

Development and Characterisation of Pumpkin Seed Coffee Drink Formulations: Nutritional, Antioxidant, and Sensory Properties

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ABSTRACT

Coffee and pumpkin seeds are both well-known sources of antioxidants, but few studies have explored their combined effects. This study investigates the potential of a coffee-pumpkin seed blend to enhance nutritional intake and offer health benefits. Three coffee formulations with varying coffee-to-pumpkin seed ratios (80:20, 70:30, and 60:40) were prepared and analyzed for their nutritional and antioxidant properties. Proximate analysis, following AOAC methods, was used to determine macronutrient content, while vitamin levels were quantified using High-Performance Liquid Chromatography (HPLC). Mineral content was assessed by Atomic Absorption Spectrometry (AAS), and antioxidant capacity was evaluated using the DPPH radical scavenging assay. Shelf life was estimated using Accelerated Shelf-Life Testing (ASLT). Results showed that Formula C (60:40 ratio) had the highest protein, fat, zinc, and iron content, while Formula A (80:20) was richest in carbohydrates, fiber, and vitamin A (β -carotene). Antioxidant activity increased with pumpkin seed content, with Formula C exhibiting the highest capacity. Sensory evaluation favored Formula A, and shelf-life testing revealed it had the longest shelf life. This study highlights the potential of pumpkin seed-infused coffee as a functional beverage, with enhanced antioxidant capacity and improved nutritional profile. However, further research, including in vivo studies and clinical trials, is needed to confirm the health benefits and identify the optimal formulation for maximum efficacy.

Key words: Antioxidant; functional drink; nutritional properties; pumpkin seed coffee.

1 INTRODUCTION

Environmental changes have caused an increase in non-communicable diseases. This is triggered by an increase in free radicals due to diet and environmental pollution (Ayoka, 2022). One of the efforts to maintain the body's homeostasis is to balance between free radicals and antioxidants. If free radicals exceed the body's ability to regulate them, oxidative stress will occur. Free radicals alter fats, proteins and DNA, leading to degenerative diseases. Antioxidants can reduce oxidative stress and thus prevent degenerative diseases (Engwa; Nweke; Nkeh-Chungag, 2022). The clinical and nutritional literature frequently discusses antioxidants and free radicals. Antioxidants are necessary to stop reactive oxygen and nitrogen species from forming and to counteract their effects. These species are produced in vivo and can harm proteins, lipids, DNA, and other macromolecules. Dietary-derived antioxidants are vital in sustaining health because endogenous antioxidant defences (superoxide dismutases, H_2O_2 -removing enzymes, and metal binding proteins) are insufficient to totally prevent harm (Bouayed; Bohn, 2012). Numerous dietary components have been proposed as significant antioxidants: Though the evidence for carotenoids and related plant pigments is less, there is considerable evidence for the critical roles played by vitamins E and C. The importance of plant phenolics, particularly flavonoids, is increasingly gaining attention (Halliwell, 1996; Dias; Pinto; Silva, 2021).

Pumpkins, members of the Cucurbitaceae family and cultivated globally, are consumed as a staple vegetable in nations like Sri Lanka and India, but they have also found extensive use as a functional food (Pooja; Preethi; Ramya, 2022). Given Indonesia's rich biodiversity, there is tremendous potential to further explore pumpkin's antioxidant properties and develop functional food products. The nutritional value and health advantages of pumpkin seeds have recently garnered significant attention. A good supply of Mg, Zn, Cu, Mo, Se, and other elements are pumpkin seeds (Marques et al., 2020). Many phytochemicals, including polysaccharides, phenolic glycosides, NEFA, and proteins, have been extracted from the leaves and germinating seeds of pumpkins (Yadav et al., 2010; Khasungu; Mwaura, 2022). Although pumpkin seeds are often seen as waste, they are actually rich in bioactive substances with nutraceutical qualities (Engwa; Nweke; Nkeh-Chungag, 2022; Montesano et al., 2018). Natural bioactive substances found in pumpkin seeds, such as carotenoids, tocopherols, and sterols, have been shown in vivo trials to have a wide range of biological action on the prevention of cancer, diabetes, and hypertension (Yadav et al., 2010; Kaur, 2020). Several studies have shown the antioxidant content of pumpkin seeds. Protein hydrolysate is an antioxidant-rich content of pumpkin seeds (Sitohy et al., 2020). Pumpkin seeds also contain phenolics such as alkaloids, saponins, and flavonoids (Mala; Kurian, 2016; Tanui, 2022). Pumpkin seeds contain the highest antioxidants compared to other pumpkin parts (Hussain et

al., 2021). Antioxidants are proven to be beneficial to help overcome free radicals that trigger the occurrence of various degenerative diseases. As the environment changes and pollution occurs, the need for antioxidants also increases.

Another popular type of food that is also rich in antioxidants is coffee. Antioxidant activity in coffee is associated with chlorogenic, ferulic, caffeic and *n*-coumaric acids. In roasted coffee, melanoidin (brown pigment) is present. In addition, some publications consider caffeine and trigonelline as antioxidants. Phenylalanine formed during the roasting process shows high antioxidant activity and high content of heterocyclic compounds (Peng et al., 2021).

Coffee is one type of beverage that is popular, and Indonesians often consume it. In 2020, coffee consumption in Indonesia was around 4.9 million packages (Utama et al., 2021). A survey of respondents aged 21-30 revealed that 52.9% consumed one cup of coffee daily, while approximately 28.9% drank two to three cups per day (Hasby; Armayuda; Haq, 2020), indicating a significant coffee consumption among young adults.

Although coffee consumption contributes to the increase in blood pressure (Surma; Oparil, 2021), however, moderate coffee consumption is safe and beneficial for individuals with normotension, hypertension, heart failure and diabetes (Chrysant 2017; Palupi, 2021). Coffee contains many bioactive compounds that have cardiometabolic effects (Mena et al., 2020; Ungvari; Kunutsor, 2024). Caffeine is the main compound responsible for increasing blood pressure. Caffeine in coffee is thought to block adenosine receptors and inhibit phosphodiesterase, thereby increasing blood pressure (Ribeiro; Sebastiao, 2010). This antagonistic effect of caffeine on adenosine receptors plays a role in the mechanism of blood pressure regulation after drinking coffee (Rivera-Oliver; Díaz-Ríos, 2014).

Previous studies have highlighted the antioxidant potential of coffee. Research conducted in Tanah Datar Regency revealed that coffee powders exhibited a significant antioxidant content, ranging from 32.79% to 39.53% (Wijayanti, 2020). Additionally, phytochemical analysis of Arabica coffee beans has identified various bioactive compounds, including tannins, alkaloids, saponins, flavonoids, and steroids, which contribute to their potent antioxidant activity (the antioxidant activity of the ethanol extract of arabica coffee beans was in the very strong category with IC₅₀ 12.427 ppm and vitamin C with IC₅₀ 0.273 ppm) (Official Methods of Analysis - AOAC, 2005).

This study aims to formulate pumpkin seeds into antioxidant-rich beverages. This research consists of several stages, including the formulation of pumpkin seed coffee in three formulas, the acceptability test, nutritional content analysis, antioxidant analysis and the determination of the

shelf-life. Robusta coffee was selected for this study due to its unique characteristics, including its higher caffeine content, stronger flavour profile, and its widespread cultivation in various regions. These attributes make it a suitable candidate for investigating the impact of processing techniques on coffee quality and consumer acceptance. Additionally, robusta coffee is often more affordable than other coffee types, making it a popular choice for consumers. This study aims to leverage the antioxidant properties of pumpkin seeds by formulating them into functional beverages. To evaluate consumer acceptance, both trained sensory panellists and a consumer panel were involved in organoleptic testing. The nutritional profile of the formulations was assessed, including the analysis of macronutrients and micronutrients. Furthermore, the antioxidant activity of the beverages was determined using the DPPH method. Finally, accelerated shelf-life testing (ASLT) was conducted to predict the product's shelf life under various storage conditions.

A combination of ground pumpkin seed and coffee to create a new coffee-based drink formula is expected as one of the alternatives to increase the antioxidant intake from food, especially for coffee lovers. It is expected that combining coffee beans with pumpkin seeds will produce a beverage rich in antioxidants. In addition, efforts to formulate pumpkin seed coffee also offer new variations on the existing coffee-based products in Indonesia.

2 MATERIAL AND METHODS

2.1 Materials

Pumpkin seed samples were the Hubbard squash, a cultivar within the species *Cucurbita maxima*, a winter squash native to the Andean region of South America. These samples were subsequently ground using a grinder. The coffee ground used was commercial robusta coffee that was freshly ground with a commercial coffee grinder (burr size 80 mm). The powder was stored at -20°C until further analysis.

For chemical reagents: absolute ethanol (Riedel-de-Haën, Germany), HPLC-grade methanol (Merck, Germany), Folin-Ciocalteu phenolic reagent (Merck, Germany), and analytical grade sodium carbonate (Merck, Germany) were employed. The free radical DPPH (2,2-diphenyl-1-picrylhydrazyl, Aldrich Sigma, Germany), analytical grade aluminium chloride (Merck, Germany), 2,4-dinitrophenylhydrazine (Merck, Germany), and analytical grade potassium hydroxide were utilised for specific analyses. Trolox (Fluka, Denmark) were employed as standard, Methanol (HPLC grade), *N*-Hexane, metaphosphoric acid solution, Distilled water, Sodium chloride, Ascorbic acid standard, and β -carotene standard.

2.2 Instrumentation

Spectrophotometric measurements were conducted using a Shimadzu UV-1700 spectrophotometer equipped with 1 cm quartz cuvettes. A flame atomic absorption spectrophotometer equipped with appropriate hollow cathode lamps for the target elements (Fe, Zn, Cu) was used for the analysis. Cuisinart Supreme Automatic Burr Mill Grinder (Dbm8) mill were used for grinding the pumpkin seed.

2.3 Methods

2.3.1 Pumpkin Seed Powder

Pumpkin seeds are first cleaned to remove any remaining flesh or fibres. Then, they are dried in a low-temperature oven or air-dried until completely dry. Next, the dried seeds are roasted and ground in a food processor or blender until a fine powder is obtained. The temperature for roasting pumpkin seeds is 325°F (160°C). Roasting at this temperature ensures that the seeds become crispy and golden brown without burning. It's important to monitor the seeds closely during the roasting process, as they can quickly go from perfectly roasted to burnt. Finally, the powdered seeds are transferred to an airtight container and stored in the refrigerator or freezer to maintain freshness.

2.3.2 Coffee formulation

Coffee formulas with varying pumpkin seed powder content (60:40, 70:30, 80:20 ratios of coffee to pumpkin seed) were prepared and packaged in suitable containers (aluminium packaging). For example, to prepare 200 g of Formulation A (60:40 ratio), 120 g of ground coffee and 80 g of pumpkin seed powder were mixed. Similar calculations were used to prepare Formulations B and C.

2.3.3 Proximate Analysis (AOAC, 2005)

Proximate analysis was conducted to determine the compositional profile of the samples. Moisture content was determined by oven drying at 105°C to constant weight. Ash content was obtained by igniting the dried samples in a muffle furnace at 550-600°C. Crude protein content was estimated from total nitrogen content, determined using the Kjeldahl method, employing a nitrogen-to-protein conversion factor of 6.25. Crude fat content was extracted using a Soxhlet apparatus with N-Hexane, while crude fibre was obtained through sequential boiling in dilute acid and alkali solutions. Nitrogen-free extract (NFE) was calculated by difference as the total carbohydrates.

2.4 Minerals determination

2.4.1 Sample Preparation

Samples (5 grams) were dried at a temperature of 105 °C until constant weight was achieved. Subsequently, samples

were ashed in a muffle furnace at 550 °C. The resulting ash was digested with HNO₃ using a heating block. The digested samples were filtered and diluted to a known volume with deionised water.

2.4.2 Analytical Procedure:

Standard solutions of the target elements were prepared within a suitable concentration range. The instrument was calibrated using these standard solutions. The prepared sample digests were aspirated into the flame atomiser of the spectrophotometer, and the absorbance for each element was recorded at its respective resonance wavelength. The concentration of each element in the sample was determined from the calibration curve.

2.5 Vitamin C and β-carotene determination

2.5.1 Sample extraction

A known weight of the coffee formula (5 grams) was homogenised and extracted with a mixture of methanol and hexane (the ratio may vary depending on the matrix). The extract was filtered, and the filtrate was partitioned between methanol and water. The methanol phase containing β-carotene was collected.

2.5.2 Vitamin C Determination (Nurhasnawati et al., 2019)

The vitamin C content was determined by titrating the extract with a standardised iodine solution using starch as an indicator. The vitamin C content was calculated based on the volume of iodine solution consumed.

2.5.3 β-Carotene Determination (Hudiyanti; Fawrin; Siahaan, 2018)

Spectrophotometric analysis: The absorbance of the β-carotene extract was measured at its characteristic wavelength (450 nm) using a spectrophotometer. A standard curve was prepared using β-carotene standards of known concentrations. The β-carotene content in the sample was determined by comparing the absorbance of the sample with the standard curve.

2.5.4 Total Flavonoid Content Determination (Nurhasnawati, 2019)

To determine total flavonoid content, 0.5 mL of sample or quercetin standard solution was added to test tubes, followed by sequential addition of 0.3 mL NaNO₂ (5%), incubation for 5 minutes, 0.3 mL AlCl₃ (10%), incubation for 6 minutes, 1 mL NaOH (1 M), and incubation for 15 minutes. Absorbance was measured at 430 nm against a methanol blank. A calibration

curve was constructed using quercetin standards to quantify total flavonoid content as mg quercetin equivalents per gram dry weight. DPPH and IC_{50} .

2.5.5 Reagent Preparation

A stock solution of DPPH (0.1 mM) was prepared in methanol. Subsequently, a working solution of DPPH (60 μ M) was obtained through dilution with methanol. Sample extracts were prepared at various concentrations using methanol as the solvent.

The percentage inhibition of the DPPH radical was calculated using the following formula:

$$\text{Percentage inhibition} = \frac{[(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100}{}$$

Where Abs control represents the absorbance of the DPPH solution and Abs sample denotes the absorbance of the sample-DPPH mixture. The IC_{50} value, indicative of the sample concentration required to inhibit DPPH radical formation by 50%, was determined from the dose-response curve. Statistical analysis was performed to assess significant differences between groups.

2.6 Sensory test

A trained panel of 10 individuals with experience in sensory evaluation was selected to assess the sensory attributes of the pumpkin seed coffee formulations. The sensory attributes evaluated included appearance, aroma, flavour, texture, and overall acceptability. Panellists were instructed to rate each attribute on a 9-point hedonic scale. Data were analyzed using ANOVA to determine significant differences among formulations, followed by Tukey's HSD test for pairwise comparisons. Additionally, a consumer acceptance test was conducted involving 40 untrained panellists. The panellists were asked to rate the overall liking of the formulations on a 9-point hedonic scale. Descriptive statistics were used to analyze the consumer acceptance data.

2.7 Shelf-Life Determination Using Accelerated Shelf Life Testing (ASLT) with Arrhenius approach (Sulaiman; Armita; Erika, 2024)

Samples (each coffee formula) were stored at elevated temperatures (25°C, 35°C, 45°C) under controlled humidity conditions. Periodically, samples were removed for analysis of water content or other relevant quality parameters.

2.8 Water Content Analysis:

Water content was determined using standard gravimetric methods, involving drying the samples at a specific temperature (e.g., 105 °C) to constant weight.

2.9 Arrhenius Equation:

The relationship between water content (as a quality indicator) and temperature was modelled using the equation as shown in Equation (1):

$$k = A * \exp(-E_a/RT) \quad (1)$$

where:

k is the reaction rate constant

A is the pre-exponential factor

E_a is the activation energy

R is the gas constant

T is the absolute temperature

By plotting the natural logarithm of the reaction rate constant ($\ln k$) against the reciprocal of temperature ($1/T$), the activation energy (E_a) can be determined from the slope of the line.

2.10 Shelf-Life Prediction

The degradation rate constant (k) for each storage temperature was determined by fitting appropriate kinetic models to the quality parameter data. The Arrhenius equation ($\ln k = \ln A - E_a/RT$) was employed to relate the rate constant (k) to temperature (T). By plotting $\ln k$ versus $1/T$, the activation energy (E_a) and Arrhenius constant (A) were calculated. Subsequently, the Arrhenius equation was used to predict the shelf life at a desired storage temperature by determining the time required for a quality parameter to reach a predefined limit.

2.11 Statistical analysis

To analyze the collected data, a combination of descriptive and inferential statistical methods will be employed. Descriptive statistics, including mean, standard deviation, and range, will be used to summarize the data for sensory evaluation, nutritional analysis, and antioxidant activity. Inferential statistics, ANOVA and Tukey's HSD test, were utilized to determine significant differences among formulations for specific sensory attributes, nutrient composition, and antioxidant activity. For shelf-life studies, kinetic modeling techniques will be applied to predict product shelf life at various storage conditions. Statistical analysis will be performed using the statistical software SPSS ver. 28.

3 RESULTS

3.1 Proximate analysis

Figure 1 presents the proximate composition of the developed coffee formulations. Carbohydrates were the

predominant macronutrient, followed by protein and fat. The results indicated that carbohydrates constituted the highest macronutrient in all three formulas, followed by protein, fat, and crude fiber. The 80:20 ratio formula exhibited the highest carbohydrate content ($54.40 \pm 1.28\%$) compared to the other two formulas (70:30 and 60:40). Since the 80:20 formula contained the highest proportion of coffee (80%), it is likely that the elevated carbohydrate levels were primarily derived from the coffee powder. Conversely, protein and fat concentrations were highest (25.78% and 13.95%, respectively) in the 60:40 ratio formula, which had the highest proportion of yellow pumpkin seeds. This suggests that the protein and fat content in the coffee formulas originated predominantly from the yellow pumpkin seeds. No significant differences were observed in moisture and ash content

among the three formulas. The 80:20 formula, characterised by a higher proportion of coffee beans, exhibited the highest carbohydrate content. Conversely, the 60:40 formula, with a greater proportion of pumpkin seeds, displayed elevated protein and fat levels. These findings highlight the influence of ingredient ratios on the overall macronutrient profile of the coffee formulations.

3.2 Mineral content

Table 1 shows the mineral content (Zn, Cu, and Fe) of three coffee formulas substituted with pumpkin seed powder. The results show that the highest mineral content in the formula was Fe (456.505 ± 9.78), followed by Zn, and Cu. The substitution ratio of pumpkin seed accounted for the increase in Fe and Zn content in the coffee formula.

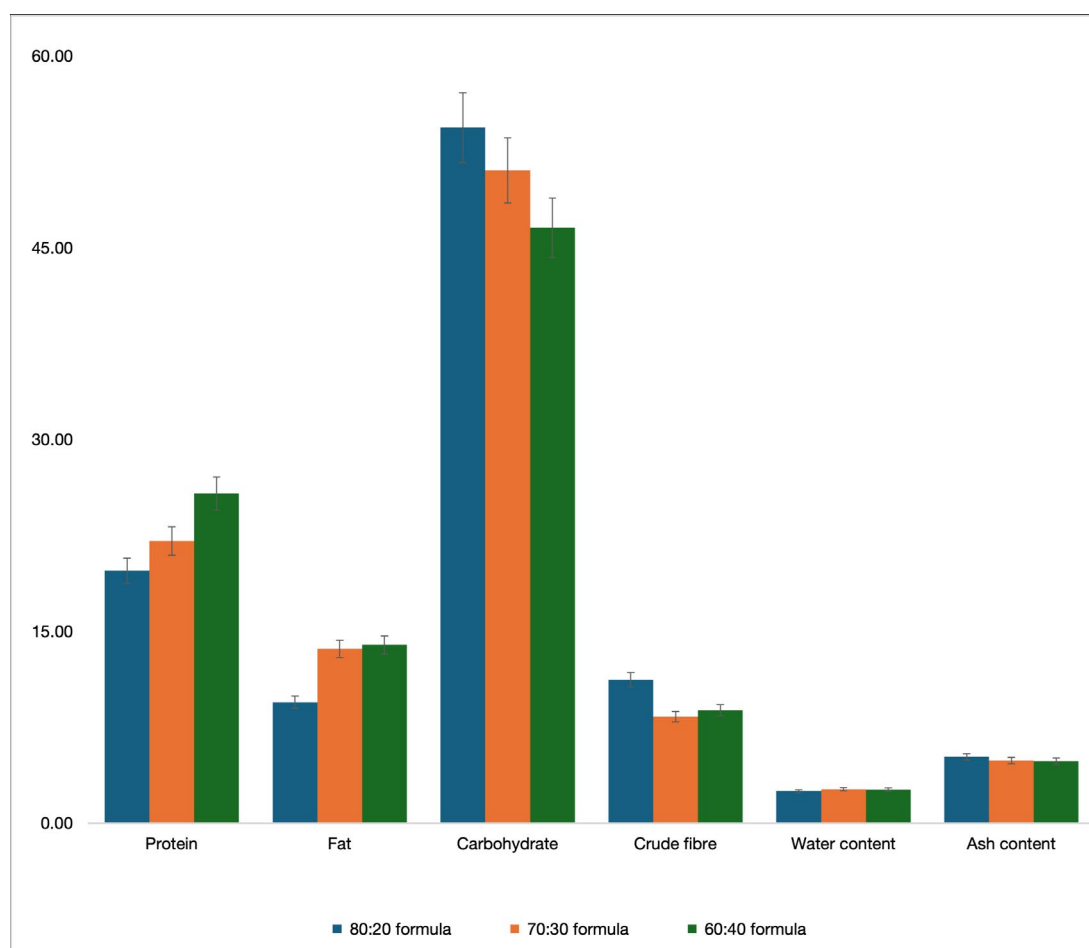


Figure 1: Proximate analysis of three pumpkin seed coffee formulas with the ratio of 80:20, 70:30, and 60:40. $n = 3$

Table 1: The mineral content (Zn, Cu, Fe) of three pumpkin seed coffee formulas with the ratio of 80:20, 70:30, and 60:40.

Formula	Zn (ppm)	Cu (ppm)	Fe (ppm)
ratio 80:20	14.55 ± 0.02	11.88 ± 1.03	138.055 ± 14.23
ratio 70:30	21.7 ± 0.07	9.79 ± 0.49	111.46 ± 12.75
ratio 60:40	22.95 ± 0.99	7.6 ± 0.17	456.505 ± 9.78

3.3 Antioxidant properties

Table 2 presents the antioxidant properties of the coffee-pumpkin seed formulations, including flavonoid content, IC50 values, and DPPH radical scavenging activity. Among the formulations, the 60:40 ratio exhibited the highest antioxidant activity, while the 70:30 ratio contained the greatest flavonoid concentration.

A comparison of β -carotene (pro-vitamin A) and vitamin C content among the three coffee formulas is presented in Figures 2 and 3. The result shows that Vitamin C content

decreased in the formula when along with the decrease in the coffee ratio in the formula, with the highest concentration in formula A (80:20) with a concentration of 45.16%. In contrast, Beta-carotene content shows an increase in the increased ratio of pumpkin seed in the formula. But the highest was in the formula 70:30 ratio.

3.4 Organoleptic analysis

Figures 4 and 5 show the organoleptic test results and the overall acceptability of the coffee formula from the trained panellists. It can be seen that based on colour, acidity, and aftertaste; formula

A is the preferred formula. Based on colour, aroma, taste, and sweetness; formula B is the preferred formula. Formula A contains the least amount of pumpkin seed mixture and the most amount of coffee. Formula B has a higher proportion of pumpkin seed mixture than formula A. Formula C, which contains the highest proportion of pumpkin seed mixture, is the least preferred formula in terms of colour, aroma, taste, and acidity. Overall, formula A (80:20) was the most preferred formula, then followed by formula B (70:30), but the difference was not significant.

3.5 Shelf life

Table 3 presents the predicted shelf life of coffee formulations based on varying water content and storage temperature. Results indicate that the 80:20 formula (highest pumpkin seed powder content) exhibited the longest shelf life at 35°C, while the 60:40 formula (lowest pumpkin seed powder content) had the shortest shelf life at the same temperature. This observation suggests a strong correlation between increased pumpkin seed powder concentration and extended shelf life. Furthermore, significant differences in shelf life were observed across the tested temperatures, emphasising the influence of storage conditions on product stability.

Table 2: The antioxidant properties of three pumpkin seed coffee formulas with the ratio of 80:20, 70:30, and 60:40

Formula	Flavonoid (%)	IC50	DPPH (%)
ratio 80:20	0.75 $\pm$ 0.05	45.17 $\pm$ 0.37	69.94 $\pm$ 0.00
ratio 70:30	0.92 $\pm$ 0.08	39.07 $\pm$ 1.69	79.16 $\pm$ 0.08
ratio 60:40	0.87 $\pm$ 0.04	32.43 $\pm$ 2.34	80.48 $\pm$ 0.33

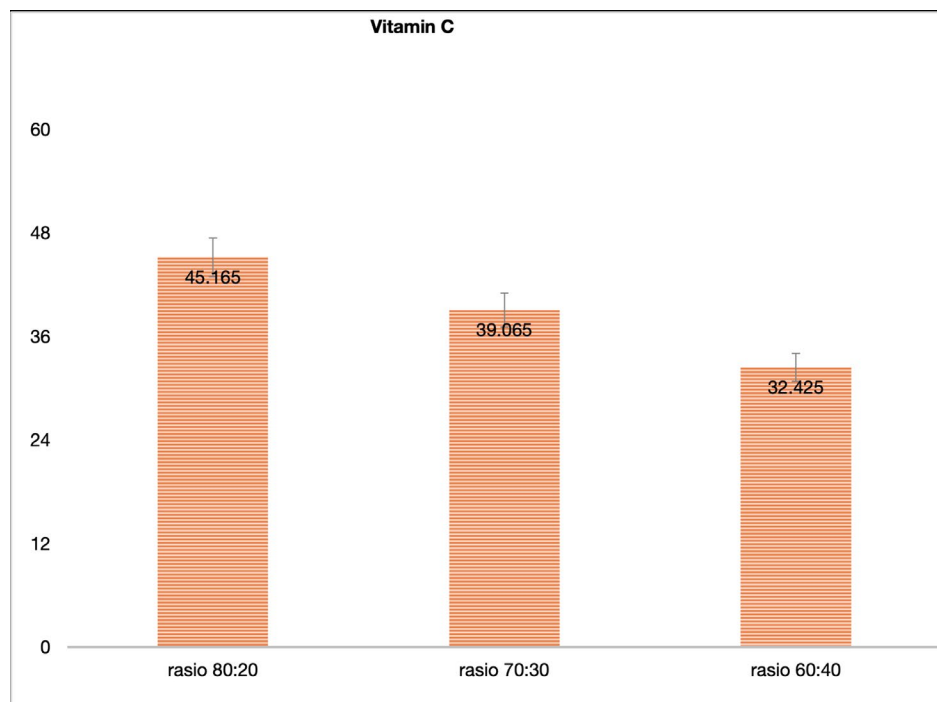


Figure 2: shows the beta-carotene content of three different pumpkin coffee formulas. The results indicate that each formula exhibits a different concentration of beta-carotene, with the 70:30 ratio having the highest concentration.

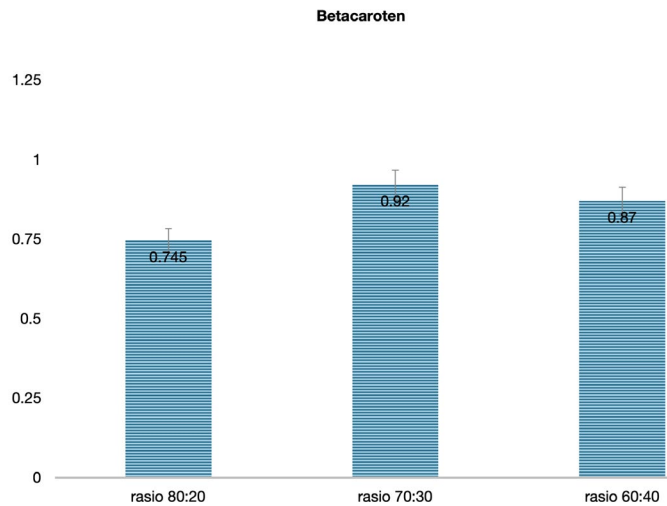


Figure 3: Beta-carotene content of pumpkin seed coffee formula with the ratio of 80:20, 70:30, and 60:40

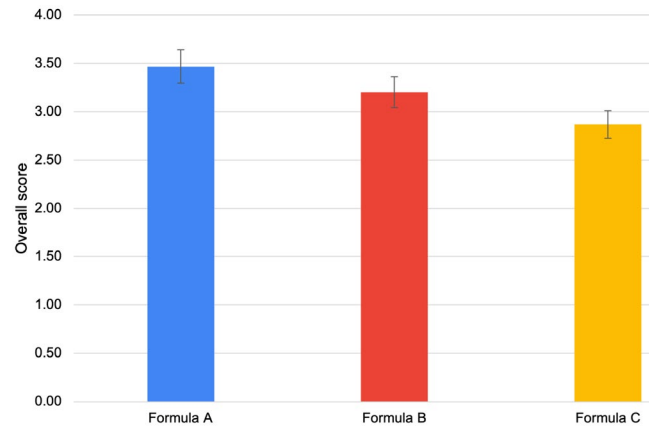


Figure 4: The overall acceptability of the three formulas of pumpkin seed coffee. Formula A (blue) represents a ratio of 80:20, Formula B (red) represents a ratio of 70:30, and Formula C represents (yellow) a ratio of 60:40.

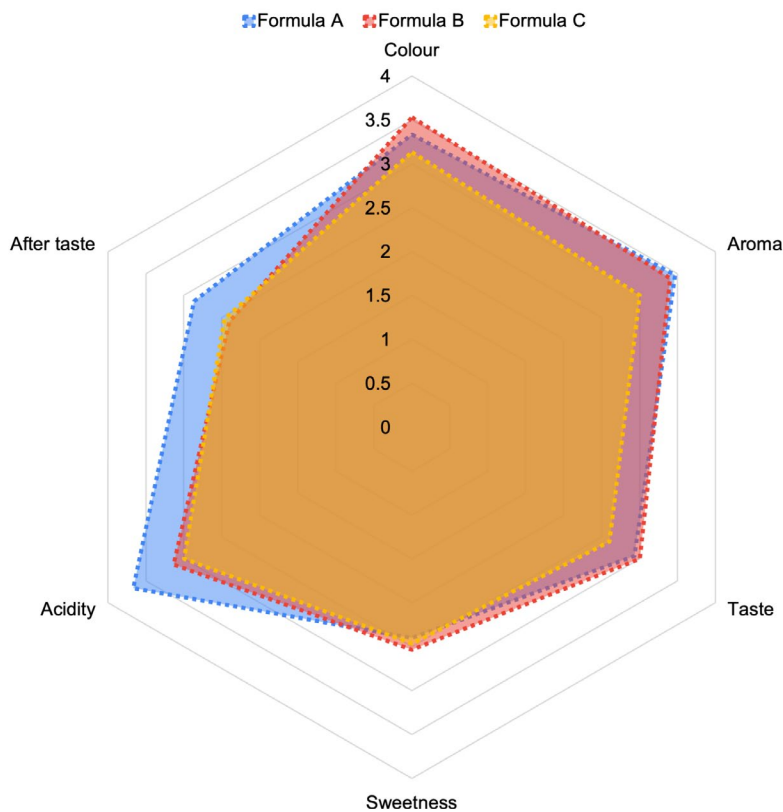


Figure 5: Trained panellists' sensory test regarding the colour, aroma, taste, sweetness, acidity and aftertaste of pumpkin seed coffee formula. Formula A (blue) represents a ratio of 80:20, Formula B (red) represents a ratio of 70:30, and Formula C (yellow) represents a ratio of 60:40.

Table 3: Determination of shelf life (days) of the coffee formulas using Arrhenius method based on the water content

Storage temperature (°C)	Formula 60:40 (days)	Formula 70:30 (days)	Formula 80:20 (days)
25	14	12	16
35	163	365	1010
45	100	300	120

4 DISCUSSION

4.1 Proximate analysis of the pumpkin seed coffee formulations

As depicted in Figure 1, carbohydrates emerged as the predominant macronutrient across all three coffee formula formulations, followed sequentially by protein, fat, and crude fiber. The 80:20 ratio formula exhibited the highest carbohydrate content ($54.40 \pm 1.28\%$) compared to the 70:30 and 60:40 ratios. This correlation is attributed to the higher proportion of coffee powder in the 80:20 formula, as coffee is primarily composed of carbohydrates. Conversely, the 60:40 ratio formula displayed the highest protein (25.78%) and fat (13.95%) concentrations, likely due to the increased proportion of yellow pumpkin seeds in this formulation. Notably, no significant variations were observed in moisture and ash content among the three formulas, suggesting that the incorporation of pumpkin seed powder had minimal influence on these parameters.

The proximate composition analysis, as presented in Figure 1, offers valuable insights into the macronutrient profile of the developed coffee formulations. Carbohydrates emerged as the predominant macronutrient across all three formulations, a finding consistent with the high carbohydrate content typically associated with coffee beans. The elevated carbohydrate content in the 80:20 formula is directly attributable to the higher proportion of coffee powder in this formulation. This observation underscores the significant contribution of coffee beans to the overall carbohydrate content of the final product.

In contrast, protein and fat content were notably higher in the 60:40 formula. This is likely due to the increased proportion of yellow pumpkin seeds in this formulation, which are known to be rich sources of both macronutrients. The results suggest that the inclusion of yellow pumpkin seeds can effectively enhance the protein and fat content of the coffee product.

The consistent moisture and ash content across all formulations indicate that the incorporation of pumpkin seed powder did not significantly alter the overall water or mineral composition of the coffee blends. This suggests that the addition of pumpkin seed powder did not adversely affect the product's physical properties or introduce extraneous mineral components.

These findings provide a foundation for understanding the nutritional composition of the developed coffee formulations. Future research could focus on the impact of these macronutrient profiles on the sensory properties and consumer acceptance of the products. Additionally, investigating the specific types of carbohydrates, proteins, and fats present in the formulations could provide further insights into their potential health benefits.

4.2 Mineral content of the pumpkin seed coffee formulations

4.2.1 Zinc (Zn)

The results of the zinc analysis clearly demonstrate a strong positive correlation between the proportion of pumpkin seeds in the coffee formulations and the resulting zinc content. This trend is particularly evident when comparing the 60:40 formula with the highest pumpkin seed proportion to the 80:20 formula with the lowest pumpkin seed ratio. The 60:40 formula, characterized by the highest pumpkin seed content, exhibited the highest zinc level ($22.95 \pm 0.99\%$). Conversely, the 80:20 formula, with the lowest pumpkin seed ratio, demonstrated the lowest zinc content. These findings strongly suggest that pumpkin seeds are the primary source of zinc in these coffee formulations. As the pumpkin seed content increases, so does the zinc concentration.

The results align with the established nutritional profile of pumpkin seeds, which are recognized as a rich source of zinc. The observed variation in zinc content among the formulas can be attributed to the differing proportions of pumpkin seeds in each formulation. While pumpkin seeds are a good source of zinc, factors such as processing methods, the presence of other dietary components, and individual physiological factors can influence zinc bioavailability. Further research is needed to assess the bioavailability of zinc in these coffee formulations.

These findings underscore the potential of coffee formulations incorporating pumpkin seeds as a valuable source of zinc. However, further research is necessary to evaluate the bioavailability of zinc in these products, optimize the formulation for maximum zinc delivery, and explore the impact of processing conditions on zinc retention during coffee production.

4.2.2 Copper (Cu)

In contrast to the Zinc mineral content, the copper (Cu) mineral content in pumpkin seed coffee formula showed a different effect with lower levels compared to Zn. Formula A with a lower ratio of pumpkin seeds in the formula contained the highest Cu compared to the other two formulas. The increasing concentration of Cu along with the increasing ratio of coffee to formula A, B, and C shows that the biggest contributor of Cu in pumpkin seed coffee formula is coffee. In contrast to zinc mineral content, copper (Cu) mineral levels exhibited a different trend within the pumpkin seed coffee formulations, displaying lower concentrations compared to zinc. Notably, Formula A, characterised by a lower proportion of pumpkin seeds, contained the highest copper content relative to the other two formulas. This inverse relationship suggests that coffee beans are the principal source of copper within these formulations.

The discrepancy between zinc and copper content warrants further investigation. Potential factors contributing to this disparity include variations in mineral composition between coffee bean and pumpkin seed cultivars, differential extraction efficiencies for these minerals during the formulation process, and potential interactions between minerals and other constituents within the coffee matrix. The inverse relationship between pumpkin seed content and copper concentration in the coffee formulations can be attributed to several factors. Firstly, coffee beans are naturally rich in copper, with the mineral content influenced by factors such as soil composition, climate, and cultivar. Secondly, while pumpkin seeds are a good source of various minerals, their copper content is relatively lower compared to coffee beans. Consequently, as the proportion of pumpkin seeds in the formulations increases, the overall copper content decreases due to the lower copper contribution from pumpkin seeds. Conversely, as the proportion of coffee beans increases, as seen in Formula A, the overall copper content increases due to the higher copper content of coffee beans. This suggests that coffee beans are the primary source of copper in these formulations.

To elucidate the underlying mechanisms driving these observations, further research is imperative. Comparative analysis of different coffee bean and pumpkin seed varieties could shed light on mineral content variability. Additionally, optimising extraction conditions and exploring the impact of processing methods on mineral retention would be beneficial.

Understanding the mineral composition of coffee-based products is crucial for evaluating their nutritional value and potential health benefits. By identifying factors influencing mineral bioavailability, it becomes possible to develop coffee formulations that contribute to adequate mineral intake within the population.

4.2.3 Iron (Fe)

Unlike the Zinc and Cu content in pumpkin seed coffee formula, the iron (Fe) content shows a different pattern, where the highest concentration is in formula C (60:40), followed by formula A (70:30) and C (80:20). This is quite interesting because the iron content in formula C is almost 4 times higher than in formulas B and C. The interaction between iron and other substances in each formula component is probably the main factor causing this. Chemical compounds such as polyphenols, tannic and chlorogenic acids, which are commonly found in coffee and tea, can form complexes with iron, especially non-heme iron (Scalbert et al., 1999), thus affecting the iron content of the coffee formula. This is relevant to the results of the analysis of flavonoid levels in the three formulas which show that the highest flavonoid compounds in formulas B and C.

The observed variations in iron (Fe) content among the

coffee formulations present an intriguing aspect of the study. Unlike zinc and copper, which exhibited a consistent increase with higher pumpkin seed powder content, iron demonstrated an inverse relationship. Formula C, containing the highest proportion of pumpkin seed powder, surprisingly exhibited the highest iron concentration. This anomalous behaviour suggests complex interactions between iron and other components within the coffee formulations.

The presence of polyphenols, including tannic and chlorogenic acids, known for their iron-binding properties, likely influenced the iron bioavailability in the different formulas. These compounds can form complexes with iron, particularly non-heme iron, potentially reducing its availability. The higher flavonoid content in formulas B and C, as evidenced by previous analysis, could explain the lower iron levels in these formulations.

To elucidate the mechanisms underlying these interactions, further investigations are warranted. Characterization of the specific iron compounds present in each formula, along with their bioavailability, would provide valuable insights. Additionally, exploring the impact of processing conditions on iron content and bioavailability is essential for optimizing the iron enrichment potential of the coffee formulations.

Understanding the factors influencing iron bioavailability in these coffee products is crucial for assessing their potential nutritional benefits. By optimizing iron content and bioavailability, it may be possible to develop coffee formulations that contribute to improved iron status, particularly in populations at risk of iron deficiency.

4.3 Antioxidant properties of the pumpkin seed coffee formulations

Flavonoids

Flavonoid content in all three formulas was quite low (<1%). The highest flavonoid concentration was in formulas B and C where the ratio of pumpkin seeds was higher than formula A. Indicating pumpkin seeds contain higher flavonoid content compared to coffee. Flavonoid compounds in coffee include chlorogenic acid, caffeic acid, ferulic acid, quercetin, campferol, and caffeine.

The low flavonoid content across all three coffee formulations is somewhat unexpected, considering the recognized flavonoid richness of both coffee and pumpkin seeds. However, the observed trend, with higher flavonoid levels in formulas B and C (containing higher proportions of pumpkin seeds), suggests that pumpkin seeds are indeed a more significant source of flavonoids compared to coffee beans in this context. The presence of chlorogenic acid, caffeic acid, ferulic acid, quercetin, campferol, and caffeine as the primary

flavonoid compounds aligns with the typical flavonoid profile of coffee.

The relatively low overall flavonoid content could be attributed to several factors, including processing conditions, extraction efficiency, and the specific varieties of coffee beans and pumpkin seeds used. Further investigation into the impact of roasting, grinding, and brewing methods on flavonoid retention is warranted to optimize flavonoid levels in the final products.

Additionally, exploring the bioavailability of the identified flavonoids in the context of the coffee matrix is crucial. The interactions between flavonoids and other coffee components may influence their absorption and utilisation in the human body.

By addressing these factors, a deeper understanding of the flavonoid composition and bioavailability in coffee-based products can be achieved, enabling the development of formulations with enhanced antioxidant properties and potential health benefits.

4.3.1 DPPH

DPPH (1,1-Diphenyl-2-picrylhydrazyl) is a stable free radical that is often used in research to measure antioxidant activity. In this study, each coffee formula was tested for DPPH levels to see the extent of antioxidant activity in the coffee formula and which formula gave the highest inhibition. In general, high DPPH inhibition indicates a coffee formula that has a high antioxidant capacity and the formula is able to neutralize free radicals more effectively. The results of the DPPH inhibition analysis of the three formulas showed that the highest DPPH inhibition was in the 60:40 ratio formula which amounted to $80.48 \pm 0.33\%$. A higher % inhibition value of DPPH indicates stronger antioxidant activity in the sample. This indicates that the sample can neutralize a larger percentage of DPPH free radicals. These results indicate that the three coffee formulas have very strong antioxidant activity in neutralizing DPPH free radicals at the concentration tested.

4.3.2 IC50

Inhibitor Concentration (IC₅₀) which is the concentration of a compound that can cause DPPH activity to decrease by 50%. The smaller the IC₅₀ value, the higher the antioxidant activity (Kuncoro; Rijai, 2018). A chemical is considered very strong in antioxidant activity if the IC₅₀ value is less than 50 ppm, the strong group IC₅₀ between 50-100 ppm, the medium group between 101-150 ppm, and the weak group between 150-200 ppm (Habibi et al., 2022).

Antioxidant activity increases with decreasing IC₅₀ value (Marsiati et al., 2023). Based on the results of the antioxidant inhibition test, it shows that the three coffee

formulas have IC₅₀ below 50%, which indicates very strong activity. The lower the IC₅₀ value, the less substance is needed to achieve 50% inhibition, so the substance is considered more effective. Based on this classification, the 60:40 ratio formula with IC₅₀ value (32.43 ± 2.34) is the formula with the strongest inhibition of antioxidant activity, followed by the 70:30 ratio coffee formula (39.07 ± 1.69) and the 80:20 ratio formula (45.17 ± 0.37). The IC₅₀ value of $32.43 \pm 2.34\%$ in the coffee formula shows the percentage of activity inhibition by the antioxidant DPPH (1,1-Diphenyl-2-picrylhydrazyl) of 50% which indicates the concentration of 60:40 coffee extract or formula that can inhibit the activity of DPPH free radicals by $32.43 \pm 2.34\%$. This result is relevant to the DPPH inhibition in Table 1, which is also high in the formula with a ratio of 60:40.

This shows that of the three formulas, the 60:40 ratio formula with a greater ratio of pumpkin seeds and with the lowest IC₅₀ value has the strongest antioxidant activity so that it can be said that pumpkin gives the greatest effect on increasing antioxidant activity in pumpkin seed coffee formula.

4.3.3 VITAMINS A AND C

A coison of β -carotene (pro-vitamin A) and vitamin C content among the three coffee formulas is presented in Figures 2 and 3. The coffee formula with an 80:20 ratio exhibited the highest β -carotene content, followed by the 70:30 and 60:40 ratio formulas. These findings suggest that coffee is the primary contributor to the overall β -carotene content in these formulations, rather than the yellow pumpkin seeds. Conversely, vitamin C concentrations were found to be negligible (<0.01%) and not significantly different among the three coffee formulas.

The significantly higher β -carotene content in the 80:20 ratio formula underscores the potential of coffee as a source of this essential pro-vitamin. These findings align with previous studies suggesting that roasted coffee beans can be a rich source of antioxidants, including carotenoids (36). However, the negligible vitamin C content in all formulas indicates that these beverages may not be a reliable source of this water-soluble vitamin. Future studies could explore the effects of different roasting profiles and brewing methods on the nutrient content of coffee-based beverage. The observed enrichment of β -carotene in the 80:20 formula due to the higher coffee bean content corroborates previous research highlighting the potential of coffee as a source of carotenoids, including pro-vitamin A. This finding underscores the coffee bean's contribution to the overall nutritional profile of the formulated beverages. Conversely, the absence of significant vitamin C levels emphasises the limitations of coffee as a source of this water-soluble vitamin.

The influence of roasting profiles and brewing methods on nutrient retention in coffee-based beverages merits further

investigation. Different processing conditions can impact the degradation of heat-sensitive compounds like carotenoids, potentially affecting the nutritional value of the final product (Gómez-Ruiz; Ames; Leake, 2008). Additionally, exploring the synergistic effects of combining coffee with other ingredients known for their vitamin C content could enhance the overall nutritional profile of the beverages (Erskine et al., 2022). By delving deeper into these aspects, a more comprehensive understanding of the nutritional potential of coffee-based products can be achieved, facilitating the development of fortified or enhanced beverages that cater to specific consumer needs.

The analysis of β -carotene and vitamin C content in the coffee formulations provides valuable insights into the nutritional composition of the products. The observed predominance of β -carotene in the 80:20 formula is consistent with the higher proportion of coffee powder in this formulation, as coffee beans are known to be a source of this carotenoid. The relatively low levels of vitamin C in all three formulas are unsurprising, as coffee beans are generally not considered a significant source of this vitamin (Torres; Farah, 2017).

The findings suggest that while the coffee formulations offer a source of β -carotene, a crucial pro-vitamin A compound, they may not contribute significantly to vitamin C intake. This information is essential for accurately labelling and marketing the products. Future research could focus on the bioavailability of β -carotene from these coffee formulations, as well as exploring other potential micronutrients present in the coffee and pumpkin seed components. Additionally, investigating the impact of processing methods on the retention of β -carotene during coffee production would be beneficial for optimizing the nutritional value of the final product.

4.4 Organoleptic Test of the pumpkin seed coffee formulations

Regarding the sensory analysis, based on this study, Formula A was selected as the optimal formula, and consumer panelists. The addition of yellow pumpkin seeds to coffee increased its antioxidant content but unfortunately decreased the acceptability of the beverage. The higher the content of pumpkin seeds in the coffee, the fewer consumers liked it, especially if the panellists had a specific preference for the taste of traditional coffee (Mala; Kurian, 2016). While the addition of yellow pumpkin seeds to the coffee formula can increase nutrient content such as protein, fibre, and essential fats, it can also disrupt the aroma and taste of the coffee beverage (Zhang et al., 2020).

Yellow pumpkin seeds contain compounds that can affect the final product's taste, such as phenolic and flavonoid compounds that give a bitter or sour taste not typically found in coffee flavour (Šamec et al., 2022). Additionally, yellow

pumpkin seeds add a coarser texture element that does not align with consumer expectations for coffee. The balance of coffee bean flavour and yellow pumpkin seeds that was still well-accepted by consumers was Formula A (80:20) (Šamec et al., 2022). Aroma is a crucial aspect of the evaluation of coffee products. The aroma of yellow pumpkin seeds may not match the aroma of coffee that consumers are familiar with, causing the product's aroma to be less appealing to panellists (Dotto; Chacha, 2020).

The results of the study showed a decrease in panellist acceptance with increasing yellow pumpkin seed content. This necessitates considering the optimal proportion of yellow pumpkin seeds to maintain significant nutritional benefits without compromising sensory acceptance. Additionally, techniques for mixing pumpkin seeds into coffee beans that minimise changes in flavour need to be considered. Furthermore, research on market segmentation is required to understand the consumer groups that may accept yellow pumpkin seed coffee products. One approach could be to educate consumers about the health benefits of yellow pumpkin seeds, so that consumers may accept the product even if the taste is different. Moreover, product variants can be developed according to market segmentation. For example, providing products with lower pumpkin seed content for consumers sensitive to changes in coffee taste and providing products with higher pumpkin seed content for consumers interested in the health benefits of yellow pumpkin seeds.

4.5 Shelf-Life of the pumpkin seed coffee formulations

The results presented in Table 3 indicate a clear relationship between water content, pumpkin seed powder concentration, and the shelf life of the coffee formula. A critical finding is that the 80:20 formula, containing the highest proportion of pumpkin seed powder, exhibited the longest shelf life at 35°C. Conversely, the 60:40 formula with a lower pumpkin seed powder content demonstrated the shortest shelf life at the same temperature. This observation strongly suggests that increasing the concentration of pumpkin seed powder in the coffee formula can significantly shorten its shelf life. The possible reason for the result is due to the high lipid content and moisture content of the formula with a higher proportion of pumpkin seed. Pumpkin seeds are rich in lipids, particularly unsaturated fatty acids. These lipids are susceptible to oxidation, which can lead to the development of off-flavours, rancidity, and ultimately, reduced shelf life. As the pumpkin seed powder concentration increases, so does the lipid content of the coffee formula, accelerating the oxidation process. Furthermore, based on its water content, in Figure 1, among three formulas, the 60:40 ratio exhibited

a higher water content compared to other formulas with a lower ratio of pumpkin seed, although the difference was not significant. The water content of a food product can influence its shelf life. Higher moisture content in food products can promote microbial growth and enzymatic reactions, leading to spoilage. While the specific mechanism is not entirely clear in this context, the increased water content in the 60:40 formula, potentially associated with the pumpkin seed powder, may have contributed to its shorter shelf life. Additionally, while pumpkin seeds contain antioxidants that can counteract oxidative processes, the increased lipid content from the higher pumpkin seed proportion may outweigh these benefits, ultimately leading to a reduction in shelf life.

Furthermore, the significant differences observed among the temperatures underscore the importance of storage conditions in preserving product quality. While the study focused on 35°C, it would be beneficial to investigate the shelf life of these formulations at other temperatures to establish a comprehensive understanding of their stability. The mechanisms underlying the observed relationship between pumpkin seed powder and shelf life require further investigation. Potential factors include the antioxidant properties of pumpkin seed powder, its ability to bind with water, or its influence on the formulation's physical and chemical properties.

These findings have practical implications for the development and optimisation of coffee formulas. By increasing the pumpkin seed powder content, it may be possible to produce coffee products with extended shelf-life, potentially reducing waste and improving product stability. However, it is crucial to balance the desired shelf life with other product attributes, such as taste, aroma, and consumer acceptance.

Future research should focus on identifying the specific components in pumpkin seed powder responsible for the observed shelf-life extension. Conducting long-term storage studies under real-world conditions is essential to validate the shelf-life predictions obtained from accelerated testing. Additionally, exploring the impact of different processing methods and storage conditions on the stability of the coffee formulas would provide valuable insights for product development. By understanding the factors influencing shelf life, the coffee industry can produce higher-quality products with extended shelf lives, benefiting both consumers and producers.

5 CONCLUSIONS

This study developed coffee formulations with varying pumpkin seed proportions, showing that pumpkin seeds enhanced nutritional content, particularly in protein,

fat, zinc, and iron. Formula C (60:40) showed the most improvement in these areas, while Formula A (80:20) excelled in carbohydrates, fiber, and vitamin A. Antioxidant activity correlated with pumpkin seed content, with Formula C demonstrating the highest capacity. Further studies should focus on optimizing formulation, storage conditions, and investigating bioavailability to balance nutritional, sensory, and shelf-life attributes.

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7 CONTRIBUTIONS TO THE AUTHORS

Conceptual Idea: Syam, A.; Methodology design: Najamuddin, U.; Data collection: Kurniaty, Y.; Amalia, M.; Data analysis and interpretation: Najamuddin, U.; Syam, A. and Writing and editing: Najamuddin, U.; Kurniati, Y.; Amalia, M.].

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