



## Physicochemical, Antioxidant, and Sensorial Properties of *Xylopiya aethiopica* (Komba) Fruit on Mish Quality

WAHEEBA ELFAKI AHMED<sup>1</sup>, KAMAL AWAD ABDEL RAZIG<sup>2</sup>,  
MOHAMMED GADALLAH ELSAYED GADALLAH<sup>1</sup>, GHADAH SALEH ALREBDI<sup>3</sup>,  
AMMAR AL-FARGA<sup>4</sup> and ISLAM RAGAB<sup>3\*</sup>

<sup>1</sup>Department of Food Science and Human Nutrition, College of Agriculture and Food, Qassim University, Buraidah, Saudi Arabia.

<sup>2</sup>Department of Food Science and Technology, Alzaiem Alazhari University, Sudan.

<sup>3</sup>Department of Chemistry, College of Science, Qassim University, Buraidah, Saudi Arabia.

<sup>4</sup>Faculty of Science, Department of Biochemistry, University of Jeddah, Saudi Arabia.

### Abstract

This study investigated the potential of *Xylopiya aethiopica* (Komba) to enhance the nutritional and functional properties of mish, a traditional Sudanese fermented dairy product. Motivated by the documented nutritional significance of both *Xylopiya aethiopica* and mish, we examined dose-response effects of Komba fruit powder (incorporated at 0.0%, 0.5%, 1.0%, and 1.5% w/w) in mish produced from Dal Dairy Factory yoghurt. Chemical composition (AOAC methods), bioactive properties (DPPH radical scavenging assay), and sensory attributes (9-point structured hedonic scale; 60 panelists) were analyzed. Results demonstrated concentration-dependent improvements: The 1.5% Komba formulation exhibited significantly higher ( $P < 0.001$ ) values than the control for total solids (17.0% vs. 13.80%), protein (5.95% vs. 4.75%), fat (5.05% vs. 3.94%), ash (2.77% vs. 1.25%), fiber (1.26% vs. 0.00%), and titratable acidity (2.06% vs. 1.70% lactic acid equivalent), alongside reduced pH (5.05 vs. 5.80). Mineral analysis confirmed elevated concentrations ( $P < 0.001$ ) in the 1.5% sample: calcium (980.22 mg/100g vs. 895.60), potassium (482.61 mg/100g vs. 455.35), magnesium (182.33 mg/100g vs. 115.37), and iron (0.26 mg/100g vs. 0.19). Antioxidant activity increased proportionally to Komba concentration, with the 1.5% sample showing maximal DPPH radical scavenging ( $84.4\% \pm 0.001$  vs. control:  $67.1\% \pm 0.001$ ), total flavonoids ( $76.7 \pm 0.1$  mg QE/100g DW vs.  $30.5 \pm 0.3$ ), and total phenolics ( $39.9 \pm 0.1$  mg GAE/100g DW vs.  $14.01 \pm 0.1$ ). These findings establish *Xylopiya aethiopica* as a functional enhancer for mish, improving nutritional density and antioxidant capacity, supporting its application in value-added fermented foods and nutraceuticals.



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**CONTACT** Islam Ragab ✉ Is.Mohamed@qu.edu.sa 📍 Department of Chemistry, College of Science, Qassim University, Buraidah, Saudi Arabia.



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## Introduction

Herbal medicine (phytomedicine) represents a cornerstone of global alternative therapeutics.<sup>1</sup> *Xylopiya aethiopica* (Dunal) A. Rich, a member of the Annonaceae family commonly known as African pepper, Ethiopian pepper, or "Komba" in Sudan, exemplifies this significance.<sup>2</sup> This tree, reaching heights exceeding 20 meters, inhabits diverse ecosystems including forests, riverbanks, and arid regions across Africa.<sup>3</sup> Its distinctive fruit consists of slightly curved cylindrical pods that transition from green to brownish-black upon drying. Traditional medicinal practices in Sudan and Nigeria extensively utilize these fruits for treating respiratory ailments (coughs, bronchitis), gastrointestinal disorders (dysentery), edema, and as carminative or purgative agents.<sup>4-8</sup> This ethnopharmacological relevance stems from its complex phytochemical profile, documented to include alkaloids, saponins, sterols, tannins, phenols, flavonoids (e.g., rutin), cardiac glycosides, volatile and fixed oils,<sup>9-13</sup> complemented by vitamins (A-E), proteins, and essential minerals (copper, manganese, zinc).<sup>12-15</sup> While previous research has thoroughly characterized *X. aethiopica*'s phytochemistry and *in vitro* bioactivities,<sup>9-13,16-19</sup> a critical knowledge gap persists regarding its functional application within traditional food systems, particularly fermented dairy products. This omission is notable for mish, a nutritionally significant Sudanese fermented milk consumed nationwide with regional variations in spicing intensity (e.g., "Haloom" among Bija tribes).<sup>20</sup> Traditionally prepared by adding soured milk to boiled milk with spices (black cumin, salt, fenugreek, red pepper) followed by 2-3 days' fermentation,<sup>20</sup> mish presents an ideal vehicle for nutritional enhancement<sup>21-25</sup> and its biofunctional properties (antioxidative, anti-diabetic, and anti-inflammatory).<sup>21, 22, 26-29</sup> To address this research void, our study systematically investigated the novel fortification of mish with *X. aethiopica* fruit powder. Specifically, we examined the dose-dependent effects (0.0%, 0.5%, 1.0%, and 1.5% w/w) on key parameters: Chemical composition (total solids, protein, fat, fiber, ash, acidity), Mineral content (calcium, potassium, magnesium, iron), Bioactive properties (DPPH radical scavenging, total phenolics, flavonoids) and Sensory acceptability (appearance, texture, flavor, overall preference).

The goal was to establish optimal fortification levels that enhance nutritional and functional

properties while maintaining organoleptic quality, thereby leveraging the documented significance of both *X. aethiopica* and mish within Sudanese dietary culture.

## Materials and Methods

This study was conducted at Alzaeem Al-Azhari University's food processing lab, which is part of the Department of Food Science and Technology, Faculty of Agriculture, Alzaeem Al-Azhari University, Sudan.

### Materials

Yoghurt was sourced from Dal Dairy Factory (Capo), and spices (cumin, salt, and *Xylopiya aethiopica* (Komba)) were procured from the central market in Khartoum, Sudan.

### Experimental Design

A total of 6 kg of Capo yoghurt was divided into four treatments, each consisting of 1.25 kg per sample, with five replicates per treatment. The treatments included a control (0%), and three experimental groups with 0.5%, 1.0%, and 1.5% *Xylopiya aethiopica* (Komba). Additionally, cumin (0.5%) and salt (1%) were added to each treatment.

### Mish Manufacturing

The mish was prepared using Capo yoghurt produced a day prior. Cumin (0.5%) and salt (1%) were mixed into the yoghurt, which was then divided into four treatments of 1.25 kg each. Each treatment was further divided into six samples of 250 grams each. The levels of *Xylopiya aethiopica* (Komba) used were as follows: the control (0.0%), 0.5% (0.75 g per 250 g of mixture), 1.0% (1.5 g per 250 g), and 1.5% (2.25 g per 250 g). The samples were incubated at room temperature for 24 hours and, then stored at 5°C on the second day. Sensory evaluation and chemical analysis were conducted on the third day of manufacturing.

### Chemical Analysis

The total solids content was measured using the drying oven method,<sup>30</sup> protein content using the Kjeldahl method,<sup>30</sup> the fat content using the Gerber method and ash content according to (AOAC, 2000).<sup>30</sup> Finally, the mish samples were subjected to sensory evaluation by sixty panelists on the third day of production. The research protocol was previously approved by the Bioethics Committee of Qassim

university (24-14-10) The panelists rated the samples on a five-point scale for appearance, texture, flavor, and overall acceptability. Each panelist was provided with water for rinsing between samples. The samples were coded before testing. The Statistical Package for Social Sciences (SPSS, 2004) was used to statistically evaluate the data. For statistical analysis, a completely randomized design was employed, and Duncan's multiple range test was utilized to separate means at  $P < 0.001$ .

## Results

### Chemical Analysis of Mish Samples

Table (1) presents the total solids, protein, fat, ash, fiber, pH value, and titratable acidity of mish samples. The sample containing 1.5% *Xylopia aethiopica* (Komba) exhibited the highest total solid content (17.0%), while the control sample (0.0%) had the lowest (13.80%). The other samples fell in between these values ( $P < 0.001$ ).

**Table 1: Impact of *Xylopia aethiopica* (Komba) fruit levels on physiological properties (%) of Mish.**

| Items             | Levels of <i>Xylopia aethiopica</i> (%) |                           |                           |                           |
|-------------------|---|---------------------------|---------------------------|---------------------------|
|                   | 1.5                                     | 1.0                       | 0.5                       | 0.0                       |
| Total solid       | 17.01 <sup>a</sup> ±0.16                | 16.61 <sup>a</sup> ± 0.13 | 14.92 <sup>b</sup> ± 0.18 | 13.80 <sup>c</sup> ± 0.15 |
| Protein content   | 5.95 <sup>a</sup> ±0.12                 | 5.83 <sup>ab</sup> ± 0.08 | 5.09 <sup>b</sup> ± 0.11  | 4.75 <sup>c</sup> ±0.09   |
| Fat content       | 5.05 ±0.06                              | 4.88± 0.03                | 4.66± 0.05                | 3.94 ±0.07                |
| Ash content       | 2.77 <sup>a</sup> ±0.03                 | 2.35 <sup>a</sup> ±0.07   | 1.86 <sup>b</sup> ± 0.04  | 1.25 <sup>b</sup> ± 0.05  |
| Fiber             | 1.26 <sup>a</sup> ± 0.05                | 0.92 <sup>b</sup> ±0.02   | 0.53 <sup>c</sup> ±0.01   | 0.00 <sup>d</sup> ±0.00   |
| pH value          | 5.05 <sup>b</sup> ±0.04                 | 5.30 <sup>ab</sup> ± 0.07 | 5.60 <sup>ab</sup> ±0.09  | 5.80 <sup>a</sup> ±0.08   |
| Treatable acidity | 2.06 <sup>a</sup> ±0.05                 | 1.95 <sup>b</sup> ±0.04   | 1.85 <sup>c</sup> ± 0.07  | 1.70 <sup>c</sup> ±0.06   |

\*Mean ± SD. Rows with different superscript letters indicate significant differences ( $P < 0.001$ ).

### Minerals Content of Mish Samples

Table (2) presents the calcium, potassium, magnesium, and iron content in mish samples. The highest concentrations of calcium (980.22 mg/100g), potassium (482.61 mg/100g), magnesium (182.33 mg/100g), and iron (0.26 mg/100g) were found in the

sample containing 1.5% *Xylopia aethiopica* (Komba). In contrast, the control sample had the lowest concentrations: calcium (895.60 mg/100g), potassium (455.35 mg/100g), magnesium (115.37 mg/100g), and iron (0.19 mg/100g). The other samples ranked in intermediate positions ( $P < 0.001$ ).

**Table 2: Impact of *Xylopia aethiopica* (Komba) fruit levels on minerals content (mg/100g) of mish.**

| Items     | Levels of <i>Xylopia aethiopica</i> (%) |                            |                            |                            |
|-----------|---|----------------------------|----------------------------|----------------------------|
|           | 1.5                                     | 1.0                        | 0.5                        | 0.0                        |
| Calcium   | 980.22 <sup>a</sup> ±0.14               | 697.0 <sup>ab</sup> ± 0.16 | 931.51 <sup>b</sup> ± 0.15 | 895 <sup>c</sup> ±0.13     |
| Potassium | 482.61 <sup>a</sup> ± 0.13              | 471.70 <sup>b</sup> ± 0.14 | 467.40 <sup>c</sup> ± 0.19 | 455.35 <sup>d</sup> ± 0.17 |
| Magnesium | 182.33 <sup>a</sup> ±0.12               | 130.74 <sup>b</sup> ±0.15  | 122.50 <sup>c</sup> ± 0.16 | 115.37 <sup>d</sup> ± 0.11 |
| Iron      | 0.26 <sup>a</sup> ±0.05                 | 0.24 <sup>b</sup> ±0.09    | 0.21 <sup>c</sup> ± 0.06   | 0.19 <sup>d</sup> ±0.08    |

\*Mean ± SD. Rows with different superscript letters indicate significant differences ( $P < 0.001$ ).

**Sensory Evaluation of Mish Samples**

Table (3) summarize the mean sensory attributes as evaluated by panelists for the four mish samples,

focusing on appearance, texture, flavor, and overall acceptability.

**Table 3: Impact of *Xylopi aethiopica* (Komba) fruit levels on organoleptic quality of mish.**

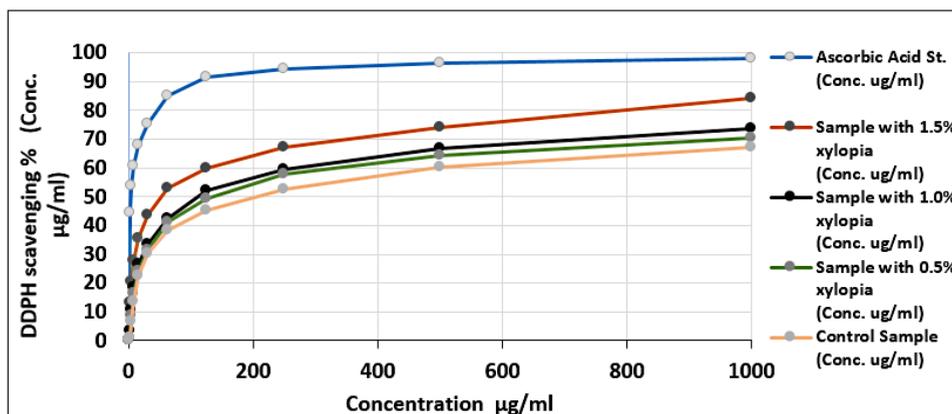
| Items                 | Levels of <i>Xylopi aethiopica</i> (%) |                         |                         |                         |
|-----------------------|--|-------------------------|-------------------------|-------------------------|
|                       | 1.5                                    | 1.0                     | 0.5                     | 0.0                     |
| Appearance            | 4.3 <sup>c</sup> ±0.05                 | 4.7 <sup>a</sup> ± 0.03 | 4.5 <sup>b</sup> ± 0.04 | 4.2 <sup>d</sup> ±0.08  |
| Texture               | 4.6 <sup>c</sup> ± 0.01                | 4.8 <sup>a</sup> ± 0.02 | 4.7 <sup>b</sup> ± 0.03 | 4.4 <sup>d</sup> ± 0.06 |
| Flavor                | 4.5 <sup>c</sup> ±0.03                 | 4.9 <sup>a</sup> ±0.01  | 4.7 <sup>b</sup> ± 0.02 | 4.1 <sup>d</sup> ± 0.07 |
| Overall acceptability | 4.4 <sup>c</sup> ±0.06                 | 4.8 <sup>a</sup> ±0.02  | 4.6 <sup>b</sup> ± 0.04 | 4.2 <sup>d</sup> ±0.05  |

Mean ± SD. Rows with different superscript letters indicate significant differences (P < 0.001).

**Antioxidant Activity of Mish Samples in Different Concentration of *Xylopi aethiopica* Fruit Seeds**

The data in Figure 1,2 and figure 3 shows the DPPH radical scavenging activity (mM Trolox equivalent ml-1), TFC (mg quercetin equivalent 100g-1 DW), and TPC (mg gallic acid equivalent 100g-1 DW) for each sample. Demonstrate how the concentration of *Xylopi aethiopica* fruits seeds affects the DPPH radical scavenging activity percentage, TFC, and TPC. The highest DPPH radical scavenging activity percentage, TFC, and TPC for mish samples with varying concentrations of *Xylopi aethiopica* fruits and

seeds (0.5, 1, and 1.5) was detected by all samples, but the sample containing 1.5% *Xylopi aethiopica* (Komba) had the highest percentage (.84.4±0.001-13.0 ±(0.001), (76.7± 0.1) and (39.9± 0.1) while the control sample had the lowest (0.67.1± 0.001-.0.9±0.005), (30.5 ± 0.3) and ( 14.01± 0.1) DPPH radical scavenging activity percentage, TFC, and TPC respectively. The remaining samples were within the range of these values (P < 0.05). However, Onyenibe<sup>47</sup> found DPPH % ug/ml) was 52.45± and Mojisola<sup>48</sup> found TFC, and TPC (mg/g) were (15.57± 0.20 and 11.71± 0.04) respectively.



**Fig. 1: Antioxidant activity (%) of *X. aethiopica* (Dose-dependent DPPH scavenging (84.4% at 1.5% Komba)**

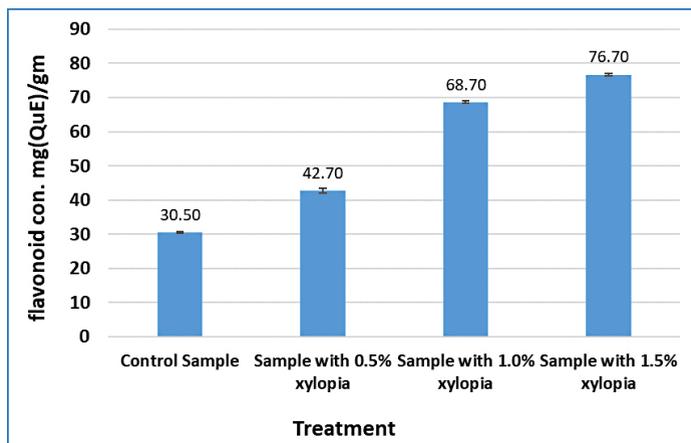


Fig. 2: Total flavonoid compounds (mg/g) of *X. aethiopica*

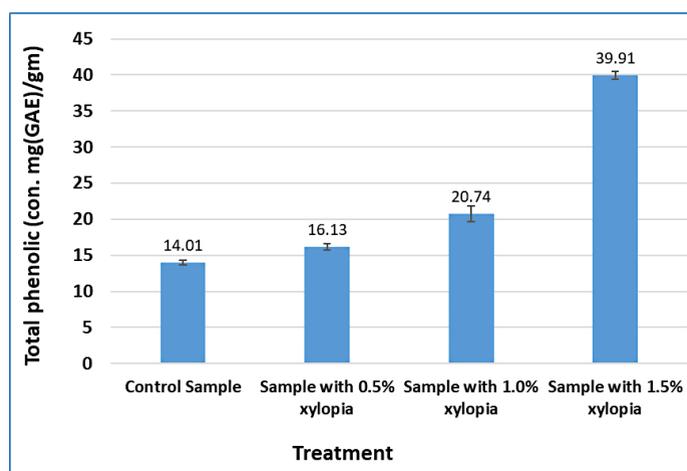


Fig. 3: Total phenolic compounds (mg/g) of *X. aethiopica*

## Discussion

### Chemical Analysis of Mish Samples

The high total solids content may be attributed to fermentation, flavoring addition, and the concentration and conversion of raw milk from liquid to gel form.<sup>31</sup>

The highest protein content (5.95%) was also observed in the sample with 1.5% *Xylopia aethiopica* (Komba), with the control sample having the lowest (4.75%). The remaining samples had intermediate rankings ( $P < 0.001$ ). These results were higher than those reported by (El-Zubeir *et al.*, 2005),<sup>32</sup> who found the protein content of mish to be  $5.09 \pm 2.80$  and (Ajibade *et al.*, 2025).<sup>33</sup> The increased protein content in the mish samples may be due to the varying levels of *Xylopia aethiopica* (Komba) used during production.

Similarly, the highest fat content (5.05%) was found in the sample with 1.5% *Xylopia aethiopica* (Komba), while the control sample had the lowest (3.94%). The remaining samples had intermediate rankings ( $P < 0.001$ ). These values were lower than those reported by (Nassib & El-Gendy, 1974)<sup>34</sup> and (El-Zubeir *et al.*, 2005 and Ajibade *et al.*, 2025)<sup>32,33</sup> for mish.

The Sudanese Standards and Metrology Organization specifies that the fat content in mish should not exceed 3%, highlighting a discrepancy between the findings of this study and the recommended values.<sup>35</sup> The sample with 1.5% *Xylopia aethiopica* (Komba) had the greatest ash concentration (2.77%), while the control sample (0.0%) had the lowest ash content (1.25%), with other samples falling in between ( $P < 0.001$ ). These results align with El Zubeir,<sup>32</sup> who reported

an ash content of 1.26% but are higher than those reported by El Erian.<sup>36</sup>

The highest fiber content (1.26%) was also found in the sample with 1.5% *Xylophia aethiopica* (Komba), whereas the control sample had no detectable fiber (0.00%). There were several samples that placed in the middle ( $P < 0.001$ ). These results are lower than those reported by Osabor,<sup>33,37</sup> who found a crude fiber content of 3.87% in *Xylophia aethiopica* (Komba) fruit, which also revealed significant mineral concentrations.

The control sample (0.0% *Xylophia aethiopica* (Komba)) had the highest pH value (5.80), while the lowest pH value (5.05) was found in the sample with 1.5% *Xylophia aethiopica* (Komba), with other samples ranking in between ( $P < 0.001$ ). These pH values are higher than those reported by Osman<sup>38</sup>, but similar to those reported by Nassib and El-Gendy.<sup>34</sup> The increase in acidity, resulting in lower pH values, is attributed to the fermentation process, which increases acid concentration.

The highest titratable acidity (2.06%) was observed in the sample with 1.5% *Xylophia aethiopica* (Komba), while the control sample had the lowest (1.70%), with other samples ranking in intermediate positions ( $P < 0.001$ ). These findings surpass those of,<sup>32</sup> who discovered that the titratable acidity of mish samples was  $1.24\% \pm 0.41$ . The increase in acidity is primarily due to the proliferation of lactic acid bacteria, which convert lactose into lactic acid.<sup>39-43</sup>

#### **Minerals Content of Mish Samples**

The results indicate a higher concentration of calcium, which is necessary for the healthy development and upkeep of teeth and bones, as well as for nerve transmission.<sup>44</sup> The daily recommended intake of calcium is 360-400 mg/day for both children and adults. However, these findings are lower than those reported by,<sup>37</sup> who found calcium levels of 2.13 mg/100g in *Xylophia aethiopica* fruit.

This study's potassium content was also less than what was reported by Osabor,<sup>37</sup> who found potassium levels of 4.80 mg/100g in *Xylophia aethiopica* fruit. Potassium ions help regulate the pH of intracellular fluids and maintain osmotic pressure within cells.<sup>45</sup> Similarly, the magnesium content was lower than that reported by Osabor,<sup>37</sup> who found magnesium levels of 2.40 mg/100g in *Xylophia aethiopica* fruit.

Magnesium is crucial for various biophysiological processes, including enzyme activation and DNA structure stabilization.<sup>46</sup>

The iron content in this study was lower than that reported by John-Dewole<sup>11</sup>, who found iron levels of 0.690 mg/100g in *Xylophia aethiopica* fruit through spectroscopic analysis of rare metals.

#### **Sensory Evaluation of Mish Samples**

The highest ratings for overall acceptability (4.8), texture (4.8), flavor (4.9), and appearance (4.7) were given to the sample that contained 1.0% *Xylophia aethiopica* (Komba) fruit. On the other hand, the sample containing 0.0% *Xylophia aethiopica* (Komba) scored the lowest in terms of general acceptability (4.2), texture (4.4), flavor (4.1), and appearance (4.2). The other samples ranked in intermediate positions ( $P < 0.001$ ).

#### **Antioxidant Activity of Mish Samples in different Concentration of *Xylophia Aethiopica* Fruit Seeds**

The rich flavonoids and phenolic compounds found in *X. aethiopica* fruit position it as a natural remedy for various conditions including cancer, heart disease, atherosclerosis, and peptic ulcers prevention. These compounds, notably flavonoids, act as potent antioxidants crucial in combatting free radicals and preventing degenerative diseases such as heart conditions.<sup>49</sup> Moreover, flavonoids play a significant role in regulating the cell cycle, inducing apoptosis, and inhibiting the growth of cancer cells.<sup>50</sup> Their multifaceted functions as anti-proliferative agents, estrogen modulators, and antioxidants offer protection against cancer and atherosclerosis. Additionally, phenolic compounds, recognized for their antioxidant properties, exhibit anti-inflammatory, anticancer, and atherosclerotic effects.<sup>51-53</sup> Studies have also highlighted the potential of plant extracts rich in flavonoids in the prevention of peptic ulcers.<sup>15,54</sup>

#### **Conclusion**

In the study, it was investigated that the effects of different levels of *Xylophia aethiopica* (Komba) on the quality of mish, a vital component of the Sudanese diet. The study utilized yoghurt from the Dal Dairy Factory and *Xylophia aethiopica* fruits from Khartoum State, Sudan. These fruits were air-dried and manually ground to create four distinct treatment groups: a control group with 0.0% *Xylophia aethiopica*

and experimental groups with concentrations of 0.5%, 1.0%, and 1.5%. Following incubation and storage, thorough assessments were conducted on the third day, including analyses of chemical composition, antioxidant activity (DPPH radical scavenging activity percentage, TFC, TPC), and sensory evaluations. The results revealed that the mish sample containing 1.5% *Xylopiya aethiopica* exhibited superior characteristics, with higher levels of total solids, protein, fat, fiber, and acidity compared to other samples. Additionally, it showed elevated levels of calcium, potassium, magnesium, and iron. In sensory evaluation, the mish sample with 1.0% *Xylopiya aethiopica* was favored for its exceptional appearance, texture, flavor, and overall acceptability. This study highlights the significant improvement in nutritional parameters and antioxidant activity in mish through the inclusion of *Xylopiya aethiopica*. It emphasizes the potential of *Xylopiya aethiopica* as a functional ingredient with nutritional and antioxidant benefits, suggesting its potential for enhancing mish quality in the food industry and potentially in medicinal applications. Future studies should explore encapsulation techniques to optimize sensory acceptance of higher concentration *Xylopiya aethiopica*-fortified mish while preserving its enhanced nutraceutical properties for commercial scalability.

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#### Conflict of Interest

The authors declare no conflict of interest.

#### Data Availability Statement

The article contains the information that was utilized to support the study's conclusions.

#### Ethics Statement

The research protocol was previously approved by the Bioethics Committee of Qassim University (24-14-10)

#### Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

#### Clinical Trial Registration

This research does not involve any clinical trials.

#### Permission to Reproduce Material from other Sources

The manuscript did not contain any materials such as figures, tables, or text excerpts that have been previously published elsewhere.

#### Author Contributions

- **Waheeba.E.Ahmed:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing
- **Kamal Awad Abdel Razig:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Resources, Writing – original draft, Writing – review & editing.
- **Mohamed G.E. Gadallah:** Conceptualization, Formal analysis, Resources, Writing – review & editing
- **Ghadah S. Alrebdi :** Conceptualization, Formal analysis, Resources, Writing – review & editing
- **Ammar AL-Farga:** Conceptualization, Formal analysis, Resources, Writing – review & editing
- **Islam Ragab:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing

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