Published: 12th May 2026

ANACHEM JOURNAL
ISSN: 2006-5221 (Online)

EFFECT OF VARYING TEMPERATURES ON THE PROPERTIES OF HONEY FROM DIFFERENT SOURCES

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DOI: https://doi.org/10.5281/zenodo.20129252

ABSTRACT

Honey is a complex natural substance whose physical and chemical characteristics are strongly affected by temperature. This study evaluated the effects of controlled heating (45, 65, 85, and 100 °C) on the proximate composition, vitamin profile, and sugar content of honey obtained from two sources: Ariaria Aba (Imo State) and a commercial supermarket in Ifite Awka (Anambra State, Nigeria). Standard analytical methods were used to determine moisture, ash, crude protein, crude fat, crude fibre, and total carbohydrate, along with vitamins A, B-complex, C, D, and E. Results showed that increasing temperature generally reduced the contents of moisture, ash, and protein, indicating thermal degradation of key constituents and showing the presence of adulterations. The carbohydrate content increased with temperature, likely due to heat-induced hydrolysis of sucrose into simpler sugars. However, sucrose and fructose levels generally decreased, suggesting concurrent degradation at higher temperatures. Vitamins A, B2, and D were particularly heat-sensitive, with noticeable reductions at elevated temperatures, while vitamins B6, C, and E remained relatively stable in the range tested. The findings suggest that excessive heating adversely affects most nutritional and quality parameters of honey, except carbohydrates and some heat-stable vitamins. These changes are likely related to the thermal breakdown of organic compounds and the loss of volatile components. No significant differences were observed between the honey sources, but temperature had a pronounced effect on composition. In general, moderate heating (at temperatures below 65 ° C) is recommended to preserve the nutritional integrity and quality of honey.

Keywords: Honey; Sugar Profile; Temperature; Proximate Composition; Vitamins.

1.0. Introduction

Honey is a naturally occurring sweet substance made by bees from nectar and honeydew and is valued not only for its nutritional benefits but also for its healing properties. Its composition includes carbohydrates (mainly fructose and glucose), proteins, vitamins, minerals, organic acids, phenolic compounds, flavonoids, and enzymes (Ghramh et al., 2020; da Silva et al., 2020). For centuries, honey has served as a medicinal product with wound healing, antimicrobial, antioxidant, and anti-inflammatory properties (Albaridi, 2019; Scepankova et al., 2021). It has also been shown to provide symptomatic relief in upper respiratory tract infections (Abuelgasim et al., 2021).

Its characteristics vary with environmental and botanical factors. Temperature is a critical ecological factor that influences honey's physicochemical and biological properties. Changes in temperature during honey storage or processing can significantly affect its viscosity, colour, enzyme activity, antioxidant capacity, and hydroxymethylfurfural (HMF) levels, which are indicators of honey degradation (Nwoko et al., 2017; Karabagias et al., 2018).

With increasing commercial interest in honey due to its health benefits and economic value, understanding the effects of temperature is vital to maintaining product integrity throughout its lifecycle. Although honey has long been used in traditional medicine, modern industrial processes, globalization, and changing climate conditions necessitate updated knowledge on how heat affects honey's stability and quality. Improper temperature handling during storage, transport, or pasteurization can degrade key bioactive compounds, reduce therapeutic benefits, and pose potential safety issues (Sajid et al., 2024).

Recent studies have examined how temperature affects the chemical composition of honey and sensory characteristics. However, most focus on a limited set of floral types or narrow temperature ranges (e.g., 25°C to 45°C). Moreover, the combined effects on multiple quality indicators, such as antioxidant activity, enzyme stability, and volatile compound retention, remain underexplored, especially at elevated processing temperatures such as 85°C or 100°C (Nair, 2016; Karabagias et al., 2018).

This study addresses the need for a comprehensive evaluation of temperature effects on different types of honey, including locally sourced samples from Nigeria, to fill critical data gaps in tropical and subtropical contexts. This study addresses these gaps by investigating how a broader range of temperatures (45°C, 65°C, 85°C, and 100°C) affects honey from multiple floral and regional sources using a multidimensional analytical approach. These findings will have direct implications for honey producers, processors, regulatory bodies, and consumers. In addition, they will contribute to the growing field of honey quality control, safety assurance, and food science.

2.0. Materials And Methods

2.1. Sample Collection

Two honey samples were examined: Sample A is Honey sourced locally from Ariaria International Market, Aba, Imo State, Nigeria. While Sample B is industrially processed honey and bought from Machpee Supermarket, Ifite, Awka, Anambra State, Nigeria.

2.1.1. Sample Appearance

The colour of the sample was assessed using the sight technique (Organoleptic Method)

2.2. Methods of Analysis

2.2.1. Proximate Composition Analysis

The proximate analysis (ash, moisture, crude fibre, crude fat, carbohydrate, and crude protein) of the samples was performed using the Association of Official Analytical Chemists (AOAC) methods (1999).

2.2.3. Vitamins Analysis

1. **Vitamin A Determination**

The vitamin A content of the honey samples was determined following the method of Roza et al. (2022). An aliquot (25 mg) of each sample was weighed and homogenized using a mortar and pestle. The extraction was carried out with 10 mL of ethanol, followed by centrifugation at 1500 rpm for 10 minutes. One millilitre (1 mL) of the floatable was transferred to a test tube, mixed with 1 mL of

0.1 M ethanolic KOH, and vigorously shaken. The mixture was heated in a 60°C water bath for 20 minutes, cooled, and 1 mL of xylene was added. Shake thoroughly, then centrifuge the tube again at 1500 rpm for 10 minutes. The absorbance of the upper xylene layer was read at 335 nm before (A_1) and after (A_2) 30 minutes of UV irradiation. The vitamin A concentration (C_x) in µM was calculated as:

$$C_x(\mu M) = (A_1 - A_2) \times 22.23 \qquad \text{(Equation I)}$$

22.23 = multiplier received based on the absorption coefficient of 1 % solution of vitamin A (as the retinol form) in xylene at 335 nm in a measuring cuvette with a thickness = 1 cm.

2. Vitamin E Determination

Vitamin E content was assessed using the procedure described by Mezaal et al. (2023). One gram (1 g) of each sample was homogenized and extracted with 10 mL of ethanol. The supernatant from centrifugation (1500 rpm, 10 min) was used. A 0.5 mL aliquot of the extract was mixed with 0.5 mL anhydrous ethanol and 3 mL xylene, shaken, and centrifuged. Then, 1.5 mL of the upper layer was reacted with 0.25 mL of 6.02 mM batophenanthroline, 0.25 mL of 0.98 mM ferric chloride, and 0.25 mL of 40 mM phosphoric acid. Absorbance was measured at 539 nm. Tocopherol concentration (µM) was calculated as stated in Equation II below:

$$Tocopherol\ (\mu M) = \frac{\text{Ax x Cs}}{\text{As}} \quad (Equation\ 2)$$

Where: Ax = Absorbance of sample, As = Absorbance of standard and Cs = Concentration of standard (23.2 µM)

3. Determination of ascorbic acid

Ascorbic acid was determined by measuring the amount in 100 mg of honey diluted with 1% metaphosphoric acid, filtered, and mixed with 0.005% 2,6-dichlorophenolindophenol. The absorbance is then measured at 515 nm within 30 minutes (Selvaraju et al., 2019)

4. Vitamin B1 and B2 Determination

The method described by Yau et al. (2020) was used. One gram (1 g) of each sample was dissolved in 100 mL of deionized water after digestion, then filtered using Whatman no. 42 filter paper into a clean volumetric flask. The filtrate was analyzed using a UV-Vis spectrophotometer at 261 nm (B_1) and 242 nm (B_2). Concentrations were calculated as stated in Equation III below:

$$concentration\ (mg\%) = \frac{|\text{A x DF x volume of cuvette (5)}}{\text{E}} \quad (Equation\ 3)$$

Where: A = absorbance, DF = dilution factor, E = extinction coefficient (25 for both B_1 and B_2),

5. Vitamin B3 (Nicotinamide) Determination

Following Okoh and Madu (2018), 5 g of each sample was dissolved in 20 mL of anhydrous glacial acetic acid and slightly warmed. Then, 5 mL of acetic anhydride and 2–3 drops of crystal violet were added. The solution was titrated with 0.1 M perchloric acid to a greenish-blue endpoint. The vitamin B_3 content was calculated as stated in Equation IV below:

$$Vitamin\ B3 = \frac{\text{titre value x 0.0122}}{0.1} \quad (Equation\ 4)$$

6. Vitamin B6 (Pyridoxine) Determination

As described by Okoh and Madu (2018), 5 g of the sample was dissolved in 5 mL of anhydrous glacial acetic acid and 6 mL of 0.1 M mercury (II) acetate. Two drops of crystal violet were added, and the solution was titrated with 0.1 M perchloric acid to a green endpoint. Each millilitre of perchloric acid is equivalent to 0.02056 g of $C_8H_{11}NO_3 \cdot HCl$.

7. Vitamin D Determination

Vitamin D was analyzed according to the

method of Rahman et al. (2019). The assay is based on the formation of a yellow chromophore from the reaction of vitamin D with trichloroacetic acid in chloroform. A standard was prepared by dissolving 25 mg of vitamin D_3 in a chloroform: methanol (1:9) mixture and diluting to a final volume of 25 mL. For sample analysis, 0.1 mL of each sample was dissolved and diluted similarly. Then, 1.6 mL of 0.25 M HCl, 0.5 mL of 15% trichloroacetic acid, and 0.5 mL of 0.375% thiobarbituric acid were added. Absorbance was measured at 464 nm against a blank.

2.2.4. Sugar Content Determination.

1. Sucrose and Fructose Estimation

The phenol-sulphuric acid method was used to determine the sugar content. One gram (1 g) of each sample was mixed with 9 mL of distilled water and vortexed. After standing for 4 hours to ensure complete sugar elution, 1 mL of the solution was mixed with 0.5 mL of 5% phenol and 2.5 mL of concentrated sulphuric acid. The absorbance was measured at 490 nm using a reagent blank. Standard curves for sucrose and fructose were used to extrapolate sample concentrations. (Alves et al., 2015).

3.1. Results and Discussion

3.2. Sample Appearance

Sample A appeared dark brown with traces of honeycomb and other particulates, while Sample B had an amber colour.

3.2. Proximate Analysis

3.2.1. Moisture Content and Ash Content

As shown in Figure 3.1 below, Sample A exhibited moisture contents ranging from 8.80% to 12.56%, with the highest value at 45°C. Sample B had a broader range, from 6.66% to 15.33%, and also decreased with rising temperature. All values fall within the Codex Alimentarius (2001) recommended moisture limit of 20–21%, indicating proper processing and low risk of fermentation. These results are consistent with Kretavičius et al. (2010), who reported moisture values between 16.2% and 18.4% in thermally treated honey. Environmental conditions and beekeeping practices are significant factors affecting moisture content (Escriche et al., 2009).

In Figure 3.2 below, the ash content in Sample A ranged from 6.46% to 7.41%, and in Sample B, from 3.1% to 6.06%. These values are significantly higher than typical values reported by Kedzierska-Matysek et al. (2016), which ranged from 0.7% to 1.9%. Such discrepancies could be due to differences in botanical and geographical origins, storage conditions, or potential adulteration (Escuredo & Seijo, 2019). Ash content is a key nutritional and quality parameter representing mineral content. While Codex Alimentarius does not specify a standard value, Chakir et al. (2011) reported an average ash content of 0.17%, with values ranging from 0.02% to 1.03%.

3.2.2. Fiber and Fat Content

As depicted in Figure 3.3 below, Sample A exhibited significantly higher fiber content than Sample B, with values ranging from 1.48% to 6.63%. The highest fiber content in Sample A was observed at 65°C, following a sharp increase from 45°C. However, a subsequent decline was noted at 85°C and 100°C, indicating thermal instability of the fiber at higher temperatures. In contrast, Sample B recorded lower fiber levels, ranging from 0.60% to 0.86%, indicating a consistent decrease with increasing temperature. These variations may be attributed to botanical origin, thermal degradation of complex polysaccharides, or matrix interaction during heating (Singh et al., 2022).

Figure 3.4 below shows that Sample A's fat content varied between 3.5% and 3.9%, while

Sample B's fat content ranged from 4.33% at 45°C to 3.40% at 100°C, indicating a temperature-induced decline. Although fats are not naturally abundant in honey, their presence in elevated quantities, as seen in both samples, could indicate contamination, adulteration, or analytical deviation (Xiantong et al., 2023). High fat levels increase the likelihood of oxidative rancidity, compromising shelf life (Martin et al., 2021). Notably, honey is expected to be fat-free or to contain only trace amounts (Xiantong et al., 2023).

3.2.3. Protein and Carbohydrate Content

As illustrated in Figure 3.5 below, the protein content in Sample A ranged from 1.2% to 2.8%, peaking at 45°C. Sample B followed a similar declining trend, ranging from 1.45% to 0.8% as the temperature increased. Both samples exceeded the standard protein range for natural honey (0.16–0.20%) as reported by Aminuwa et al. (2024), suggesting possible botanical variation or contribution from other nitrogenous compounds (Amabye & Mekonen, 2016).

Figure 3.6 below shows that the carbohydrate content increased with temperature in both samples. Sample A recorded values between 70.03% and 74.56%, while Sample B showed a more marked increase from 71.95% to 85.42%. Only values at 85°C and 100°C in Sample B aligned with the carbohydrate concentration (80–82.71%) reported by Ndife et al. (2014) for imported U.S. honey. Carbohydrates represent the primary energy-yielding component of honey. The increase may result from moisture loss at elevated temperatures, which concentrates the sugar content. For optimal preservation, honey should contain ≥83% carbohydrates and <17% moisture when stored below 11°C to prevent fermentation (European Union Commission, 2001).

3.2.4. Metabolizable Energy

As shown in Figure 3.7 below, metabolizable energy in Sample A ranged from 322.03 to 335.76 kcal/100g, while Sample B showed a wider range from 332.60 to 375.50 kcal/100g. These results underscore honey's role as a high-energy, carbohydrate-rich food suitable for both infants and adults due to its easily digestible sugar profile, which resembles that of fruits (Bogdanov et al., 2008).

3.3. Sugar content determination

Figures 3.1 and 3.2 below show the graphical representation of sucrose and fructose contents, respectively, in the analyzed honey samples.

3.3.1. Sucrose Content

In Figure 3.8 below, the sucrose values for Sample A ranged from 2.08% to 2.49%, indicating a relatively narrow variation that decreased with increasing temperature. In contrast, Sample B showed a wider variation, ranging from 1.47% to 2.56%. The consistent sucrose level in Sample A may suggest uniform processing and minimal adulteration. This could also reflect differences in nectar sources, processing techniques, or partial sucrose inversion during storage (Waworuntu, 2024).).

Higher sucrose levels in honey can indicate early harvesting or incomplete enzymatic processing by bees (Codex Alimentarius, 2001). However, both samples fall within the acceptable sucrose threshold (<5%) according to Codex Alimentarius, the European Union, and Ethiopian Standards. Elevated sucrose levels can affect crystallization behavior, yet the measured values suggest natural, unadulterated honey.

3.3.2. Fructose Content

In Figure 3.9 below, Sample A showed a fructose content ranging from 30.51% to 23.28%, with the value declining as the temperature increased. Sample B showed similar values, ranging from 29.76% to 23.32%. The observed variation can be attributed to differences in floral source, seasonal influence, or processing method (Cherian et al., 2010).

The higher fructose content contributes to increased sweetness, reduced crystallization, and enhanced shelf life due to improved moisture retention (Ferdinando & Batia, 2008). Honey with higher fructose levels is generally preferred for culinary use due to its smooth texture and stability. The decreasing trend in fructose with increasing temperature aligns with the thermal degradation and Maillard reaction pathways that affect sugar stability.

3.4. Vitamins

3.4.1. Vitamin A and B1

In Figure 3.10 below, Sample A consistently maintained a Vitamin A content of 0.8 mg/100 g across all temperature treatments. Sample B exhibited a drop to 0.67 mg/100g at 85°C, followed by a return to 0.8 mg/100g at 100°C. This fluctuation in Sample B suggests possible degradation, partial recovery, or analytical variability. Honey is not a good source of Vitamin A, so the high content observed may result from adulterations (Fakhlaei et al., 2020).

As indicated in Figure 3.11 below, the vitamin B1 content of Sample A decreased from 0.6 to 0.5 mg/ 100 g and remained stable at the higher temperature points. Sample B also showed a progressive decline as temperature increased, confirming the thermal sensitivity of Vitamin B1 (thiamine), which degrades with prolonged exposure to heat (Rychlik et al., 2016).

3.4.2. Vitamin B2 and B3

Figure 3.12 below shows that Vitamin B2 in Sample A decreased sharply from 1.02 mg/100g at 45°C to 0.4 mg/100g at higher temperatures, where it stabilized. Sample B followed a similar decline trend. The decrease confirms the thermal instability of riboflavin under prolonged heat exposure. Honey is not a good source of Vitamin B2, so the high content observed may be due to adulteration (Fakhlaei et al., 2020).

As shown in Figure 3.13 below, Sample A consistently had higher Vitamin B3 levels (0.3 to 0.24 mg/100 g) than Sample B (0.1 to 0.04 mg/100 g), and both samples showed gradual degradation as the temperature increased. This is consistent with previous findings on niacin sensitivity in honey matrices (Juszczak et al., 2020).

3.4.3. Vitamin B6 and C

Both vitamins in Samples A and B showed an apparent temperature-dependent decrease in Figures 3.14 and 3.15 below. This result is expected, as both B6 and C are water-soluble and thermolabile, prone to oxidation and degradation under elevated thermal conditions (Iqbal et al., 2006). The similarity in the range of values between the two samples suggests comparable vitamin stability.

3.4.4. Vitamin D and E

Figure 3.16 below shows that both samples displayed identical Vitamin D values—0.88, 0.86, 0.78, and 0.71 mg/100g—from 45°C to 100°C. Demonstrating uniform degradation across treatments. This might indicate similar sources or a processing history. Honey is not a good source of Vitamin D, so the high content observed may be due to adulteration (BiologyInsights Team, 2025).

Figure 3.17 below shows that the Vitamin E content decreased with increasing temperature in both samples. However, Sample A consistently maintained higher levels than Sample B, suggesting either better oxidative stability or a higher initial concentration.

4.0. Conclusions

The findings of this study demonstrate that the physicochemical and nutritional properties of honey are significantly influenced by heat treatment at varying temperatures. Specifically, parameters such as sucrose, fructose, moisture content, ash content, crude fat, crude protein, crude carbohydrate, metabolizable energy, crude fiber, and vitamins (A, B1, B2, B3, B6, C, D, and E) were all affected by increasing temperature.

A consistent decline in the levels of sugars (sucrose and fructose), moisture, and ash content was observed as the temperature increased, indicating the thermal sensitivity of these constituents. In addition, the high values of protein, fat, and ash might be a result of adulteration with fat- and protein-rich substances. In particular, a high fat content increases the risk of rancidity over time if honey is improperly stored or exposed to excessive heat. Therefore, the choice of temperature during honey processing or use should be carefully considered, depending on its intended application.

Both honey samples analyzed were rich in carbohydrates, metabolizable energy, and vitamins C and E. Their high fructose and sucrose content confirms their sweetness. However, this may decrease with heat exposure, leading to flavour degradation. However, their low moisture and high carbohydrate content suggest good shelf stability, minimizing the likelihood of fermentation.

Regarding vitamin composition, Sample A exhibited higher levels of vitamins B1, B3, C, and E, while Sample B had higher concentrations of vitamins B2 and B6. Vitamins A and D were found in similar amounts in both samples. The results, especially for Vitamins A, B1, and D, indicate that the analysed honey may not be pure, suggesting adulteration or contamination.

In summary, while honey remains a nutritious, energy-rich natural product, its quality and functional attributes are sensitive to thermal processing, underscoring the need for temperature control during storage, processing, and application.

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GRAPHICAL REPRESENTATIONS OF THE RESULTS

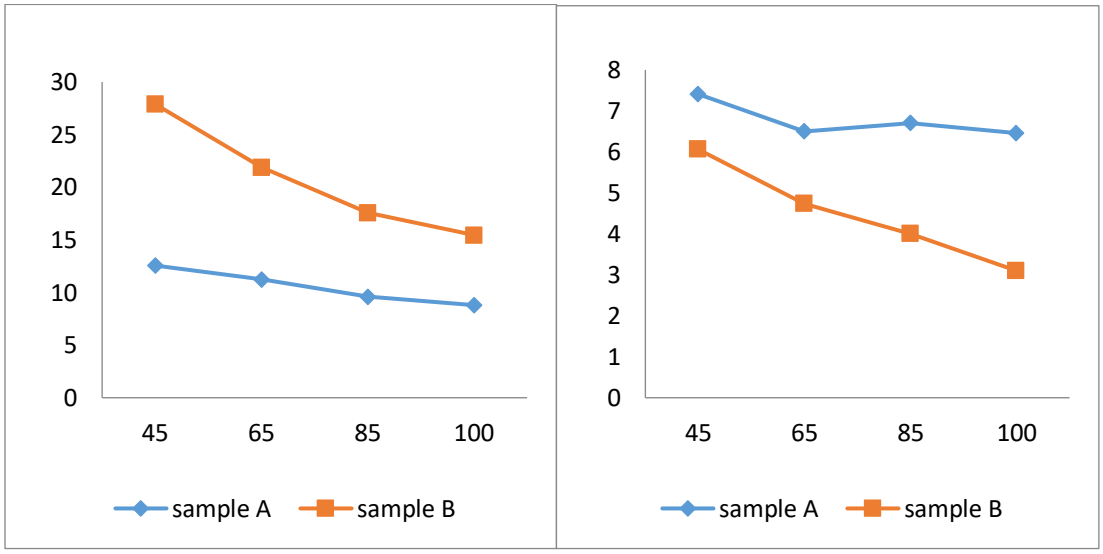


Figure 3.1: Graphical Representation of Moisture Content **Figure 3.2**: Graphical Representation of Ash Content

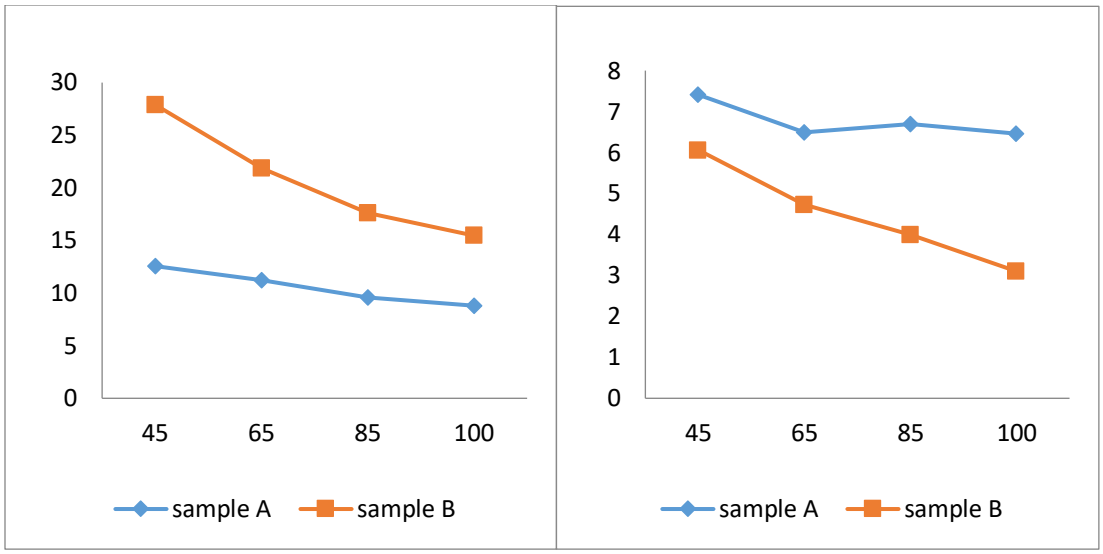


Figure 3.3: Graphical Representation of Fiber Content **Figure 3.4**: Graphical Representation of Fat Content

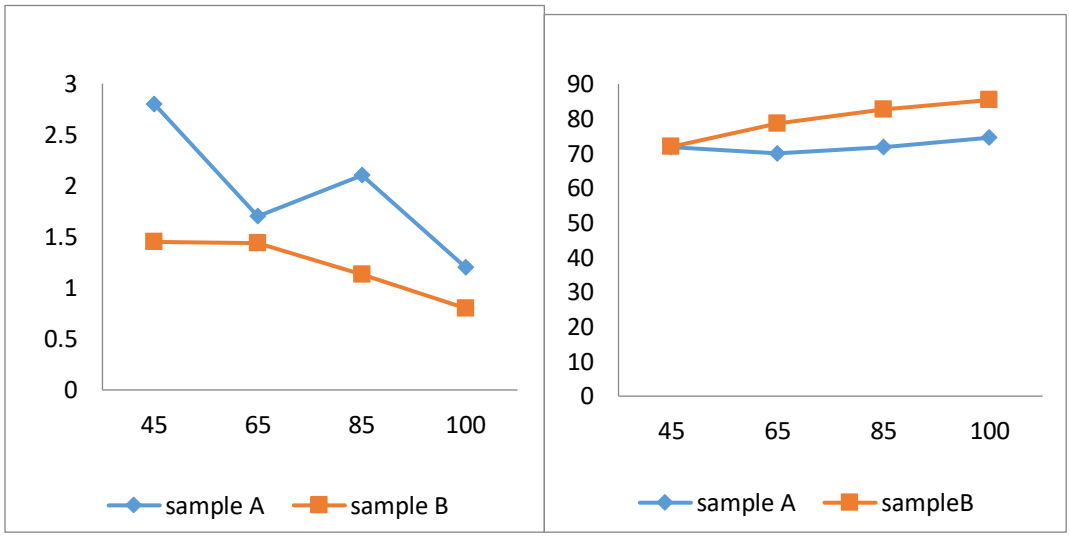


Figure 3.5: Graphical Representation of Protein Content **Figure 3.6**: Graphical Representation of Carbohydrate Content

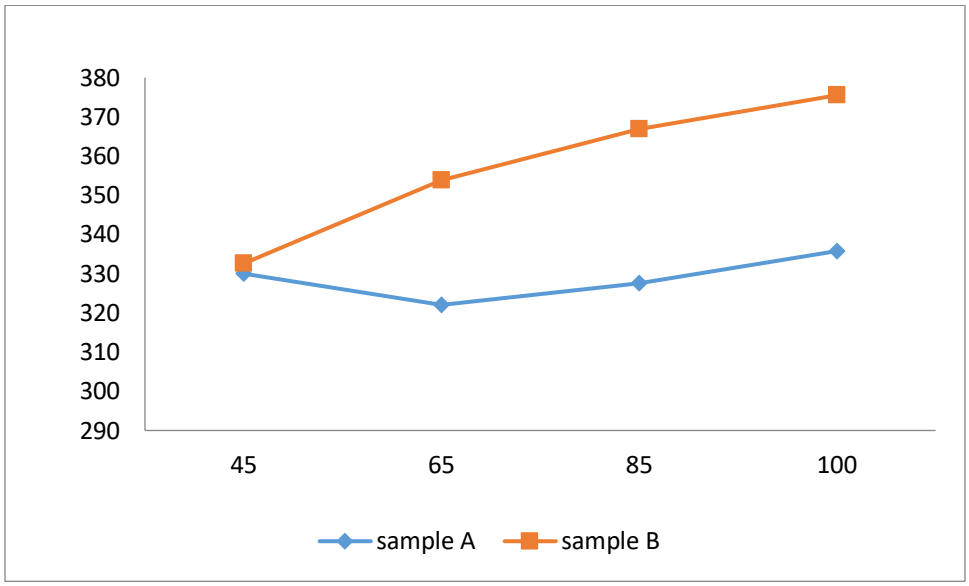


Figure 3.7: Graphical Representation of Metabolizable Energy

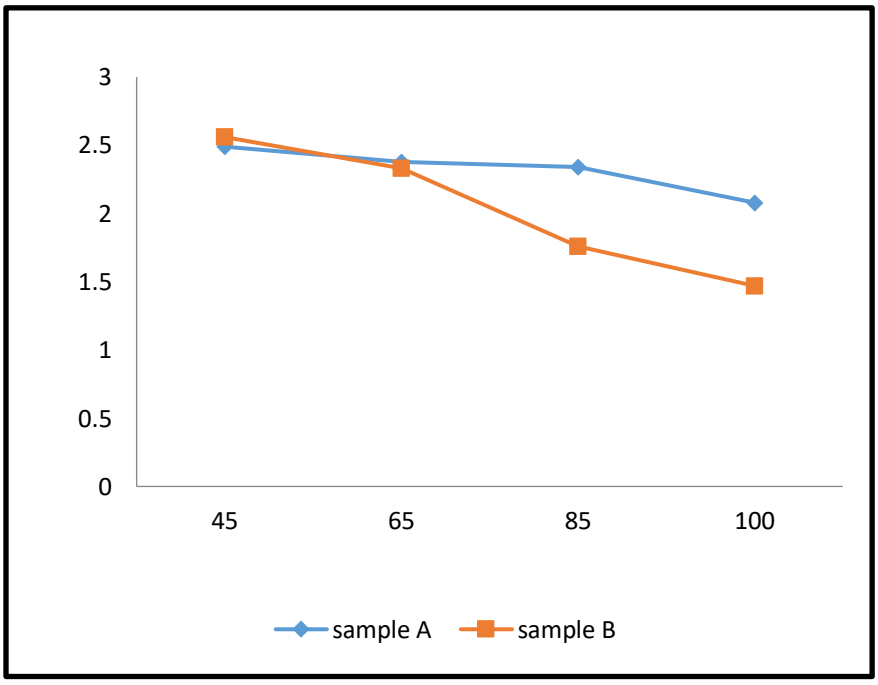


Figure 3.8: Graphical Representation of Sucrose Content

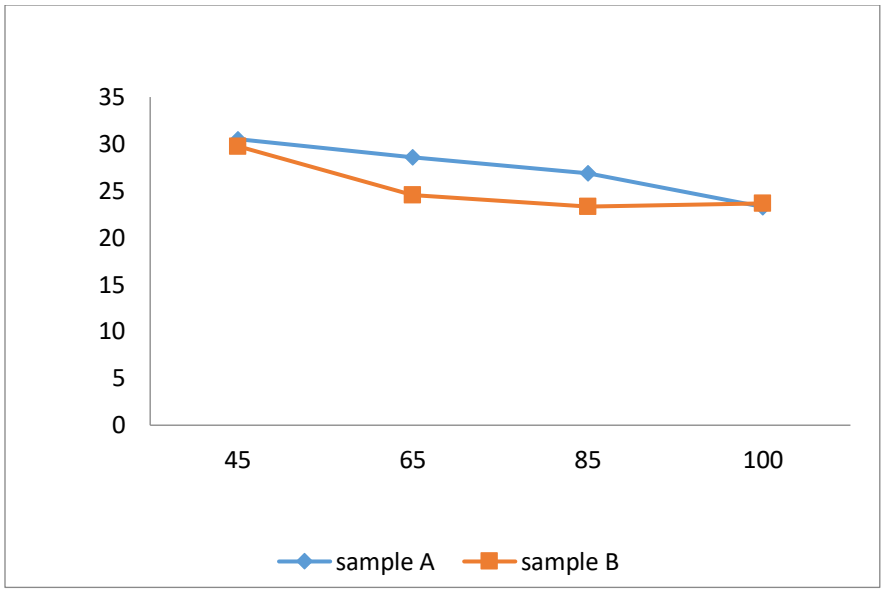


Figure 3.9: Graphical Representation of Fructose Content

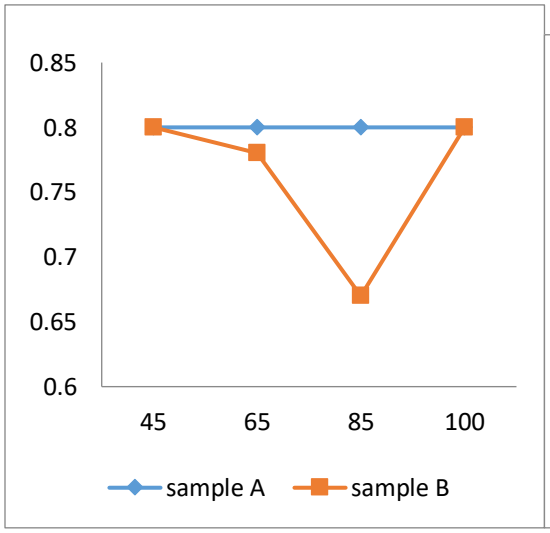


Figure 3.10: Graphical Representation of Vitamin A

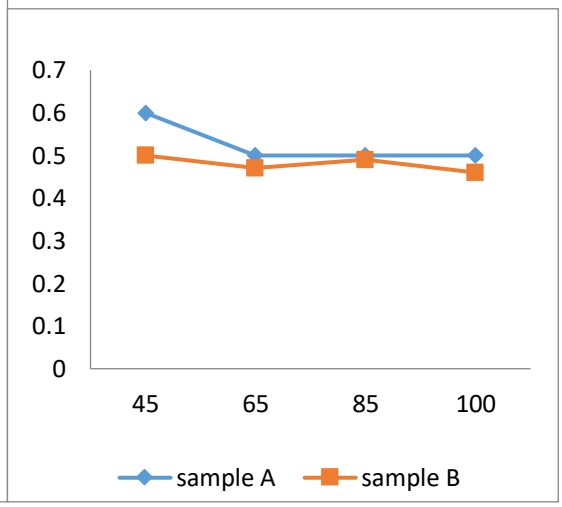
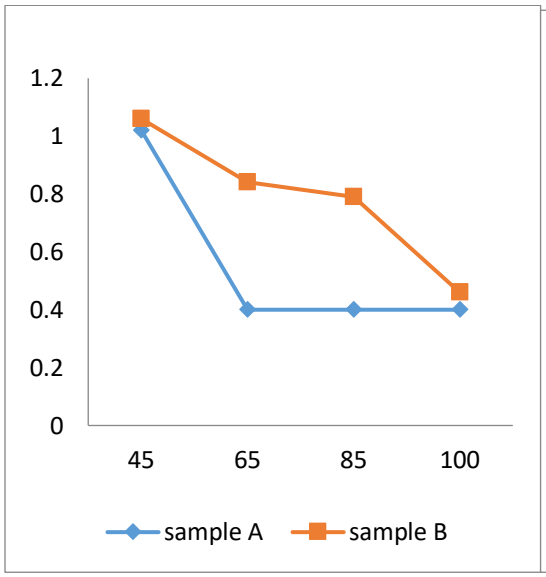


Figure 3.11: Graphical Representation of Vitamin B1



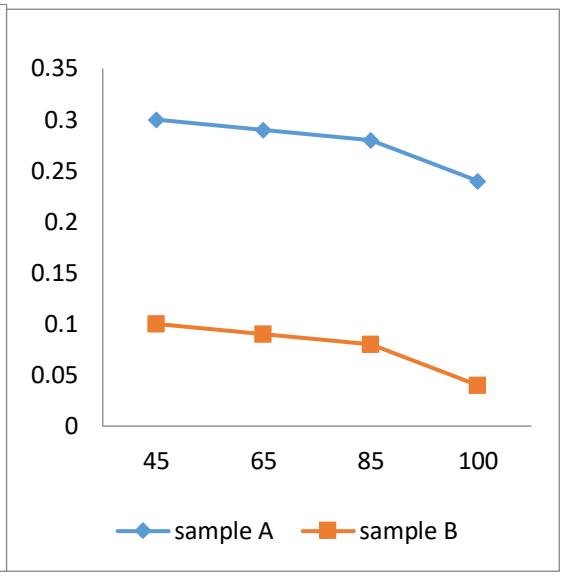
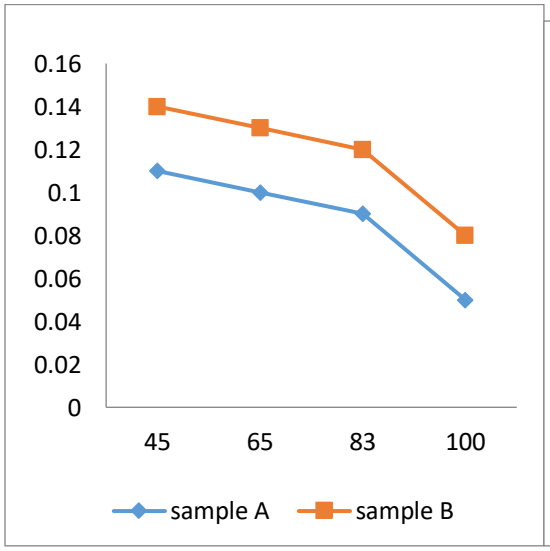


Figure 3.12: Graphical Representation of Vitamin B2 **Figure 3.13**: Graphical Representation of Vitamin B3



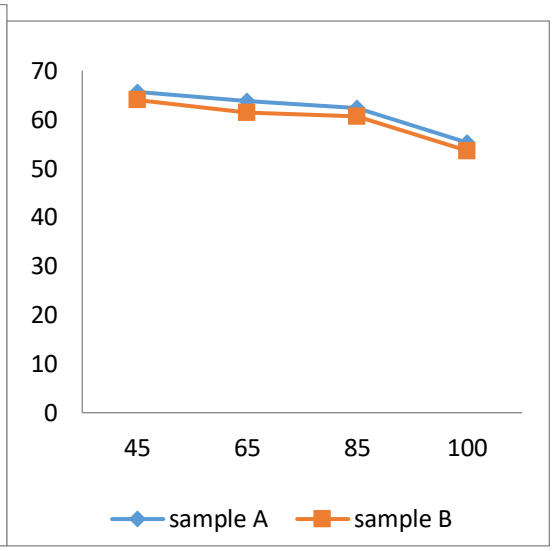
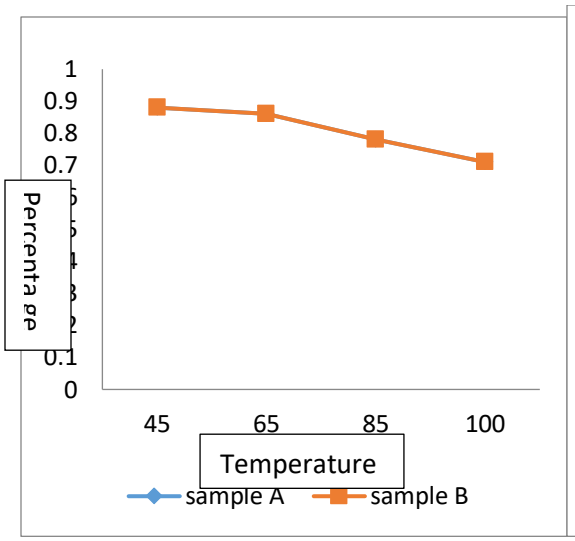


Figure 3.14: Graphical Representation of Vitamin B6 **Figure 3.15**: Graphical Representation of Vitamin C



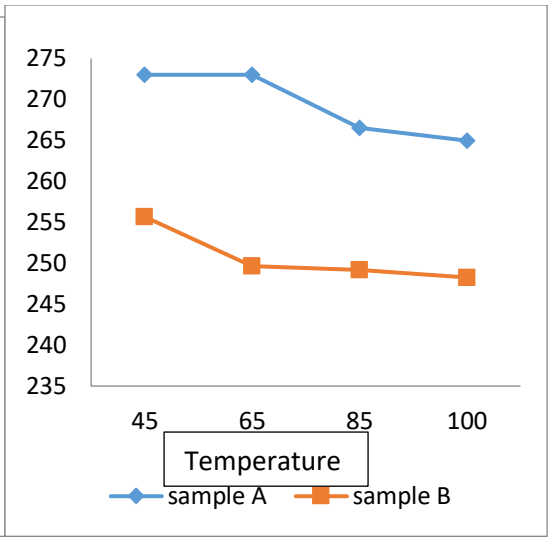


Figure 3.16: Graphical Representation of Vitamin D **Figure 3.17**: Graphical Representation of Vitamin E