ORIGINAL ARTICLE

Food Microbiology, Safety and Toxicology

Quantification of caffeine and carbohydrates in chocolate and confectionery products from Dhaka, Bangladesh: A UV-Visible spectrophotometric approach

Md. Mazharul Islam 🛛 🕫 Sanjida Tanjid 💿 Mohammad Shoeb

Department of Chemistry, Faculty of Sciences, University of Dhaka, Dhaka-1000, Bangladesh. mazharulchdu@gmail.com / sanjida-2018426782@chem.du.ac.bd / shoeb@du.ac.bd

ABSTRACT

Background: Caffeine and carbohydrates are significant components in chocolate and confectionery products, influencing both their sensory appeal and health impact. Accurate quantification of these components is essential to provide consumers with reliable nutritional information and to inform healthier dietary choices.

Aims: This study aims to quantify the moisture and ash content, as well as determine the concentrations of caffeine and carbohydrates, using UV-Visible spectrophotometry, in selections of chocolates and candies commercially available in Dhaka, Bangladesh. Additionally, the study seeks to elucidate variations in these components across different brands and product types, thereby contributing to a better understanding of their compositional profiles.

Methods: Samples from six chocolate brands and two coffee candy brands were procured from local markets and supermarkets in Dhaka. Standard analytical methodologies were employed to determine moisture and ash content. For the quantification of caffeine and carbohydrates, standard solutions were prepared, and absorbance measurements were conducted using a UV-Visible spectrophotometer at specified wavelengths. Moisture and ash content were calculated using standard formulas, while caffeine and carbohydrate concentrations were derived from calibration curves.

Results: The moisture content in the analyzed samples ranged from 0.25% to 1.88%, while ash content varied between 5.22% and 6.90%. Caffeine concentrations were found to range from 24.18 \pm 2.51 mg.kg⁻¹ in Perk chocolate to 60.12 \pm 0.85 mg.kg⁻¹ in KitKat (70% Dark). Carbohydrate content exhibited considerable variation, with values ranging from 2.03 \pm 0.01 g/100 g in Coffee Bite to 41.05 \pm 0.46 g/100 g in Perk chocolate per 100 g. Dark chocolate samples demonstrated higher caffeine levels compared to milk and white chocolate varieties, consistent with their elevated cocoa solid content.

Conclusions: The study revealed significant variability in moisture, ash, caffeine, and carbohydrate content across different chocolate and candy brands. These findings emphasize the necessity of rigorous compositional analysis for quality assurance and public health considerations.

Keywords: Caffeine, Carbohydrates, Chocolate, Candy, UV-Visible Spectrophotometry.

1 INTRODUCTION

The global consumption of chocolates and confectionery products, particularly among children and young adults, continues to rise, including in Bangladesh. In 2022, Bangladesh's chocolate exports reached USD 6.94 million, ranking it 83rd globally. Chocolate constituted the 135th most exported product from the nation that year, with primary destinations including India (USD 3.98 million), Vietnam (USD 838,000), Australia (USD 610,000), Nepal (USD 543,000), and Bolivia (USD 196,000) (OEC, 2022). In





Corresponding author: Md. Mazharul Islam E-mail: mazharul@du.ac.bd Tel. +88 (01737341540)

> **Received:** July 11, 2024 **Revised:** January 09, 2025 **Accepted:** January 19, 2025 **Published:** February 06, 2025

Article edited by:

Prof. Khaled Méghit Boumédiène

Article reviewed by:

Dr. Md. Iqbal Rouf Mamun Dr. Sabiha Achat

Cite this article as: Islam, M.M., Tanjid, S., & Shoeb, M. (2025). Quantification of caffeine and carbohydrates in chocolate and confectionery products from Dhaka, Bangladesh: A UV-Visible spectrophotometric approach. *The North African Journal of Food and Nutrition Research, 9* (19): 57–66. https://doi.org/10.51745/najfnr.9.19.57-66

© 2025 The Author(s). This is an open-access article. This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third-party material in this article are included in the article's Creative Commons license, unless indicate otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is no permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.

2022, Bangladesh imported USD 17.9 million worth of chocolate, positioning it as the 104th largest importer worldwide. Chocolate represented the 396th most imported product within the country. Major supplying nations comprised Malaysia, India, Sri Lanka, Singapore, and the Netherlands with the most rapid import growth observed from Malaysia, India, and Sri Lanka (OEC, 2022). The primary consumers of chocolate and confectionery are children, adolescents, and young adults, driven by a preference for sweet flavors. Urban populations and middle-



to-high-income groups also contribute significantly to consumption, especially during celebrations and festive occasions. This substantial consumption can be attributed to chocolate's capacity to elicit sensory pleasure and induce positive emotional responses (Kusumadevi et al., 2021). Chocolate's distinctive qualities contribute to its widespread appeal. It is available in three varieties: dark chocolate, white chocolate, and milk chocolate. Milk chocolate constitutes the largest segment of consumption accounting for 40% of the total (Nafingah et al., 2019). The olfactory profile of chocolate is determined not solely by the specific cocoa variety utilized but also, significantly by the presence of amorphous sugar. This interaction between cocoa type and sugar morphology is crucial in defining the aromatic characteristics of the final chocolate product (Saputro et al., 2017).

Moisture content is a key determinant of food quality, preservation, and shelf-life (Nielsen, 2017). Accurate moisture measurement is essential for the analysis of other food components; the remaining residue after analysis, termed total solids, is vital for carbohydrate calculation. Elevated moisture levels can accelerate spoilage and reduce shelf life, making its assessment crucial for storage and processing considerations (Kirk & Sawyer, 1991). Although various methods exist for moisture determination, achieving precise and reliable results can be challenging. Ash content, the inorganic residue remaining after the complete oxidation or ignition of organic matter, primarily reflects the mineral content of food (Pomeranz & Meloan, 1994). Ash content measurement is an integral aspect of proximate analysis for nutritional evaluation (Ismail, 2017), serving as an indicator of product quality, potential adulteration, and nutritional value (Harris & Marshall, 2017). Collectively, these measurements offer valuable insights into a sample's characteristics, supporting quality control procedures and ensuring compliance with industry standards (Del Río-Celestino & Font, 2020).

Caffeine (1,3,7-Trimethylxanthine; $C_8H_{10}N_4O_2)$ is a naturally occurring xanthine alkaloid predominantly found in *Coffea arabica* (coffee), *Camellia sinensis* (tea), and *Theobroma cacao* (cocoa) (Institute of Medicine CMNR, 2001). Characterized by its stimulant, antioxidant, antiinflammatory, and analgesic properties, caffeine is a key ingredient in several over-the-counter medications (Vieira *et al.*, 2020). As the most prominent compound in coffee, it is also prevalent in soft drinks, chocolates, confectionery, and energy drinks, making it one of the most consumed substances globally (Saraiva *et al.*, 2023). Cocoa beans, the source of cocoa mass and cocoa butter, are fundamental to the production of dark, milk, and white chocolate, with regional variations in ingredient proportions (Katz *et al.*, *al.*, 2011). Additionally, caffeine is present in over 60 other plant species (Ashihara *et al.*, 2017). Coffee, valued for its distinctive flavor and significant stimulant and psychoactive effects, is a globally ubiquitous beverage (Roehrs & Roth, 2008). This widespread consumption is driven by coffee's unique organoleptic attributes and its capacity to enhance physiological alertness and cognitive performance (Bhandari *et al.*, 2023). Health and regulatory bodies have recently emphasized the potential risks associated with caffeine consumption for specific populations, including pregnant and breastfeeding women, children, adolescents, young adults, and individuals with pre-existing cardiovascular conditions and other specific health concerns, underscoring the significant disparity in effects between normal and excessive doses (Temple *et al.*, 2017).

Chocolate contains substantial amount of carbohydrates, primarily from added sugars and smaller quantities of naturally occurring starches, the content of which varies by type. While cocoa beans naturally contain carbohydrates, additional sugars and other carbohydrate sources are frequently incorporated during chocolate production to achieve the desired organoleptic characteristics (Khowala et al., 2008). Carbohydrates, the most abundant organic compounds, are essential nutrients and the primary energy source. Even non-digestible carbohydrates are crucial for a balanced diet (Belitz et al., 2009). Carbohydrates constitute a major component of chocolates, confectionery, and energy drinks. Determination of total carbohydrate content is essential for several reasons, including the assessment of food quality based on sweetness, stability, appearance, and texture. This information is also vital for energy evaluation, nutritional labeling, and detecting adulteration. Moreover, understanding carbohydrate content facilitates the correlation of dietary intake with health outcomes (Menezes et al., 2004).

While advanced analytical techniques such as High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) (Müller et al., 2014) are commonly employed for caffeine and carbohydrate analysis, their accessibility I soften limited to their complexity and cost. Caffeine concentrations are typically measured using HPLC-Mass Spectrometry/Mass Spectrometry (HPLC-MS/MS) in human biological samples (Geraghty et al., 2015), and GC with Nitrogen-Phosphorus Detection (GC-NPD) (Sereshti & Samadi, 2014) or HPLC with Photodiode Array Detection (HPLC-PDAD) (Zhao et al., 2014) for beverages such as coffee and tea. GC-MS/MS is also employed for caffeine analysis in chocolate and other matrices, although research on caffeine in chocolates and confectionery in Bangladesh is limited (Lisko et al., 2016). Conversely, UV-Visible spectrophotometry offers a cost-effective and straightforward method for determining caffeine and total carbohydrate

content, providing high accuracy and reproducibility even with small sample quantities (Al-Bratty *et al.*, 2020). The limitations of UV-Visible spectrophotometry in Bangladesh include outdated equipment, substandard reagents, and inadequate maintenance, all of which compromise analytical accuracy. Adverse environmental conditions further degrade instrument performance and sample stability, reducing the method's reliability for rigorous studies. This study aims to analyze moisture and ash content, and to quantitatively determine caffeine and carbohydrate levels in various chocolates and confectionery, including dark, milk, and white varieties, available in Dhaka, Bangladesh, using UV-Visible spectrophotometry. The study will elucidate variations in caffeine and carbohydrate compositions, providing insights into the diverse profiles of these confectionery products.

2 METHODS

2.1 Samples

In the investigation of caffeine and carbohydrate content in chocolate and confectionery products sourced from Dhaka city, Bangladesh, a rigorous and systematic sample collection protocol was implemented. Six brands of chocolate, namely Amul Milk Chocolate, Cadbury Dairy Milk, Snickers, KitKat (70% Dark), Perk and Toblerone, alongside two brands of coffee-flavored candies. Coffee Bite and Kopiko, were procured from local markets and supermarkets. To preserve their chemical integrity, the collected samples were immediately refrigerated, thereby preventing potential alterations or degradation in their composition (Ouhakki et al., 2024). Additionally, meticulous verification of batch numbers and expiration dates was conducted to ensure that only fresh and valid products in the analysis. This step was critical to eliminate any potential biases arising from the use of expired or compromised samples.

2.2 Instrumentation and chemicals

This study utilized a comprehensive array of laboratory instruments and chemical reagents to quantify the caffeine and carbohydrate content in the chocolate and candy samples. Distilled water was utilized as the primary solvent throughout the analysis. Concentrated sulfuric acid (H₂SO₄, 98%) and phenol were procured from Merck, Germany. Standard caffeine was obtained from Beximco-Pharma Limited; Bangladesh and extra pure D-glucose was purchased from Aldrich Chemical Co. Ltd and stored at 0°C in a refrigerated environment. All glassware and plasticware were thoroughly cleaned and sterilized prior to use to avoid contamination. The analytical instruments employed included an analytical balance (Model AL 104, Mettler Toledo, USA), an Electric balance (Model FR-200, NDO-450ND, Japan), a double-beam UV spectrophotometer (Model UV-1800, Shimadzu), an oven and furnace (Model



2.3 Moisture content and ash content

The Moisture and ash content of the samples were determined in accordance with established standard methods (Ahn et al., 2014; AOAC, 2001). For moisture content determination, pre-clean and dried porcelain crucibles were weighed empty and subsequently filled with a measured quantity of powdered sample. The crucibles were then placed in an oven and dried at 105°C for three hours. After cooling in a desiccator, the final weight of the crucible containing the dried sample was recorded to calculate the moisture content. For ash content determination, the crucibles were heated at 105°C for three hours, followed by further heating at 700°C for about four hours. After cooling, the weight of the crucible containing the residual ash was measured to determine the ash content. The moisture and ash content were calculated using the following equations provided, ensuring precise quantification of the samples' composition:

 $Moisture \ content(\%) = \frac{weight \ before \ heating-weight \ after \ heating}{weight \ before \ heating} \times 100$ $Ash \ content(\%) = \frac{weight \ of \ ash}{weight \ of \ the \ sample} \times 100$

2.4 Preparation of standard caffeine solutions

The standard caffeine, stored at 0°C under refrigerated conditions, was prepared for analytical purposes by dissolving 0.01 g of the compound in 100 mL of distilled water. This primary standard solution was meticulously labeled with details including the identity of the standard, the solvent used, and its concentration. The primary solution served as the foundation for preparing secondary standard solutions through precise volumetric transfers. Working standard solutions were subsequently generated via serial dilution of the secondary standard solution, yielding concentrations of 1, 2, 4, 10, 20, 40, and 60 mg L⁻¹. The absorbance of these solutions was measured using a double-beam UVspectrophotometer to construct a calibration curve, which facilitated the accurate quantification of caffeine concentrations in the analyzed confectionery products. The maximum absorbance wavelength (λ -max) for the working standard solutions was identified at 273 nm (Habtamu & Belay, 2020). The resulting calibration curve is illustrated in Figure 1.



M. Islam et al



Figure 1. Calibration curve of standard solutions of caffeine



For the determination of caffeine, 0.50 g of each finely powdered chocolate and candy sample was dissolved in 100 mL of distilled water. A vortex mixer was employed to ensure the formation of a homogenous mixture. The resulting solution was carefully filtered, and the filtrate was used to measure absorbance with a double-beam UV spectrophotometer equipped with a 1 cm cell made of quartz cuvette. Prior to sample analysis, the spectrometer was calibrated using a blank solution, which consisted of distilled water in the case of caffeine determination. All sample solutions were prepared following this protocol, and absorbance measurements were recorded in triplicate for each sample to calculate the mean caffeine concentration, standard deviation (SD), and relative standard deviation (RSD). The absorbance measurements were conducted within a wavelength range of 240 – 350 nm, as depicted in Figure 2.





Figure 2. UV-Spectrum of two chocolate samples Abs. Absorbance

2.6 Preparation of standard D-Glucose solutions

A 2 mg quantity of D-glucose was combined with 3 mL of concentrated sulfuric acid (H₂SO₄, 98%). The mixture was thoroughly agitated using a vortex for one minute to ensure complete dissolution. Following the preparation of the standard solution, a series of diluted solutions with varying concentrations were prepared by withdrawing precise volumes of the initial solution and diluting them with concentrated H₂SO₄. The concentrations of the resulting solutions were 12.5, 60, 100, 150, and 200 mg L⁻¹. Subsequently, 3 mL aliquots from each volumetric flask were



Figure 3. Calibration curve for standard D-glucose at different concentration

transferred into separate test tubes, and 50 μL of 80% aqueous phenol was added to each tube. The mixtures were vortexed for 1 minute to ensure homogeneity. The λ -max for

the working standard solutions was identified at 489 nm (Sultana *et al.*, 2012). The calibration curve derived from these measurements is presented in Figure 3.

2.7 Quantification of total carbohydrate content

For the determination of total carbohydrate content, 0.10 g of each sample was dissolved in 100 mL of distilled water. A homogenous mixture was achieved using a combination of sonication and vortex mixing. A 50 mL aliquot of this solution was further diluted with distilled water to a final volume of 100 mL. From this diluted solution, 3 mL was transferred to a test tube, and 50 µL of 80% aqueous phenol and 3 mL of concentrated H₂SO₄ (98%) were added. Each sample solution was prepared uniformly following this standardized protocol. The Absorbances of the solutions was measured using a double-beam UV spectrophotometer equipped with a 1 cm quartz cuvette. Prior to sample analysis, the spectrophotometer was calibrated using a blank solution, which consisted of sulfuric acid for carbohydrate determination. Absorbance measurements were conducted within a wavelength range of 400 - 600 nm. At these



Figure 4. UV-Spectrum of two chocolate samples containing D-glucose Abs. Absorbance

specified wavelengths, the absorbance of each sample solution was recorded, as depicted in (Figure 4).

3 RESULTS AND DISCUSSION

Chocolate is characterized as a low-moisture food product with high concentrations of sugar and fat (Sun et al., 2023). To ensure optimal quality and safety, moisture levels in chocolate are meticulously monitored and regulated (AZoM, 2021). Moisture content plays a critical role in determining the texture of confectionery products, influencing whether they exhibit a hard or soft consistency (Jackson, 1995). Confections generally possess lower moisture content compared to various other food categories; for instance, the moisture content in hard candies ranges from 1% and 2% (Ergun et al., 2010). When moisture levels fall below 0.60%, microbial growth is effectively inhibited, rendering products such as caramel, toffee, jellies, gum, hard candy, and chocolate resistant to microbial contamination (Minifie, 1999). Moisture levels between 0.5% and 1.5% do not significantly affect the rheological properties of chocolate. However, when water content exceeds 2%, the product exhibits anomalous rheological behavior. Elevated moisture levels cause chocolate particles to adhere, leading to agglomeration, which increases viscosity and hardness, ultimately compromising the flow properties of the chocolate matrix (Afoakwa et al., 2008).

As illustrated in Figure 5, the moisture content of the analyzed samples varied slightly, with none exceeding 2%. This indicates that the quality, texture, and hardness of the chocolate and candy samples were maintained within acceptable limits. The highest moisture content was observed in Toblerone (1.88%), while the lowest was recorded in Cadbury Dairy Milk (0.25%). These variations in moisture content can be attributed to differences in the ingredients



Figure 5. Graphical representation of moisture content in different chocolate and candy



used by various brands, highlighting the influence of formulation on moisture levels.

The analysis of ash content in chocolate and candy samples revealed minor variations among the products, as illustrated in Figure 6. Ash content, which reflects the total mineral content, is a critical factor for assessing the nutritional value and quality of food products (Ismail, 2017). The results



Figure 6. Graphical representation of Ash content in different chocolate and candy

was detected in KitKat (70% Dark), a dark chocolate sample. This finding aligns with numerous studies indicating that dark chocolates contain higher caffeine levels compared to other chocolate varieties (Ludwig *et al.*, 2014; Jeon *et al.*, 2019). In contrast, the lowest caffeine content was observed in Perk, a wafer chocolate. Wafer chocolates typically contain additional ingredients such as sugar, hydrogenated vegetable fat, refined wheat flour, milk solids, starch, cocoa solids, and emulsifiers, which contribute to their lower caffeine content. Milk chocolate intermediate caffeine levels.

The European Food Safety Authority (EFSA) has established that a daily caffeine intake of up to 400 mg for adults, 200 mg for pregnant or lactating women, and 100 mg for children is considered safe (EFSA, 2015; AACAP, 2020). Exceeding these thresholds or abruptly discontinuing caffeine consumption can lead to adverse physiological effects, including anxiety, insomnia, hallucinations, hypertension, headaches, gastrointestinal disturbances, diuresis, dehydration, tremors, palpitations, and cardiac arrhythmias due to the stimulant properties of caffeine (Addicott *et al.*, 2009; Jahrami *et al.*, 2020; Weibel *et al.*, 2021). The EFSA has also noted a lack of sufficient data regarding the safety of

Brand Name (n=8)	Caffeine con	tent (mgkg ⁻¹)	Carbohydrate content (g/100g)		
	Mean ± SD	RSD (%)	Mean ± SD (g/100g)	RSD (%)	
Amul Milk Chocolate	36.93 ± 1.13	2.06	18.94 ± 0.68	1.44	
Cadbury Dairy Milk	28.60 ± 5.38	1.88	13.86 ± 0.35	2.54	
Snickers	33.29 ± 1.68	1.56	15.54 ± 0.17	1.09	
KitKat (70% Dark)	60.12 ± 0.85	1.42	12.03 ± 0.33	2.76	
Perk	24.18 ± 2.51	1.33	41.05 ± 0.46	1.12	
Toblerone	49.19 ± 0.46	0.93	7.78 ± 0.14	1.84	
Coffee Bite	30.24 ± 0.13	0.42	2.03 ± 0.01	0.99	
Kopiko	28.80 ± 0.72	2.50	2.68 ± 0.05	1.93	

Note. Three replicates were done for each type of brand

indicated that Toblerone chocolate samples exhibited the highest ash content (6.90%), indicative of a higher mineral composition. These variations can be attributed to differences in product composition and the inclusion of various additives. The ash content across all samples ranged from 5.22 - 6.90%. The observed variability in ash content among different types of chocolates and candies underscores the influence of ingredients and processing methods on mineral composition. Higher ash content is often associated with enhanced nutritional benefits, such as elevated levels of essential minerals including magnesium, iron, and potassium.

The study revealed that the average caffeine content in the chocolate and candy samples ranged from 24.18 ± 2.51 to 60.12 ± 0.85 mg kg⁻¹ (Table 1). The highest caffeine content

caffeine consumption in children and adolescents (Saraiva *et al.*, 2023). None of the analyzed samples contained caffeine levels exceeding the permissible limit of 400 mg kg⁻¹ (EFSA, 2015). These regulatory limits are in place to ensure that chocolates maintain safe caffeine levels, especially given the potential for cumulative caffeine intake from other dietary sources. Excessive caffeine consumption can lead to adverse health effects, necessitating careful monitoring of caffeine content in food products. The standard deviation (SD) values ranged from 0.13 to 5.38, reflecting the variability and diversity of the measured caffeine concentrations.

Carbohydrates play a vital role in providing energy, influencing blood sugar levels, and supplying dietary fiber. An analysis on chocolate and candy samples revealed



carbohydrate contents ranging from 2.03 ± 0.01 to 41.05 ± 0.46 g/100 g. Wafer chocolates, such as Perk, exhibited the highest carbohydrate content which can be attributed to the inclusion of additional ingredients. In contrast, dark chocolates generally contained lower carbohydrate content compared to milk chocolates. Among the samples, Kopiko and Coffee Bite, both coffee-flavored candies, recorded the lowest carbohydrate content. The low standard deviations (ranging from 0.01 to 0.68) indicate consistent and reproducible measurement results, underscoring the reliability of the analytical methodology employed. The observed variability in carbohydrate content is attributed to the inclusion of ingredients such as nuts, caramel, or fillings, which contribute to significant differences in the total carbohydrate content of chocolates and candies.

The relative standard deviation (RSD) values, presented in Table 1, demonstrate the precision and reproducibility of the analytical methods used to determine caffeine and carbohydrate content in the chocolate and candy samples. For caffeine analysis, RSD values ranged from 0.42% in Coffee Bite, indicating high precision, to 2.50% in Kopiko, suggesting greater variability. All RSD values for caffeine were below 3%, confirming the reliability of the results. For carbohydrate analysis, RSD values varied from 0.99% in Coffee Bite, reflecting excellent precision, to 2.76% in KitKat (70% Dark), which exhibited more variability likely due to the complex composition of dark chocolate. Overall, the analytical method employed in this study demonstrated robustness and consistency, ensuring accurate quantification of the target analytes.

The analysis of caffeine content across various chocolate and candy brands revealed significant variability in the maximum permissible daily consumption levels for different demographic groups, as detailed in Table 2.

For adults, Amul Milk Chocolate can be consumed in quantities of up to 271 packs daily, while Toblerone, which contains the highest caffeine content per pack, has a lower limit of 81 packs. Pregnant women and children, being more sensitive to caffeine, have substantially lower thresholds. Coffee Bite and Kopiko, owing to their minimal caffeine content, allow the highest number of packs per day for these groups. Importantly, the recommended consumption limits for each brand far exceed the actual caffeine content, which is a positive indicator for consumer safety. This variability highlights the importance of monitoring caffeine intake, particularly for sensitive populations, to prevent potential health risks associated with excessive consumption.

The comprehensive analysis of moisture and ash content, as well as caffeine and carbohydrate levels, in various chocolate and candy samples from Dhaka, Bangladesh, revealed significant variability influenced by product type and composition. Dark chocolates were found to contain the highest caffeine levels, while wafer chocolates exhibited elevated carbohydrate levels due to additional ingredients. The UV-Visible Spectrophotometric method proved to be an effective and reliable technique for the accurate quantification of these components. These findings emphasize the importance of monitoring these parameters for quality control and public health, especially given the widespread consumption of these products. This study underscores the necessity for stringent regulatory standards to ensure consumer safety and product consistency in the confectionery industry.

4 CONCLUSIONS

The analysis of chocolate and candy samples revealed minor variations of moisture and ash content, alongside significant differences in caffeine and carbohydrate contents, which were primarily influenced by product type and ingredient composition. Moisture content ranged from 0.25% in Cadbury Dairy Milk to 1.88% in Toblerone, ensuring that all samples maintained appropriate texture and quality standards. Ash content was highest in Toblerone (6.90%), indicative of its superior mineral composition relative to other

	Packing		Adult		Pregnant women		Children	
Brand Name	(Volume) (g)	Caffeine (mg/pack)	Daily intake	Max. Cons. (pack)/ day	Daily intake	Max. Cons. (pack)/ day	Daily intake	Max. Cons. (pack)/ day
Amul Milk Chocolate	40.0	1.477		271		135		68
Cadbury Dairy Milk	20.0	0.572		699		350		175
Snickers	53.0	1.765		227		113		57
KitKat (70% Dark)	41.0	2.465	400 mg	163	200 mg	81	100 mg	41
Perk	25.0	0.605	maximum	661	maximum	330	maximum	165
Toblerone	100.0	4.919		81		41		20
Coffee Bite	4.0	0.121		3305		1653		826
Kopiko	4.0	0.115		3478		1739		869

Table 2. Maximum consumption level of the targeted chocolates and candy for the adult, pregnant women and children

Note. Max. Cons.: maximum consumption



samples. Caffeine content was most concentrated in dark chocolates, such as KitKat (70% Dark), with a value of 60.12 mg kg⁻¹, whereas wafer chocolates, such as Perk exhibited the lowest caffeine levels at 24.18 mg kg⁻¹, underscoring the influence of ingredient composition on caffeine concentration.

With respect to carbohydrate content, Perk, a wafer chocolate, recorded the highest value at 41.05 g/100g, while products such as Coffee Bite and Kopiko demonstrated significantly lower levels, reflecting the variability in ingredient profile. These differences highlight the distinct characteristics of each product type and emphasize the importance of considering ingredient composition in determining nutritional value and quality.

Overall, the current study underscores the necessity of monitoring these components to ensure product consistency and safeguard consumer safety. The findings provide valuable insights that can enhance consumer awareness, inform health considerations, and support regulatory compliance within the food industry. By offering detailed nutritional insights, this research contributes to a deeper understanding of the compositional differences among chocolate and candy products, ultimately promoting informed decision-making and improved product standards.

Acknowledgment: The authors are grateful to International Science Programme (ISP), Uppsala University, Sweden for financial support.

Source of funding: Currently not available.

Previous submissions: Not applicable.

Authors' Contribution: Md. Mazharul Islam: Conceptualization, Data curation, Investigation, Methodology, Software, Supervision, Visualization and Writing-original draft; Sanjida Tanjid: Formal analysis, Visualization, Writing– review & editing; Mohammad Shoeb: Data curation, Funding acquisition, Project administration, Resources, Writing – review & editing.

Conflicts of Interest: The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Preprint deposit: None.

REFERENCES

- AACAP (American Academy of Child and Adolescent Psychiatry). (2020). Caffeine and children. Retrieved 9 June 2024 from https://www.aacap.org/AACAP/Families_and_Yout h/Facts_for_Families/FFF-Guide/Caffeine_and_Children-131.aspx [Publisher]
- Addicott, M. A., Yang, L. L., Peiffer, A. M., Burnett, L. R., Burdette, J. H., Chen, M. Y., Hayasaka, S., Kraft, R. A.,

Maldjian, J. A., & Laurienti, P. J. (2009). The effect of daily caffeine use on cerebral blood flow: How much caffeine can we tolerate? *Human Brain Mapping*, *30*(10), 3102–3114. https://doi.org/10.1002/hbm.20732 [Crossref] [PubMed] [Google Scholar] [Publisher]

- Afoakwa, E. O., Paterson, A., Fowler, M., & Ryan, A. (2008).
 Flavor Formation and Character in Cocoa and Chocolate:
 A Critical review. *Critical Reviews in Food Science and Nutrition*, 48(9), 840–857.
 https://doi.org/10.1080/10408390701719272 [Crossref]
 [PubMed] [Google Scholar] [Publisher]
- Ahn, J. Y., Kil, D. Y., Kong, C., & Kim, B. G. (2014). Comparison of Oven-drying Methods for Determination of Moisture Content in Feed Ingredients. *Asian-Australasian Journal of Animal Sciences*, 27(11), 1615– 1622. https://doi.org/10.5713/ajas.2014.14305 [Crossref] [PubMed] [Google Scholar] [Publisher]
- Al-Bratty, M., Alhazmi, H. A., Rehman, Z. U., Javed, S. A., Ahsan, W., Najmi, A., Khuwaja, G., Makeen, H. A., & Khalid, A. (2020). Determination of caffeine content in commercial energy beverages available in Saudi Arabian market by gas Chromatography-Mass spectrometric analysis. *Journal of Spectroscopy*, 2020, 1–9. https://doi.org/10.1155/2020/3716343 [Crossref] [Google Scholar] [Publisher]
- AOAC Official Method 2001.12Water/Dry matter (Moisture) in animal feed, grain, and forage (Plant tissue). (2023). In Oxford University Press eBooks. https://doi.org/10.1093/9780197610145.003.1388 [Crossref] [Publisher]
- Ashihara, H., Mizuno, K., Yokota, T., & Crozier, A. (2017). Xanthine Alkaloids: Occurrence, Biosynthesis, and Function in Plants. *Progress in the chemistry of organic natural products*, 105, 1–88. https://doi.org/10.1007/978-3-319-49712-9_1 [Crossref] [PubMed] [Google Scholar] [Publisher]
- AZoM. (2014). *Moisture and fat analysis in chocolate*. Retrieved 9 June 2024 from https://www.azom.com/article.aspx?ArticleID=11472 [Publisher]
- Belitz, H.-D., Grosch, W., & Schieberle, P. (2009). Food Chemistry (4th ed.). Springer. https://doi.org/10.1007/978-3-540-69934-7 [Crossref] [Google Scholar] [Publisher]
- Bhandari, B., Bansal, N., Zhang, M., & Schuck, P. (2013). Handbook of Food Powders: Processes and Properties. Woodhead Publishing. http://dx.doi.org/10.1533/9780857098672 [Crossref] [Google Scholar] [Publisher]
- Del Río-Celestino, M., & Font, R. (2020). The Health Benefits of Fruits and Vegetables. *Foods (Basel, Switzerland)*, 9(3), 369. https://doi.org/10.3390/foods9030369 [Crossref] [PubMed] [Google Scholar] [Publisher]
- EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). (2015). Scientific Opinion on the safety of



caffeine. *EFSA* Journal, 13(5). https://doi.org/10.2903/j.efsa.2015.4102 [Crossref] [Google Scholar] [Publisher]

- Ergun, R., Lietha, R., & Hartel, R. W. (2010). Moisture and shelf life in sugar confections. *Critical Reviews in Food Science and Nutrition*, 50(2), 162–192. https://doi.org/10.1080/10408390802248833 [Crossref] [PubMed] [Google Scholar] [Publisher]
- Geraghty, S. R., McNamara, K., Kwiek, J. J., Rogers, L., Klebanoff, M. A., Augustine, M., & Keim, S. A. (2015). Tobacco Metabolites and Caffeine in Human Milk Purchased via the Internet. *Breastfeeding Medicine: The Official Journal of the Academy of Breastfeeding Medicine*, 10(9), 419–424. https://doi.org/10.1089/bfm.2015.0096 [Crossref] [PubMed] [Google Scholar] [Publisher]
- Habtamu, D., & Belay, A. (2020). First order derivative spectra to determine caffeine and chlorogenic acids in defective and non-defective coffee beans. *Food Science & Nutrition*, 8(9), 4757–4762. https://doi.org/10.1002/fsn3.1723 [Crossref] [PubMed] [Google Scholar] [Publisher]
- Harris, G. K., & Marshall, M. R. (2017). Ash Analysis. In *Food Science Text Series* (pp. 287–297). Springer International Publishing. https://doi.org/10.1007/978-3-319-45776-5_16 [Crossref] [Google Scholar] [Publisher]
- Institute of Medicine (US) Committee on Military Nutrition Research. (2001). Caffeine for the Sustainment of Mental Task Performance: Formulations for Military Operations. National Academies Press (US). https://doi.org/10.17226/10219 [Crossref] [PubMed] [Google Scholar] [Publisher]
- Ismail, B. P. (2017). Ash Content Determination. In Food Analysis Laboratory Manual (pp. 117–119). Springer International Publishing. https://doi.org/10.1007/978-3-319-44127-6_11 [Crossref] [Google Scholar] [Publisher]
- Jackson, E.B. (1995). Sugar confectionery manufacture. (2nd ed.). Springer. [Google Scholar] [Publisher]
- Jahrami, H., Al-Mutarid, M., Penson, P. E., Al-Islam Faris, M., Saif, Z., & Hammad, L. (2020). Intake of Caffeine and Its Association with Physical and Mental Health Status among University Students in Bahrain. *Foods (Basel, Switzerland)*, 9(4), 473. https://doi.org/10.3390/foods9040473 [Crossref] [PubMed] [Google Scholar] [Publisher]
- Jeon, J. S., Kim, H. T., Jeong, I. H., Hong, S. R., Oh, M. S., Yoon, M. H., Shim, J. H., Jeong, J. H., & Abd El-Aty, A. M. (2019). Contents of chlorogenic acids and caffeine in various coffee-related products. *Journal of Advanced Research*, 17, 85–94. https://doi.org/10.1016/j.jare.2019.01.002 [Crossref] [PubMed] [Google Scholar] [Publisher]
- Katz, D. L., Doughty, K., & Ali, A. (2011). Cocoa and chocolate in human health and disease. Antioxidants & Redox Signaling, 15(10), 2779–2811.

https://doi.org/10.1089/ars.2010.3697 [Crossref] [PubMed] [Google Scholar] [Publisher]

- Khowala, S., Verma, D., & Banik, S. P. (2008). Biomolecules: (Introduction, Structure and Function): Carbohydrates. *National Science Digital Library*, 6, 1-93. [Google Scholar] [Publisher]
- Kirk, R. S., Sawyer, R., & Egan, H. (Eds.). (1991). Pearson's Composition and Analysis of Foods (9th ed.). Longman. http://ci.nii.ac.jp/ncid/BA13386742 [Google Scholar] [Publisher]
- Kusumadevi, Z., Saputro, A. D., Dewi, A. K., Irmandharu, F., Oetama, T., Setiowati, A. D., Rahayoe, S., & Bintoro, N. (2021). Physical characteristics of compound chocolate made with various flavouring agents produced using melanger as a small-scale chocolate processing device. IOP Conference Series. *Earth and Environmental Science*, *653*(1), 012036. https://doi.org/10.1088/1755-1315/653/1/012036 [Crossref] [Google Scholar] [Publisher]
- Lisko, J. G., Lee, G. E., Kimbrell, J. B., Rybak, M. E., Valentin-Blasini, L., & Watson, C. H. (2017). Caffeine Concentrations in Coffee, Tea, Chocolate, and Energy Drink Flavored E-liquids. Nicotine & Tobacco Research: Official Journal of the Society for Research on Nicotine and Tobacco, 19(4), 484–492. https://doi.org/10.1093/ntr/ntw192 [Crossref] [PubMed] [Google Scholar] [Publisher]
- Ludwig, I. A., Mena, P., Calani, L., Cid, C., Del Rio, D., Lean, M. E., & Crozier, A. (2014). Variations in caffeine and chlorogenic acid contents of coffees: what are we drinking? *Food & function*, 5(8), 1718–1726. https://doi.org/10.1039/c4fo00290c [Crossref] [PubMed] [Google Scholar] [Publisher]
- Menezes, E. W., de Melo, A. T., Lima, G. H., &Lajolo, F. M. (2004). Measurement of carbohydrate components and their impact on energy value of foods. *Journal of Food Composition and Analysis*, 17(3-4), 331– 338. https://doi.org/10.1016/j.jfca.2004.03.018 [Crossref] [Google Scholar] [Publisher]
- Minifie, B. W. (1989). Chocolate, cocoa, and confectionery: Science and Technology (3rd ed.). Van Nostrand Reinhold. https://doi.org/10.1007/978-94-011-7924-9 [Crossref] [Google Scholar] [Publisher]
- Müller, C., Vetter, F., Richter, E., & Bracher, F. (2014). Determination of caffeine, myosmine, and nicotine in chocolate by headspace solid-phase microextraction coupled with gas chromatography-tandem mass spectrometry. *Journal of Food Science*, 79(2), T251–T255. https://doi.org/10.1111/1750-3841.12339 [Crossref] [PubMed] [Google Scholar] [Publisher]
- Nafingah, R., Kurniasari, J., Cahyani, A., Harmayani, E., & Saputro, A. D. (2019). Investigating the impact of Palm Sap Sugar proportion and fat content on heat stability of Milk Chocolate. IOP Conference Series. *Earth and Environmental Science*, 355(1), 012043.



https://doi.org/10.1088/1755-1315/355/1/012043 [Crossref] [Google Scholar] [Publisher]

- Nielsen, S. S. (2017). Moisture content determination. In Food science text series, 105–115. https://doi.org/10.1007/978-3-319-44127-6_10 [Crossref] [Google Scholar] [Publisher]
- Observatory of Economic Complexity (OEC) Chocolate in Bangladesh. (2022). The Observatory of Economic Complexity. Retrieved June 10, 2024, from https://oec.world/en/profile/bilateralproduct/chocolate/reporter/bgd [Crossref] [Publisher]

Ouhakki H, Elfallah K, Adiba A, Hamid T, Elmejdoub N. (2024). Investigation of the water quality in Oum Er Rbia River (Morocco): A multifaceted analysis of undesirable physicochemical, substances, toxic compounds, and bacteriological traits. Tropical Journal of Product Natural Research, 8(4). https://doi.org/10.26538/tjnpr/v8i4.11 [Crossref] [Google Scholar] [Publisher]

- Pomeranz, Y., & Meloan, C. E. (1994). Ash and Minerals. In Food Analysis, (pp. 602–624). Springer US. https://doi.org/10.1007/978-1-4615-6998-5_35 [Crossref] [Google Scholar] [Publisher]
- Roehrs, T., & Roth, T. (2008). Caffeine: sleep and daytime sleepiness. *Sleep Medicine Reviews*, 12(2), 153–162. https://doi.org/10.1016/j.smrv.2007.07.004 [Crossref] [PubMed] [Google Scholar] [Publisher]
- Saputro, A. D., Van De Walle, D., Aidoo, R. P., Mensah, M. A., Delbaere, C., De Clercq, N., Van Durme, J., & Dewettinck, K. (2017). Quality attributes of dark chocolates formulated with palm sap-based sugar as nutritious and natural alternative sweetener. *European Food Research & Technology*, 243(2), 177–191. https://doi.org/10.1007/s00217-016-2734-9 [Crossref] [Google Scholar] [Publisher]
- Saraiva, S. M., Jacinto, T. A., Gonçalves, A. C., Gaspar, D., & Silva, L. R. (2023). Overview of Caffeine Effects on Human Health and Emerging Delivery Strategies. *Pharmaceuticals (Basel, Switzerland)*, 16(8), 1067. https://doi.org/10.3390/ph16081067 [Crossref] [PubMed] [Google Scholar] [Publisher]
- Sereshti, H., & Samadi, S. (2014). A rapid and simple determination of caffeine in teas, coffees and eight

beverages. *Food* chemistry, 158, 8–13. https://doi.org/10.1016/j.foodchem.2014.02.095 [Crossref] [PubMed] [Google Scholar] [Publisher]

- Sultana, A., Haque, M. S., Shoeb, M., Islam, M. S., Mamun, M. I. R., & Nahar, N. (2012). Presence of yellow 6, an artificial colour additive in orange juice. *Journal of the Bangladesh Chemical Society*, 25(1), 80–86. https://doi.org/10.3329/jbcs.v25i1.11778 [Crossref]
 [Google Scholar] [Publisher]
- Sun, S., Xie, Y., Yang, R., Zhu, M., Sablani, S., & Tang, J. (2023). The influence of temperature and water activity on thermal resistance of Salmonella in milk chocolate. *Food Control*, 143, 109292. https://doi.org/10.1016/j.foodcont.2022.109292 [Crossref] [Google Scholar] [Publisher]
- Temple, J. L., Bernard, C., Lipshultz, S. E., Czachor, J. D., Westphal, J. A., & Mestre, M. A. (2017). The Safety of Ingested Caffeine: A Comprehensive Review. *Frontiers in psychiatry*, 8, 80. https://doi.org/10.3389/fpsyt.2017.00080 [Crossref] [PubMed] [Google Scholar] [Publisher]
- Vieira, A. J., Gaspar, E. M., & Santos, P. M. (2020). Mechanisms of potential antioxidant activity of caffeine. *Radiation Physics and Chemistry*, 174, 108968. https://doi.org/10.1016/j.radphyschem.2020.108968 [Crossref] [Google Scholar] [Publisher]
- Weibel, J., Lin, Y. S., Landolt, H. P., Berthomier, C., Brandewinder, M., Kistler, J., Rehm, S., Rentsch, K. M., Meyer, M., Borgwardt, S., Cajochen, C., & Reichert, C.
 F. (2021). Regular Caffeine Intake Delays REM Sleep Promotion and Attenuates Sleep Quality in Healthy Men. *Journal of Biological Rhythms*, 36(4), 384–394. https://doi.org/10.1177/07487304211013995 [Crossref] [PubMed] [Google Scholar] [Publisher]
- Zhao, F., Lin, H. T., Zhang, S., Lin, Y. F., Yang, J. F., & Ye, N. X. (2014). Simultaneous determination of caffeine and some selected polyphenols in Wuyi Rock tea by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry*, 62(13), 2772–2781. https://doi.org/10.1021/jf4056314 [Crossref] [PubMed] [Google Scholar] [Publisher]