inflammatory activities, as well as the physicochemical quality and safety, of instant coffee and chocolate powder formulations enriched with selected herbal extracts, turmeric (*Curcuma longa*), temulawak (*Curcuma xanthorrhiza*) and ginger (*Zingiber officinale*) supplied by an industry partner. Antioxidant capacity of both raw herbal extracts and formulated products was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) assays, together with determination of total phenolic content (TPC). Anti-inflammatory activity was assessed using an *in vitro* protein denaturation assay. Product quality and safety were examined through solubility testing, moisture content determination and heavy metal analysis (lead, cadmium and arsenic). The herbal-enriched formulations demonstrated significantly enhanced antioxidant and anti-inflammatory activities relative to non-enriched controls. DPPH radical scavenging activity ranged from 44.8–86.5% for the chocolate formulation and 94.6–98.4% for the coffee formulation, while FRAP values ranged from 0.105–0.144 mg AAE/ml and 0.143–0.178 mg AAE/ml, respectively. Concentrations of all analysed heavy metals were within permissible regulatory limits. These findings indicate that herbal-enriched instant coffee and chocolate powders exhibit favourable functional properties and support their potential application as functional beverages for mitigating oxidative stress and inflammation associated with gout.

1. Introduction

Hyperuricemia is a metabolic disorder characterised by elevated serum uric acid concentration due to increased production, impaired renal excretion or combination of both. Persistent hyperuricemia is the primary precursor to gout, a condition in which monosodium urate (MSU) crystals accumulate in joints, triggering inflammation and severe pain. In recent decades, the prevalence of hyperuricemia and gout has risen substantially, due to lifestyle factors such as high-purine diets, obesity, physical inactivity and psychological stress [15].
Current treatments such as nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids and urate-lowering

therapies are effective but may cause gastrointestinal disturbances, renal impairment and cardiovascular risks. These limitations have driven interest in complementary strategies, particularly functional beverages enriched with antioxidant and anti-inflammatory compounds. Traditional and medicinal herbs like turmeric (*Curcuma longa*), temulawak (*Curcuma xanthorrhiza*) and ginger (*Zingiber officinale*) are rich sources of bioactive compounds including curcumin, xanthorrhizol and gingerols which have been shown to protective effects against oxidative stress and inflammation [2]. Coffee and cocoa-based products, contain polyphenols and flavonoids, may further enhance these effects when combined with herbal extracts [11].

Despite the commercial availability of functional beverages, many products lack scientific validation regarding bioactivity, safety and stability. Therefore, study seeks to determine whether instant coffee and chocolate powders enriched with selected herbal extracts possess validated antioxidant and anti-inflammatory functional properties, are safe for consumption based on heavy metal assessment and exhibit acceptable physicochemical stability. Accordingly, this study aims to evaluate the functional properties of raw herbal extracts, analyse the bioactive composition and safety of the formulated products, characterise their physicochemical properties and establish a standardised analytical framework for quality assessment.

2. Methodology

This section describes the materials used, experimental procedures, characterisation techniques and data analysis strategies employed in this study. The methodology was designed to evaluate the functional properties, safety, physicochemical characteristics and stability of herbal extract–enriched instant coffee and chocolate powders using standardised analytical methods.

2. 1 Materials

The functional instant coffee and chocolate powders were supplied by an industry partner and herbal extracts which are turmeric (*Curcuma longa*), temulawak (*Curcuma xanthorrhiza*) and ginger (*Zingiber officinale*), were obtained from KIZA HERBS, Temerloh, Pahang, Malaysia. All the experiments were performed at the laboratory of Universiti Tun Hussein Onn Malaysia (UTHM), Pagoh campus. All the analytical-grade chemicals and reagents were provided by the university. Prior to analysis, samples were stored at 4 °C to maintain stability and prevent degradation of bioactive compounds.

2.2 Functional Properties Analysis

The functional properties of the samples were assessed for antioxidant activity, anti-inflammatory ability and total phenol content. All the experiments were carried out under controlled laboratory conditions, and the data were collected using the UV-Vis Spectrophotometry method.

2.2.1 DPPH Radical Scavenging Assay

The DPPH radical scavenging activity was evaluated using a 0.1 mM DPPH solution prepared in methanol. Ascorbic acid (0–1.0 mg/mL) was used as the reference standard. An aliquot of 1 mL of sample or standard solution was mixed with 2 mL of DPPH solution and incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a UV–Vis spectrophotometer [4]. All measurements were conducted in triplicate, and results were expressed as percentage radical scavenging activity.

2.2.2 Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was performed using freshly prepared FRAP reagent consisting of acetate buffer (300 mM, pH 3.6), TPTZ solution (10 mM in 40 mM HCl) and ferric chloride solution (20 mM) mixed in a ratio of 10:1:1 (v/v/v). Ascorbic acid (0.2–1.0 mg/mL) was used for calibration curve. For the assay, 100 µL of sample or standard solution was added to 3.0 mL of FRAP reagent and incubated at 37 °C for 30 minutes. Absorbance was recorded at 593 nm, [10] and results were expressed as mg ascorbic acid equivalents per mL (mg AAE/mL). All analyses were performed in triplicate.

2.2.3 Anti-inflammatory Activity

Anti-inflammatory activity was assessed using the bovine serum albumin (BSA) protein denaturation method. A 1% (w/v) BSA solution was prepared in phosphate-buffered saline (PBS, pH 6.4). Sample solutions (0.8 mg/mL) were prepared from the stock solutions, while aspirin was used as the positive control. A reaction mixture containing 0.5 mL of sample or standard solution, 0.5 mL of BSA solution and 3.5 mL of PBS were incubated at room temperature for 15 minutes, followed by heating at 70 °C for 5 minutes. After cooling, absorbance was measured at 660 nm [12]. Anti-inflammatory activity was expressed as percentage inhibition of protein denaturation. All measurements were conducted in triplicate.

2.2.4 Total Phenolic Content (TPC)

Total phenolic content (TPC) was determined using the Folin–Ciocalteu colorimetric method with gallic acid as the standard. Gallic acid calibration solutions (0.2–1.0 mg/mL) were prepared. Sample solutions (0.5 and 1.0 mg/mL) were obtained from the stock solutions. A volume of 0.5 mL of sample or standard solution was mixed with 2 mL of diluted Folin–Ciocalteu reagent (1:10, v/v). After 5 minutes, 4 mL of 7.5% (w/v) sodium carbonate solution was added. The mixture was incubated in the dark at room temperature for 30 minutes, and absorbance was measured at 765 nm [5]. TPC was expressed as gallic acid equivalents based on the calibration curve.

2.3 Safety Assessment

Heavy metal content was determined using inductively coupled plasma–mass spectrometry (ICP-MS) that was described in method [14]. Approximately 0.25 g of each powdered sample was digested with 10 mL of concentrated nitric acid (65%) on a hot plate for 2–3 hours until a clear solution was obtained. Hydrogen peroxide (30%) was added when necessary to complete digestion. The digested samples were diluted with 1% (v/v) nitric acid to obtain appropriate working concentrations. Calibration standards ranging from 0.2 to 4.0 ppb were prepared from a multi-element standard solution. Heavy metal concentrations were calculated using the corresponding calibration curves and dilution factors and expressed as μg/kg.

2.4 Physicochemical Analysis

Solubility was evaluated by dispersing 5 g of sample in 10 mL of distilled water and stirring continuously at 37 °C for 3 hours using a magnetic stirrer. The mixture was filtered to remove undissolved particles, and the absorbance of the filtrate was measured using a UV–Vis spectrophotometer as an indicator of dissolved solids [9].

2.5 Stability Assessment

Moisture content was determined using the oven-drying method. Approximately 3 g of sample was dried at 105 °C for 3 hours, cooled in a desiccator and weighed. Moisture content was calculated based on weight loss. For stability assessment, samples were stored at 4 °C [6] and moisture content measurements were conducted weekly over a two-week storage period.

2.6 Standardised Assessment

All analyses of the chocolate and coffee powders were conducted following Standard Operating Procedures (SOPs) with quality assurance and quality control guidelines. Functional properties (antioxidant, anti-inflammatory activity and total phenolic content), safety (heavy metals), physicochemical properties (solubility) and stability (moisture content) were assessed as per the SOPs. Regarding these procedures ensured reliable and traceable evaluation of product quality, safety and functionality.

2.7 Data Analysis Strategy

This study employed a quantitative data analysis approach. All experiments were conducted in triplicate, and the results were expressed as mean ± standard error. Data processing and graphical presentation were performed using Microsoft Excel.

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3. Results and Discussion

3.1 Functional Properties of Raw Material

3.1.1 Antioxidant Activity

The antioxidant activity of ginger (*Zingiber officinale*), turmeric (*Curcuma longa*) and temulawak (*Curcuma xanthorrhiza*) was evaluated using the DPPH radical scavenging assay (Table 1). All extracts exhibited strong antioxidant activity, with inhibition values exceeding 90%. Turmeric showed the highest scavenging activity (95.3%), followed by temulawak (94.5%) and ginger (91.7%). These results were consistent with their absorbance values at 517 nm, where lower absorbance corresponded to higher radical scavenging capacity.

Table 1: DPPH Radical Scavenging Activity of Selected Raw Herbal Extracts (n=3)

Ingredient Extract	Absorbance (517 nm) (mean ± SE)	DPPH scavenging activity (% Scavenging)
Ginger	0.077 ± 0.001	91.7
Turmeric	0.043 ± 0.002	95.3
Temulawak	0.051 ± 0.001	94.5

The superior antioxidant performance of turmeric and temulawak is likely attributable to their high curcuminoid content, which is known for efficient electron-donating and free-radical neutralisation properties [3]. Ginger, while slightly less active in this assay, contains phenolic compounds such as gingerols and shogaols that also contribute to its antioxidant function. The low standard errors indicate good assay reproducibility. Overall, the high DPPH scavenging activities of all three extracts confirm their suitability as antioxidant ingredients in functional food formulations. The particularly strong activity of turmeric and temulawak supports their selection as primary bioactives for products targeting oxidative stress and inflammation-associated conditions such as gout.

Next, FRAP analysis was conducted to corroborate the antioxidant findings. The reducing antioxidant capacity of ginger (*Zingiber officinale*), turmeric (*Curcuma longa*) and temulawak (*Curcuma xanthorrhiza*) were assessed based on mean absorbance values (± standard error) measured at 593 nm (Table 2). Turmeric exhibited the highest ferric reducing antioxidant power (0.269 mg AAE/ml), followed by ginger (0.216 mg AAE/ml) and temulawak (0.205 mg AAE/ml), indicating a superior electron-donating capacity for turmeric. These values corresponded with absorbance at 593 nm, where higher absorbance reflects greater reducing activity. The enhanced reducing power of turmeric is likely attributable to its higher content of redox-active polyphenols, particularly curcuminoids, which are well recognised for their strong electron-donating ability and capacity to stabilise reactive oxygen species [7].

Table 2: Ferric Reducing Antioxidant Power (FRAP) of Selected Raw Herbal Extracts (n=3)

Ingredient Extract	Absorbance (593 nm) (Mean ± SE)	FRAP Value (mg AAE / ml)
Ginger	0.371 ± 0.001	0.216
Turmeric	0.530 ± 0.001	0.269
Temulawak	0.339 ± 0.001	0.205

Ginger and temulawak, while exhibiting slightly lower reducing power, also demonstrated substantial antioxidant capacity due to the presence of phenolic compounds such as gingerols, shogaols and related curcuminoids. An inverse relationship between extract turbidity and FRAP values was observed, whereby clearer extracts exhibited higher reducing activity, suggesting that improved solubility and dispersion of phenolic compounds enhance ferric ion reduction efficiency. The low standard error values further indicate good analytical precision and reproducibility. Overall, the FRAP results confirm that all three herbal extracts possess appreciable reducing antioxidant capacity, with turmeric showing the greatest potential, supporting their suitability as antioxidant ingredients in functional food formulations targeting oxidative stress and inflammation-associated conditions.

3.1.2 Anti-Inflammatory Activity

The anti-inflammatory activity of raw herbal extracts was assessed using the BSA protein denaturation assay. As shown in Table 3, among the raw materials analysed, ginger exhibited the lowest absorbance value (0.027), corresponding to the highest percentage inhibition of protein denaturation (96.7%). This was followed by turmeric, which showed an absorbance of 0.039 and a percentage inhibition of 95.2%. In contrast, temulawak recorded the highest absorbance value (0.043) and the lowest inhibition percentage (82.7%). Since lower absorbance values indicate greater inhibition of protein denaturation and hence stronger anti-inflammatory activity [1], these results demonstrate that all three raw materials possess notable anti-inflammatory potential.

Table 3: Inhibition of Protein Denaturation of Selected Raw Herbal Extracts Determined by the BSA Assay (n=3)

Ingredient Extract	Absorbance (660 nm) (Mean ± SE)	Protein Denaturation (% Inhibition)
Ginger	0.027 ± 0.001	96.7
Turmeric	0.039 ± 0.001	95.2
Temulawak	0.043 ± 0.001	82.7

3.1.3 Total Phenolic Content

As presented in Table 4, ginger exhibited the highest total phenolic content (55.2 mg GAE/g), followed by turmeric (49.4 mg GAE/g) and temulawak (41.8 mg GAE/g). This trend is consistent with the functional assay results, in which ginger and turmeric demonstrated stronger antioxidant and anti-inflammatory activities in the DPPH, FRAP and BSA assays. These findings indicate a strong association between total phenolic content and biofunctional activity, supporting the role of phenolic compounds as key contributors to the observed antioxidant and anti-inflammatory effects.

Table 4: Percentage of Total Phenolic Content of raw herbal extract using standard of gallic acid (n=3)

Ingredient Extract	Absorbance (765 nm) (mean ± SE)	TPC Value (mg GAE/g)
Ginger	0.468 ± 0.004	55.2
Turmeric	0.970 ±0.004	49.4
Temulawak	0.427 ±0.006	41.8

3.2 Functional Properties of Formulated Instant Coffee and Chocolate Powder

3.2.1 Antioxidant Activity

The formulated instant coffee and chocolate powders exhibited enhanced antioxidant activity. As shown in Table 5, the coffee formulations demonstrated higher DPPH radical scavenging activity (94.6–98.4%) compared with the chocolate formulations (44.8–86.5%). Among the chocolate samples, CH S1 showed greater antioxidant activity (86.5%) than CH S2 (44.8%), while CO S1 exhibited the highest scavenging activity (98.4%) among the coffee formulations. The superior performance of the coffee-based products suggests more effective retention and possible synergistic interactions of antioxidant compounds within the coffee matrix. The observed variations between samples are likely attributable to differences in bioactive compound concentration and formulation composition.

Table 5: DPPH scavenging activity of formulated coffee and chocolate powder samples (n=3)

Sample by Industry	Absorbance (517 nm) (mean ± SE)	DPPH scavenging activity (% Scavenging)
Sample 1 Chocolate (CH S1)	0.125 ± 0.009	86.5
Sample 2 Chocolate (CH S2)	0.509 ± 0.008	44.8
Sample 1 Coffee (CO S1)	0.015 ± 0.0028	98.4
Sample 2 Coffee (CO S2)	0.050 ± 0.0057	94.6

As shown in Table 6, FRAP values of the formulated samples ranged from 0.105 to 0.178 mg AAE/ml, with coffee formulations exhibiting higher ferric reducing power that chocolate-based formulations. CO S1 recorded the highest FRAP value (0.178 mg AAE/ml), followed by chocolate sample 2 (0.144 mg AAE/ml) and CO S2 (0.143 mg AAE/ml) while CH S1 showed the lowest reducing capacity (0.105 mg AAE/ml). The higher FRAP values observed in coffee formulations are attributed to the presences of redox-active compounds such as phenolic and chlorogenic acids, which enhance electron-donating capacity. Overall, FRAP results are consistent with the DPPH findings, confirming that coffee-based formulations possess superior antioxidant reducing power.

Table 6: FRAP Value of formulated coffee and chocolate powder sample (n=3)

Sample by industry	Absorbance (593 nm) (mean ± SE)	FRAP Value (mg AAE / ml)
Sample 1 Chocolate (CH S1)	0.043 ± 0.007	0.105
Sample 2 Chocolate (CH S2)	0.156 ± 0.009	0.144
Sample 1 Coffee (CO S1)	0.260 ± 0.011	0.178
Sample 2 Coffee (CO S2)	0.155 ± 0.008	0.143

3.2.2 Anti-inflammatory

The anti-inflammatory activity of the formulated coffee and chocolate powder samples is presented in Table 7. The highest inhibitory activity was observed for Sample 1 Chocolate (CH S1), which inhibited the inflammatory response by 94.0 %, followed by Sample 2 Chocolate (CH S2) at 90.5 %. In contrast, Sample 1 Coffee (CO S1) and Sample 2 Coffee (CO S2) exhibited slightly lower inhibition values of 83.3% and 82.1%, respectively. As lower absorbance values indicate stronger inhibition, these results demonstrate that the chocolate formulations reduced the inflammatory response more effectively than the coffee formulations, likely due to higher levels of bioactive compounds.

Table 7: Anti-Inflammatory Activity of Formulated Coffee and Chocolate Powders Determined by the BSA Protein Denaturation Assay (n=3)

Sample by industry	Absorbance (660 nm) (mean ± SE)	Protein Denaturation (% Inhibition)
Sample 1 Chocolate (CH S1)	0.049 ± 0.005	94.0
Sample 2 Chocolate (CH S2)	0.077 ± 0.006	90.5
Sample 1 Coffee (CO S1)	0.136 ± 0.009	83.3
Sample 2 Coffee (CO S2)	0.146 ± 0.009	82.1

3.2.3 Total Phenolic Content

The total phenolic content (TPC) of chocolate and coffee powder samples was determined using the Folin-Ciocalteu assay and expressed as mg GAE/g in Table 8. Coffee samples exhibited higher TPC, with CO S2 at 113.80 mg GAE/g and CO S1 at 83.40 mg GAE/g, compared to chocolate samples CH S1 and CH S2 (28.20 and 15.40 mg GAE/g) respectively. The elevated TPC in coffee correlates with higher polyphenol levels, such as chlorogenic acid, which are known to mitigate oxidative stress and inflammation, which are key factors in gout pathophysiology. Differences in TPC between formulations likely reflect variations in composition and processing, which can affect phenolic retention [8]. While chocolate samples had lower TPC, other bioactives like flavonoids and methylxanthines may provide complementary antioxidant and anti-inflammatory effects. Overall, coffee-based formulations demonstrate greater potential as functional ingredients for gout management due to their higher phenolic content.

Table 8: TPC Value of formulated coffee and chocolate powder sample (n=3)

Sample by industry	Absorbance (765 nm) (mean ± SE)	TPC Value (mg GAE/g)
Sample 1 Chocolate (CH S1)	0.346 ± 0.009	15.40
Sample 2 Chocolate (CH S2)	0.385 ± 0.008	28.20
Sample 1 Coffee (CO S1)	0.555 ± 0.014	83.40
Sample 2 Coffee (CO S2)	0.648 ± 0.016	113.80

3.3 Safety Assessment

The concentrations of heavy metals (Cd, Pb, and As) in the formulated chocolate and coffee powders are presented in Table 9. This indicates they are safe for consumption. Among the samples, CH S1 had the highest Cd, Pb, and As levels in chocolate, while CO S2 showed the highest Pb among coffee samples. Variations in heavy metal content likely reflect differences in raw material sources, formulation composition and processing conditions. Overall, the low levels across all samples confirm minimal contamination risk, demonstrating that both chocolate- and coffee-based powders are safe for dietary use.

Table 9: Concentration of Heavy Metals (Pb, Cd, As) in Formulated Chocolate and Coffee Powder Samples (mg/kg) for safety assessment at 0.4 ppb (n=3)

Sample by industry	Cd (µg/kg) (mean ± SE)	Pb (µg/kg) (mean ± SE)	As (µg/kg) (mean ± SE)
Sample 1 Chocolate (CH S1)	2.104 ± 0.008	2.824 ± 0.008	1.363 ± 0.007
Sample 2 Chocolate (CH S2)	0.490 ± 0.004	2.313 ± 0.007	1.225 ± 0.004
Sample 1 Coffee (CO S1)	1.234 ± 0.006	2.039 ± 0.007	1.181 ± 0.007
Sample 2 Coffee (CO S2)	0.092 ± 0.002	2.694 ± 0.007	0.604 ± 0.003

The graph shows a clear trend in Figure 1 in heavy metal concentrations among the samples. CH S1 exhibited the highest level of Cd, Pb and As, while CH S2 was lower particularly for Cd and As. In coffee sample, CO S1 had higher Cd and As, whereas CO S2 showed the lowest Cd and As but slightly higher than Pb. Overall, chocolate samples tend to have higher Pb, while coffee samples vary more in Cd and As. Despite these trends, all values remain below the 0.4 ppb safety limit, confirming the powders are safe for consumption.

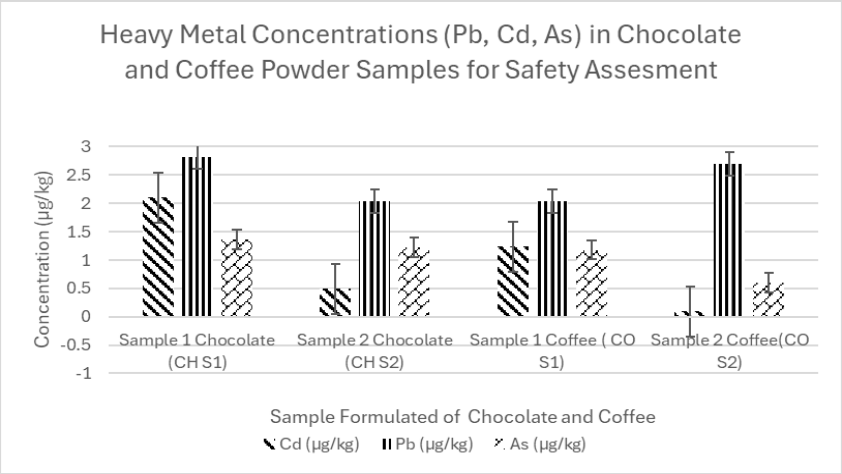

Fig. 1: The heavy metal concentrations in the formulated samples for safety assessment (n=3)

3.4 Physicochemical Analysis

Solubility is a physicochemical property which plays a significant role in determining the ability of a powdered product to dissolve in a solvent. The solubility property affects the reconstitution characteristics, appearance and consumer acceptability of a product. The solubility of the four formulated samples was determined to examine their ability for dissolution.

The solubility of the formulated chocolate and coffee powders, measured by absorbance at 280 nm, is presented in Table 10. Among chocolate samples, CH S2 (0.275 ± 0.005) showed higher solubility than CH S1 (0.205 ± 0.005). For coffee powders, CO S2 (0.277 ± 0.005) exhibited slightly higher solubility than CO S1 (0.264 ± 0.006). Overall, coffee samples demonstrated marginally higher solubility than chocolate samples, suggesting formulation and composition influence the physicochemical behaviour of the powders.

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Table 10: The solubility measurement for physicochemical characterization (n=3)

Sample by industry	Absorbance (280 nm) (mean ± SE)
Sample 1 Chocolate (CH S1)	0.205 ± 0.005
Sample 2 Chocolate (CH S2)	0.275 ± 0.005
Sample 1 Coffee (CO S1)	0.264 ± 0.006
Sample 2 Coffee (CO S2)	0.277 ± 0.005

Figure 2 illustrates the relative solubility of chocolate and coffee powders. Among chocolate samples, CH S2 showed higher solubility compared to CH S1, indicating that its formulation or particle characteristics may promote better dispersion in the solvent. In coffee formulations, CO S2 exhibited the highest solubility, slightly exceeding CO S1, suggesting that coffee powders generally have better dissolution properties than chocolate powders [6]. Overall, the trend shows that sample 2 formulations, both chocolate and coffeedemonstrate superior solubility compared to sample 1. This pattern may be attributed to differences in formulation composition, particle size distribution, or the presence of more water-soluble components. Improved solubility is desirable for physicochemical performance, as it can enhance dispersion, bioactive availability, and ease of reconstitution for the powders.

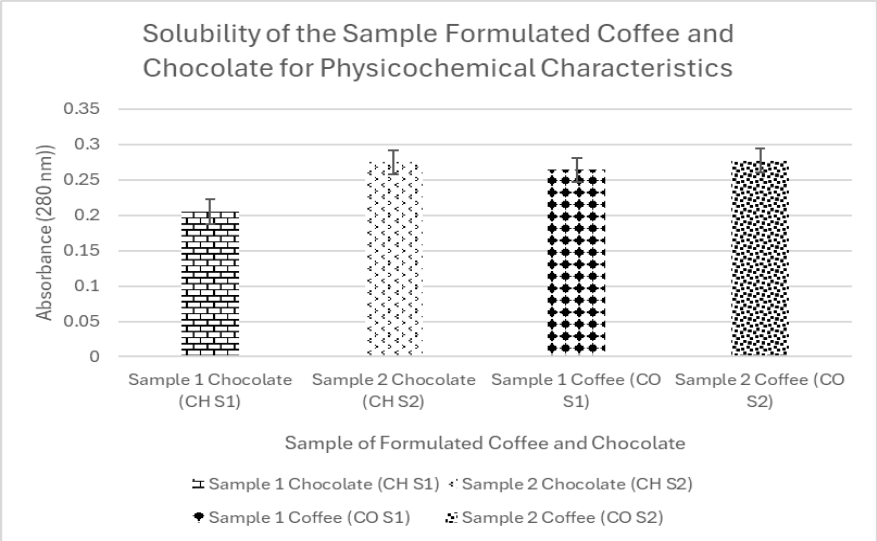

Fig. 2: The solubility testing is measured at absorbance of 280 nm for evaluation of physicochemical (n=3)

3.5 Stability Assessment

Figure 3 below shows the trend in moisture content of chocolate and coffee powders during storage. Among chocolate samples, CH S1 exhibited a slight increase, while CH S2 showed a slight decrease, indicating minor differences in moisture retention. Both coffee formulations, CO S1 and CO S2, displayed slight increases over time, suggesting small moisture uptake during storage. Overall, coffee powders tended to retain slightly more moisture than chocolate powders, but all samples maintained relatively stable moisture levels. These trends indicate good physical stability, with minor variations likely influenced by formulation differences, hygroscopic properties, or interaction with ambient humidity.

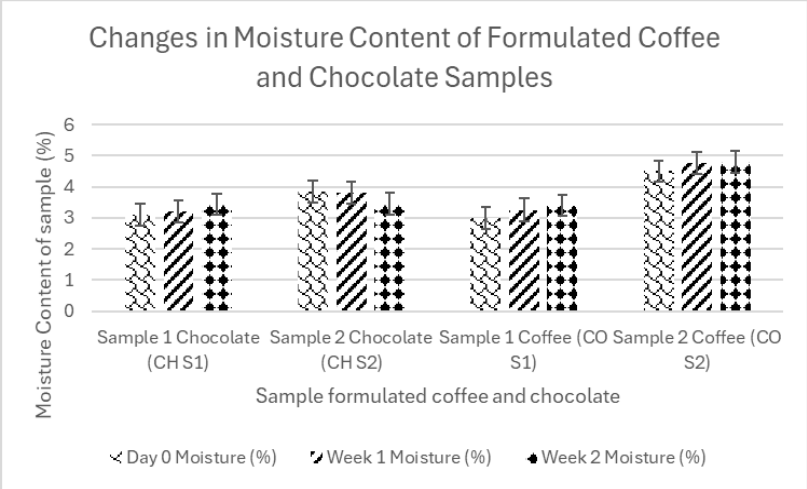

Fig. 3: Moisture content of the formulated coffee and chocolate samples during storage at 4 °C, monitored over the stability testing period

3.6 Standardised Assessment

The standard operating procedures (SOPs) applied in this study are summarised in Table 11 and were selected to comprehensively evaluate the quality, safety, physicochemical characteristics and stability of the formulated chocolate and coffee powders. Functional properties were assessed through antioxidant activity (DPPH and FRAP assays), anti-inflammatory activity using the BSA protein denaturation method and total phenolic content determination using the Folin–Ciocalteu assay. Safety evaluation was conducted via heavy metal analysis using ICP-MS, while physicochemical behaviour was assessed through solubility measurement. Stability of the formulations was evaluated by monitoring moisture content using the oven-drying method.

The use of these SOPs ensures that the analyses performed are systematic and aligned with established analytical practices. Collectively, the selected assessments provide reliable information on product functionality, safety, and stability, supporting a holistic evaluation of the formulated chocolate and coffee powders.

Table 11: Summary of SOPs used for the quality and safety evaluation of chocolate and coffee powders

No.	Assessment Category	Analysis	SOP No.
1	Functional Properties	Antioxidant Activity	
		i. DPPH Assay	SOP/AA/DPPH
		ii. FRAP Assay	SOP/AA/FRAP
		Anti- Inflammation Activity	
		i. BSA Protein Denaturation	SOP/AI/BSA
		Total Phenolic Content	
		i. Folin-Ciocalteu Assay	SOP/TPC/FCA
2	Safety Assessment	Heavy Metal Analysis	
		i. Inductively Coupled Plasma Mass Spectrometry (ICP-MS)	SOP/HM/ICPMS
3	Physicochemical Analysis	Solubility measurement	SOP/SM
4	Stability Testing	Moisture Content Determination	
		i. Oven Drying Method	SOP/MC/ODM

Conclusion

This study demonstrated that turmeric, ginger and temulawak exhibit strong antioxidant and anti-inflammatory activities, supporting their suitability as functional ingredients. Formulated instant coffee and chocolate powders effectively retained bioactivity, with antioxidant activity of 94.6% and 44.8% and anti-inflammatory potential of 82.1% and 90.5%, respectively, alongside substantial phenolic content. Physicochemical evaluation confirmed good solubility and moisture stability, while heavy metal levels were within permissible limits, ensuring product

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safety. Overall, the findings highlight the potential of herbal-enriched instant coffee and chocolate powders as evidence-based functional beverages for managing gout-related oxidative stress and establish a standardized framework for future assessment of functional powdered products.

Acknowledgement

The authors would also like to thank the Faculty of Engineering Technology, Universiti Tun Hussein Onn Malaysia (UTHM) for its support.

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