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## Standardisation and quality evaluation of garlic fermented with dammar bee honey: A functional fermentation approach

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### Abstract

Garlic fermented in honey is a traditional preparation long valued for its anti-inflammatory and immune-boosting effects. However, it remains scientifically underexplored and lacks standardisation in terms of processing and quality evaluation. This study aimed to develop a functional, shelf-stable version of this product using dammar bee (*Tetragonula iridipennis*) honey, a rare, bioactive-rich variety, by optimising the honey-to-garlic ratio through a completely randomised design (CRD) comprising seven treatments (T<sub>1</sub>-T<sub>7</sub>) and three replications. Preliminary trials determined optimal fermentation conditions, followed by organoleptic evaluation using a 9-point hedonic scale (parameters: appearance, colour, flavour, taste, texture, and overall acceptability). T<sub>6</sub> (60% honey + 40% garlic) was identified as the most acceptable treatment and was subjected to refrigerated storage (4 ± 1 °C) for three months. Organoleptic evaluations were conducted on monthly basis, while physicochemical, and microbiological analyses were conducted at the beginning and end of the storage period. Evaluated quality parameters included moisture, TSS, titratable acidity, pH, total sugar, reducing sugar, non-reducing sugar, thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, vitamin C, calcium, phosphorus, potassium, iron, zinc, total phenolic content (TPC), and total flavonoid content (TFC). Functional attributes were assessed by measuring total antioxidant and anti-inflammatory activity, alongside microbial enumeration of fungi, yeast, and bacteria. No microbial spoilage was observed during storage. While sensory and functional parameters remained consistently high, slight reductions were observed in certain physicochemical attributes such as pH, vitamin C and B, likely due to natural fermentation dynamics and refrigerated storage. The results underscore the efficacy of dammar bee honey not only as a natural fermentation medium but also as a functional agent that enhances organoleptic properties, stabilizes bioactives, and significantly suppresses the harsh flavour and odour of garlic, yielding a more palatable, shelf-stable product with improved sensory and therapeutic appeal.

**Keywords:** Dammar bee honey, functional pastilles, process optimization, organoleptic evaluation, storage stability

### Introduction

Fermentation has long been employed as a traditional method to enhance the nutritional, functional, and sensory properties of foods (Sionek *et al.*, 2023) <sup>[40]</sup>. Among the substrates used in food fermentations, natural sweeteners such as honey have recently garnered increasing interest due to their prebiotic potential, antimicrobial activity, and ability to support beneficial microbial growth (Thipraksa *et al.*, 2023) <sup>[41]</sup>. Garlic (*Allium sativum*), known for its potent antimicrobial, antioxidant, and anti-inflammatory properties, is a staple in traditional medicine and functional food applications [Garlic therapeutic properties]. However, its pungent aroma and intense flavour often limit its direct incorporation into consumer-friendly formats (Baquiran *et al.*, 2023) <sup>[6]</sup>.

Honey, especially from stingless bees, serves as a unique natural medium for fermentation. Its low pH, high osmolality, and diverse phytochemical profile provide a selective environment that supports the growth of specific fermentative microbes while suppressing spoilage organisms (Fentie *et al.*, 2022) <sup>[17]</sup>. Among stingless bee honeys, *Tetragonula iridipennis* (dammar bee honey) stands out due to its rare polyphenolic compounds,

heightened antioxidant activity, and superior therapeutic potential compared to *Apis mellifera* honey (Zaldivar-Ortega *et al.*, 2024; Bhatta) [47]. Despite its value in traditional medicine, its application as a fermentation base remains largely unexplored in scientific literature, especially in structured food systems.

Fermented honey garlic preparations have been used in many indigenous healing practices across Asia for their immunity-boosting and anti-inflammatory benefits (Kumar *et al.*, 2024) [31]. However, the lack of standardisation and scientific validation restricts their acceptance in mainstream functional food industries. Previous attempts at garlic fermentation have largely focused on black garlic or brine-based methods, which may alter the nutritional profile or introduce unwanted sensory changes (Ryu *et al.*, 2017; Jones *et al.* 1994) [38, 28]. Using dammar bee honey as a fermentative medium not only leverages its natural preservative and therapeutic qualities but also holds the potential to retain or even enhance the bioactive components of garlic while reducing its pungency and bitterness (Dezmirean *et al.*, 2012) [15].

Considering the well-established therapeutic potential of both garlic and honey, their combination through fermentation represents a novel and synergistic strategy for developing value-added functional foods (Wolde *et al.*, 2024) [45]. This study was undertaken to standardise the fermentation of garlic using dammar bee honey, evaluate its physicochemical, sensory, and functional characteristics, and assess its shelf life under refrigerated conditions. The work seeks to validate and optimise a traditional formulation through rigorous scientific methodology, with implications for future development of stable, consumer-acceptable, health-promoting fermented products.

## Materials and Methods

### Materials

Fresh garlic bulbs (*Allium sativum*), uniform in size and free from physical damage or microbial contamination, were procured from a certified organic vendor in Thrissur, Kerala. Dammar bee honey (*Tetragonula iridipennis*) was sourced from the Department of Agricultural Entomology, Kerala Agricultural University, Vellanikkara, Thrissur. The raw honey was filtered through sterile muslin cloth to remove physical impurities and stored in amber glass bottles at  $4 \pm 1^\circ\text{C}$  to preserve thermolabile and photosensitive bioactive compounds. Authenticity was confirmed by evaluating standard physicochemical parameters including moisture content, HMF, and sugar profile. All analyses were performed within four weeks of collection to retain bioactivity.

Additional formulation ingredients included food-grade isomalt and cinnamon extract, both procured from local certified food ingredient suppliers. High-bloom strength gelatin (300 Bloom) was obtained from Nitta Gelatin India Ltd. (Cochin, Kerala) to ensure optimal texture and structural integrity of the product. A food-grade antimicrobial agent, potassium metabisulfite (KMS), was also sourced locally and reserved for use based on microbial stability outcomes during storage trials. All ingredients used were food-grade and compliant with FSSAI specifications.

### Standardisation of fermentation treatments

Seven different formulations of honey fermented garlic were developed using a completely randomized design (CRD)

with honey concentrations ranging from 10% to 70% (w/w%), each replicated thrice (Table 1). Garlic cloves were peeled, weighed, and mixed with pre-measured volumes of dammar bee honey. The mixture was transferred to sterile amber glass jars, sealed, and kept at room temperature ( $28 \pm 2^\circ\text{C}$ ) for spontaneous fermentation.

**Table 1:** Treatment combinations of dammar bee honey and garlic (w/w%)

Treatment	Dammar bee honey (%)	Garlic (%)
T <sub>1</sub>	10	90
T <sub>2</sub>	20	80
T <sub>3</sub>	30	70
T <sub>4</sub>	40	60
T <sub>5</sub>	50	50
T <sub>6</sub>	60	40
T <sub>7</sub>	70	30

### Preliminary standardization of fermentation duration

To determine the optimal duration for honey-garlic fermentation, a series of preliminary trials were conducted using three representative treatment combinations: low (T<sub>1</sub> - 10% honey), medium (T<sub>4</sub> - 40% honey), and high (T<sub>7</sub> - 70% honey) honey concentrations. Each treatment was monitored over a 30-day period at ambient temperature ( $28 \pm 2^\circ\text{C}$ ), and changes in physicochemical parameters, including pH, titratable acidity, total soluble solids (TSS), and visual indicators of fermentation such as gas formation and color change, were recorded at regular intervals. Based on these observations, a fermentation duration of 21 days was identified as optimal, balancing sufficient acidification and flavor development without compromising organoleptic quality or causing excessive deterioration in product appearance or texture.

### Organoleptic evaluation

All seven honey-fermented garlic treatments (T<sub>1</sub> to T<sub>7</sub>) were subjected to organoleptic evaluation following fermentation. A semi-trained sensory panel of 20 judges was selected and oriented according to the method outlined by Jellinek (1985) [27]. Using a 9-point hedonic scale (1 - dislike extremely to 9 - like extremely), the samples were evaluated for appearance, colour, flavour, taste, texture, and overall acceptability. The test was conducted in a controlled setting, with samples coded and presented in randomized order to minimize bias. The treatment with the highest overall acceptability score was selected for further storage and quality analysis.

### Storage study and quality evaluation of selected treatment

The treatment exhibiting the highest overall sensory acceptability was selected for further study. The sample was packed in sterile, airtight glass bottles and stored under refrigerated conditions ( $4 \pm 1^\circ\text{C}$ ) for a period of three months. Quality evaluation was conducted at two time points, initially (0th day) and after three months of storage. The analyses included the following:

### Physico-chemical parameters

#### Moisture

The moisture content of sample was determined using the A.O.A.C method (2023). The moisture content of the sample was evaluated by taking its known weight and

drying it in a hot air oven at 60 °C to 70 °C, after which it was cooled in a desiccator and weighed. The heating and cooling operation was repeated until a steady weight was obtained. The moisture content of the sample was determined by using the weight loss during drying.

#### Total Soluble Solids (TSS)

Total soluble solids of the sample were determined using hand refractometer. The readings were taken at room temperature and expressed as degree brix Ranganna (2017) [37].

$$\% \text{ Titrateable acidity} = \frac{\text{Titre value} \times \text{Normality of NaOH} \times \text{Volume made up} \times \text{Equivalent weight of acid} \times 100}{\text{Volume of sample taken for estimation} \times \text{Weight of sample taken} \times 1000}$$

#### pH

pH was directly determined by the pH meter (Mi 150 pH/temperature Bench meter) after standardization with a buffer (pH 4.0 at 20°C and pH 7.0)

#### Sugar profile

##### Total sugar

The total sugars were determined using the method suggested by Ranganna (2017) [37]. From the clarified solution used for the estimation of reducing sugar, 50 mL was taken this solution was gently boiled after adding citric acid and water. The volume was made upto 250 mL after neutralizing the solution with sodium hydroxide. The aliquot of this solution was titrated against fehling's solution A and B. The total sugar content was expressed as a percentage.

$$\text{Total sugars (\%)} = \frac{\text{Fehling's factor} \times 250 \times \text{dilution} \times 100}{\text{Titre value} \times 50 \times \text{weight of the sample}}$$

##### Reducing sugar

The reducing sugar content in the sample was estimated according to the method described by Ranganna (2017) [37]. A known volume of the sample was clarified using lead acetate, followed by the addition of potassium oxalate to precipitate excess lead. The filtrate obtained was used for the analysis. An aliquot (50 mL) of this clarified solution was titrated against Fehling's solutions A and B in a 1:1 ratio. The solution was gently heated to boiling, and methylene blue was added as an internal indicator. The endpoint was noted as the disappearance of the blue colour, indicating the reduction of cupric ions to cuprous oxide. The reducing sugar content was calculated using the following formula and expressed as a percentage on a fresh weight basis:

$$\text{Reducing sugar (\%)} = \frac{\text{Fehling's factor} \times \text{Dilution factor} \times 100}{\text{Titre value (ml)} \times \text{Weight of the sample (g)}}$$

##### Non-reducing sugar

Non-reducing sugar content was determined by subtracting the percentage of reducing sugars from the total sugars. It was calculated using the following formula:

#### Titrateable acidity

To determine acidity of the sample, the method suggested by Ranganna (2017) [37] was followed. Titrateable acidity was determined by titrating the fruit juice against 0.1 N sodium hydroxide (NaOH) using one per cent phenolphthalein solutions as an indicator. The titre values were recorded when the solution turned pink. Titrateable acidity was expressed as per cent citric acid equivalent using the formula;

$$\text{Non-reducing sugar (\%)} = \text{Total sugars (\%)} - \text{Reducing sugars (\%)}$$

#### Vitamin content

##### Thiamine (B<sub>1</sub>) and Riboflavin (B<sub>2</sub>)

Thiamine (B<sub>1</sub>) and Riboflavin (B<sub>2</sub>) were estimated according to the method described by Fernando and Murphy (1990) [18]. The samples were subjected to acid hydrolysis followed by enzymatic treatment to release bound vitamins. The extract was then filtered and injected into an HPLC system equipped with a fluorescence detector. Separation was achieved using a reversed-phase C18 column, and quantification was done by comparing peak areas with those of known standards.

##### Niacin (B<sub>3</sub>)

Niacin (B<sub>3</sub>) was determined following the method of Tyler and Shrago (1980) [42]. After acid hydrolysis and sample clarification, niacin was quantified using an HPLC system fitted with a UV detector. The separation was carried out on a reversed-phase column under isocratic conditions, and niacin content was calculated based on standard calibration curves.

##### Pantothenic acid (B<sub>5</sub>) and Pyridoxine (B<sub>6</sub>)

Pantothenic acid (B<sub>5</sub>) and Pyridoxine (B<sub>6</sub>) were estimated using reversed-phase HPLC as outlined by Ciulu *et al.* (2011) [13]. Samples were subjected to acid extraction and filtration, then analyzed using an HPLC system with UV detection. Separation was performed on a C18 column using an appropriate mobile phase. Quantification was done by comparing sample peaks to external standards.

#### Vitamin C

Ascorbic acid was determined via titration using 2,6-dichlorophenol indophenol dye, following the modified method of Xiao *et al.* (2012) [46]. A known weight of sample was homogenized with 3% metaphosphoric acid, filtered, and titrated with standard dye until a persistent pink endpoint. Vitamin C content (mg/100 g FW) was calculated using the formula:

$$\text{Vitamin C} = \frac{\text{Burette reading for sample} \times \text{dye factor} \times \text{final volume after dilution}}{\text{Aliquot taken} \times \text{weight of sample}}$$



## Mineral content

### Calcium

Calcium content was estimated following the method described by Perkin-Elmer (1982). One gram of the sample was pre-digested using 10 mL of a diacid mixture consisting of nitric acid and perchloric acid in a 9:4 ratio. The resulting clear extract was used for the estimation of calcium using an atomic absorption spectrophotometer (AAS). The calcium content was expressed as milligrams per 100 grams of the sample.

### Phosphorus

The phosphorus content was analysed calorimetrically as suggested by Jackson (2005) [26], which gives yellow colour with nitric acid vanadate molybdate reagent. To 5 mL pre-digested aliquot, 5 mL of nitric acid vanadate molybdate reagent was added and made up to 50 mL with distilled water. After 10 minutes, the OD was read at 420 nm. A standard graph was plotted by serial dilution of standard phosphorus solution. The phosphorus content was expressed in mg per 100 g.

### Potassium

Potassium in the sample was estimated using the method suggested by Jackson (2005) [26] with the help of a flame photometer. One gram of sample was digested using a diacid solution. The pre-digested sample was used to measure potassium content in the flame photometer and was expressed as mg per 100 g of the sample.

### Iron and zinc

The content of iron and zinc in the sample were determined following the method proposed by Perkin-Elmer (1982). One gram of the sample was accurately weighed and subjected to wet digestion using a 10 mL acid mixture composed of nitric acid and perchloric acid in a 9:4 ratio. The mixture was heated gently until a clear solution was obtained. After cooling, the digested sample was filtered and the final volume was made up with deionized distilled water. The resulting clear diacid extract was used for analysis. The concentrations of zinc, selenium, and iron were determined using an atomic absorption spectrophotometer (AAS). The results were expressed as milligrams per 100 grams of sample.

## Bioactive compounds

### Total Phenolic Content (TPC)

Total phenolic compounds were measured using a modified method by Albano and Miguel (2011). Methanolic extracts (330 µL) were mixed with 3 mL of 10% Na<sub>2</sub>CO<sub>3</sub> and 16 µL of Folin-Ciocalteu reagent in a 50 mL test tube. After 30 minutes of incubation in the dark at room temperature, absorbance was recorded at 760 nm. Gallic acid was used as the standard, and results were expressed as mg gallic acid equivalents (GAE) per 100 g fresh weight.

### Total Flavonoid Content (TFC)

Flavonoid content was determined using a modified gravimetric method (Harborne, 1973). 5 g of the sample was mixed with 50 mL of 80% methanol and extracted in a water bath at 40 °C for 10 hours. After filtration and evaporation, the residue was dried and weighed. The total flavonoid content was expressed as mg per g dry weight.

## Functional properties

### Total antioxidant activity (% inhibition)

The total antioxidant capacity (TAC) of the honey-fermented garlic samples was determined using the phosphomolybdate assay, as described by Prieto *et al.* (1999) [36], with modifications. Ascorbic acid was used as the standard. A stock solution of ascorbic acid (10 mg/mL) was prepared in distilled water, and serial dilutions were made for standard curve construction.

In a test tube, 300 µL of the sample extract was mixed with 3 mL of phosphomolybdate reagent (composed of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were covered with aluminum foil and incubated at 95 °C for 90 minutes. After cooling to room temperature, the absorbance was measured at 765 nm against a blank containing methanol instead of the sample. The antioxidant capacity was expressed as micrograms of ascorbic acid equivalents per milliliter (µg AAE/mL), based on the standard curve.

### Total anti-inflammatory activity (% inhibition)

The nitric oxide (NO) radical scavenging activity was determined using the method described by Marcocci *et al.* (1994) [33], with slight modifications. Sodium nitroprusside (5 mM) in phosphate-buffered saline (PBS, pH 7.4) was mixed with various concentrations of the sample and incubated at 25 ± 2 °C for 150 minutes under light to promote NO generation. After incubation, 1.0 mL of the reaction mixture was treated with 1.0 mL of Griess reagent (equal volumes of 1% sulfanilamide in 5% phosphoric acid and 0.1% N-(1-naphthyl) ethylene diamine dihydrochloride). The mixture was incubated at room temperature for 30 minutes in the dark, and absorbance was measured at 546 nm. Ascorbic acid served as the standard. The percentage inhibition of nitric oxide was calculated using the formula:

$$\text{NO inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control} \times 100}$$

## Microbial enumeration

The microbial quality of the sample was assessed by estimating the total bacterial, yeast, and fungal counts using the standard pour plate technique as described by Harrigan and McCance (1976) [24]. One gram of the sample was homogenised in 9 mL of sterile physiological saline (0.85% NaCl) to obtain the initial dilution, followed by serial dilutions up to 10<sup>-6</sup>. For total bacterial count, Plate Count Agar (PCA) was used, and the inoculated plates were incubated at 37 °C for 24-48 hours. The number of colonies was recorded and expressed as colony-forming units per gram (CFU/g).

To estimate yeast and fungal counts separately, Yeast Extract Glucose Chloramphenicol Agar (YGC Agar) was used for yeast enumeration and Potato Dextrose Agar (PDA) acidified with 1% lactic acid was used for fungi. Plates for both were incubated at 28 °C, with yeast plates observed for 3-5 days and fungal plates for 5-7 days. After incubation, colonies were counted and expressed as CFU/g. These tests provided insights into the hygienic and microbial safety status of the product.

### Organoleptic evaluation

Organoleptic evaluation was conducted initially and then on at monthly intervals, for a period of 3 months, to monitor changes in sensory attributes over time. The same panel and procedures described earlier were employed. Sensory parameters assessed included appearance, colour, flavour, taste, texture, and overall acceptability.

### Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics software, version 22 and KAU Grapes. Sensory evaluation data for the honey-fermented garlic treatments (T<sub>1</sub>-T<sub>7</sub>) were analyzed using Kendall's coefficient of concordance (W) to assess panelist agreement and identify the most preferred formulation. The treatment with the highest overall acceptability (T<sub>6</sub>) was selected for further analysis. For the storage study of T<sub>6</sub>, paired *t*-tests were conducted to compare physico-chemical parameters

between the initial day (0th) and after 3 months of refrigerated storage. Statistical significance was considered at  $p < 0.05$ .

## Results and Discussion

### Selection of Optimized Treatment

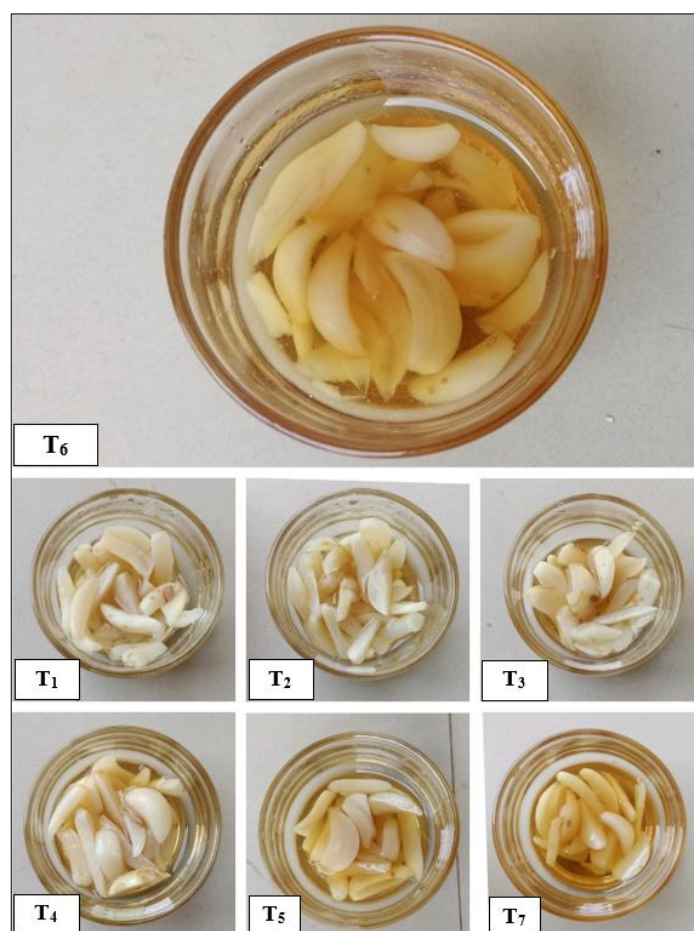
Among the seven treatment combinations developed, T<sub>6</sub> (60% honey: 40% garlic) was selected as the optimized formulation based on its superior organoleptic attributes. It received the highest mean scores for flavour, texture, and overall acceptability on a 9-point hedonic scale. The balance of sweetness from honey and pungency from garlic in T<sub>6</sub> was perceived as most harmonious by the sensory panel. Its appearance was also noted to be visually appealing with a uniform golden hue and minimal sedimentation, suggesting good dispersion and fermentation dynamics. The detailed sensory scores are presented in Table 2.

**Table 2:** Mean scores of organoleptic evaluations of different treatments of honey fermented garlic

Parameters	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>	T <sub>7</sub>	W
Appearance	6.91 (1.18)	7.45 (2.03)	7.78 (3.21)	8.02 (3.84)	8.19 (4.30)	8.62 (5.01)	8.33 (4.65)	0.621**
Colour	7.10 (1.31)	7.62 (2.21)	7.84 (3.06)	8.08 (3.77)	8.25 (4.32)	8.66 (5.02)	8.38 (4.81)	0.608**
Flavour	6.82 (1.22)	7.41 (2.34)	7.76 (3.19)	7.98 (3.68)	8.34 (4.44)	8.69 (5.15)	8.12 (4.07)	0.589**
Texture	7.05 (1.40)	7.48 (2.05)	7.89 (3.18)	8.11 (3.89)	8.39 (4.61)	8.74 (5.27)	8.19 (4.93)	0.643**
Taste	6.89 (1.28)	7.35 (2.14)	7.74 (3.08)	8.04 (3.73)	8.32 (4.22)	8.68 (4.92)	8.25 (4.50)	0.601**
Overall Acceptability	7.02 (1.50)	7.58 (2.44)	7.92 (3.33)	8.19 (3.88)	8.48 (4.41)	8.85 (5.26)	8.42 (4.67)	0.675**
Total Score	41.79	44.89	47.93	48.42	49.97	52.24	49.69	

(T<sub>1</sub> - 100% garlic: 0% honey; T<sub>2</sub> - 90% garlic: 10% honey; T<sub>3</sub> - 80% garlic: 20% honey; T<sub>4</sub> - 70% garlic: 30% honey; T<sub>5</sub> - 60% garlic: 40% honey; T<sub>6</sub> - 40% garlic: 60% honey; T<sub>7</sub> - 30% garlic: 70% honey)

W - Kendall's Coefficient of Concordance; \*\*Significant at 1% level; Values in parentheses indicate mean rank scores.



**Fig 1:** Standardised honey fermented garlic (T<sub>1</sub> - 100% garlic: 0% honey; T<sub>2</sub> - 90% garlic: 10% honey; T<sub>3</sub> - 80% garlic: 20% honey; T<sub>4</sub> - 70% garlic: 30% honey; T<sub>5</sub> - 60% garlic: 40% honey; T<sub>6</sub> - 40% garlic: 60% honey; T<sub>7</sub> - 30% garlic: 70% honey)

### Storage study and quality evaluation of selected treatment

To evaluate the stability of the optimized honey-fermented garlic formulation, the selected treatment (T<sub>6</sub> - 60% honey) was stored under refrigerated conditions (4 ± 1 °C) for a period of 3 months.

**Table 3:** Physicochemical parameters of T<sub>6</sub> honey-fermented garlic at 0 and 90 days (mean ± SD, *n* = 3)

Parameter	0 Day (Mean ± SD)	90 Days (Mean ± SD)	<i>t</i> -value	<i>p</i> -value
Moisture (%)	29.54 ± 0.48	30.76 ± 0.51	6.142	0.002 **
TSS (°Brix)	42.10 ± 0.29	41.65 ± 0.23	3.412	0.026 *
Titrateable acidity (%)	0.42 ± 0.03	0.49 ± 0.02	4.001	0.016 *
pH	3.81 ± 0.06	3.65 ± 0.08	5.259	0.004 **

Significance levels: *p* < 0.05 (\*), *p* < 0.01 (\*\*), NS = Not Significant

The physicochemical changes observed in the selected treatment (T<sub>6</sub> - 60% honey) over the 90-day storage period reflect the dynamic transformations occurring during and after the fermentation process. The significant increase in moisture content (from 29.54% to 30.76%) may be attributed to osmotic interactions between garlic and honey, which is hygroscopic in nature. This slight elevation in moisture could also result from water release due to garlic tissue softening during fermentation, as supported by earlier studies on fruit- and vegetable-based ferments (Kartik, 2021) [29].

The decrease in total soluble solids (TSS) (from 42.10°Brix to 41.65°Brix) is consistent with microbial metabolism utilizing fermentable sugars present in honey and garlic. This observation aligns with trends reported in other lactic acid fermentation systems, where sugars are converted into organic acids and other metabolites (Zhao *et al.*, 2022) [48]. While the reduction was statistically significant, the change was marginal, suggesting a relatively stable sugar profile owing to the high honey concentration, which limits microbial proliferation.

A notable increase in titrateable acidity (from 0.42% to 0.49%) and a corresponding decline in pH (from 3.81 to 3.65) were observed, indicating successful acidogenesis during the fermentation and storage phases. These acidification trends are typical of garlic submerged in high-

### Physicochemical parameters

The physicochemical parameters including moisture content, total soluble solids (TSS), titrateable acidity, and pH were assessed initially and post-storage, and the results are presented in Table 3.

sugar mediums like honey, which may support the growth of acid-tolerant microbes in early fermentation and yield increased levels of organic acids such as lactic and acetic acids. Such acidification not only contributes to the safety and shelf stability of the product but also enhances its organoleptic complexity (Hilgendorf *et al.*, 2024) [25].

These results demonstrate the chemical stability and microbial safety potential of the optimized treatment over a 3-month storage period under refrigeration, suggesting suitability for commercialization with minimal additive use. However, the absence of non-significant trends across parameters suggests that T<sub>6</sub> may represent a balance point between flavor development and stability under the selected storage conditions.

Given the limited prior research on honey fermented garlic, particularly with stingless bee honey or dammar bee honey as a substrate, these findings contribute valuable insights into optimizing fermentation conditions and shelf-life behavior. Further comparative studies using different types of honey and garlic varieties could help refine the fermentation profile for targeted functional properties.

### Sugar Profile

The total, reducing, and non-reducing sugar contents of the selected treatment (T<sub>6</sub>) were analysed at initial and final storage periods, and the results are presented in Table 4.

**Table 4:** Sugar profile of honey-fermented garlic (T<sub>6</sub>) during storage (mean ± SD, *n* = 3)

Parameter	Initial (Day 0)	Final (Day 90)	<i>t</i> -value	<i>p</i> -value
Total sugars (%)	56.42 ± 0.12	55.68 ± 0.14	7.211	0.002 **
Reducing sugars (%)	30.25 ± 0.15	32.79 ± 0.13	18.408	0.007 **
Non-reducing sugars (%)	26.17 ± 0.08	22.89 ± 0.09	29.855	0.004 **

Significance levels: *p* < 0.05 (\*), *p* < 0.01 (\*\*), NS = Not Significant

The sugar composition of the honey-fermented garlic product (T<sub>6</sub>) exhibited statistically significant changes during the 90-day storage period. A slight but significant reduction in total sugar content (from 56.42% to 55.68%) may be attributed to the ongoing fermentation process, wherein non-reducing sugars such as sucrose are hydrolyzed and subsequently metabolized by native microbes (Chua *et al.*, 2022) [12].

This is supported by the significant increase in reducing sugar content (from 30.25% to 32.79%), likely due to the conversion of sucrose to glucose and fructose, catalyzed either by natural enzymatic action from honey (e.g., invertase activity) or microbial invertases developed during fermentation (Čaušević *et al.*, 2017) [9]. The increase in reducing sugars aligns with reports from similar studies in

fruit fermentations where acidification promotes sugar inversion (Elfirta *et al.*, 2023) [16].

Concurrently, the non-reducing sugar content decreased markedly (from 26.17% to 22.89%), reinforcing the hypothesis that hydrolysis and microbial activity were key contributors to this transformation. This shift in sugar profile may also positively influence flavor development and antioxidant potential, as reducing sugars participate in Maillard-type reactions during storage, particularly under mild acidic conditions (Chen *et al.*, 2023) [10].

The elevated levels of reducing sugars post-storage suggest that the product retains fermentative activity even under refrigerated conditions, albeit at a slower rate. These results collectively point to the stability and biochemical progression of the sugar matrix in the product, potentially



contributing to its functional and sensorial qualities over time (Wang *et al.*, 2021)<sup>[44]</sup>.

Given the limited literature specifically on sugar dynamics in honey fermented garlic, particularly with stingless bee honey like dammar, these findings fill a significant gap and provide a framework for sugar profiling in similar value-added products.

**Table 5:** Vitamin content of T<sub>6</sub> honey-fermented garlic at 0 and 90 days (mean ± SD, n = 3)

Vitamin	0 Day (Mean ± SD)	90 Days (Mean ± SD)	t-value	p-value
Thiamine (B <sub>1</sub> ) (mg/100 g)	0.89 ± 0.03	0.74 ± 0.04	5.594	0.005 **
Riboflavin (B <sub>2</sub> ) (mg/100 g)	0.56 ± 0.02	0.49 ± 0.02	4.320	0.012 *
Niacin (B <sub>3</sub> ) (mg/100 g)	1.12 ± 0.05	0.98 ± 0.03	4.984	0.007 **
Pantothenic acid (B <sub>5</sub> ) (mg/100 g)	0.91 ± 0.04	0.84 ± 0.04	2.581	0.063 NS
Pyridoxine (B <sub>6</sub> ) (mg/100 g)	0.74 ± 0.03	0.66 ± 0.02	4.386	0.011 *
Vitamin C (mg/100 g)	7.38 ± 0.21	5.86 ± 0.17	11.331	0.002 **

Significance levels: p < 0.05 (\*), p < 0.01 (\*\*), NS = Not Significant

The vitamin profile of T<sub>6</sub> honey-fermented garlic revealed a storage-induced decline across most measured parameters, with the exception of pantothenic acid, which showed a non-significant decrease (p > 0.05).

Significant reductions in thiamine (B<sub>1</sub>), riboflavin (B<sub>2</sub>), niacin (B<sub>3</sub>), and pyridoxine (B<sub>6</sub>) are in line with literature indicating the vulnerability of water-soluble vitamins to oxidative degradation, low pH environments, and prolonged storage (Walther and Schmid, 2017)<sup>[43]</sup>. The acidic conditions fostered by the fermentation process (as supported by increasing titratable acidity and decreasing pH in Table 3) likely contributed to hydrolytic breakdown or destabilization of these labile vitamins (Bajaj *et al.*, 2020)<sup>[5]</sup>. The moderate decline in riboflavin and pyridoxine mirrors similar findings in acidified or fermented vegetable systems, particularly those stored under refrigeration for extended periods (Chepkoech *et al.*, 2022)<sup>[11]</sup>.

Interestingly, pantothenic acid (B<sub>5</sub>) did not show a significant decline over the 90-day period, indicating relative stability under the refrigerated, low-light, and mildly acidic conditions of this study. This is consistent with prior findings suggesting that B<sub>5</sub> exhibits greater resistance to hydrolysis compared to other B vitamins (Gonzalez-Lopez *et al.*, 2016)<sup>[22]</sup>.

The sharp decrease in vitamin C content (from 7.38 to 5.86 mg/100g; p < 0.01) aligns with the known sensitivity of ascorbic acid to oxidative stress and low pH storage. Given its role as a primary antioxidant, vitamin C is highly susceptible to degradation in the presence of metal ions,

### Vitamin Content of Honey-Fermented Garlic (T<sub>6</sub>)

Table 5 presents the vitamin content of the selected treatment (T<sub>6</sub>) at 0 and 90 days of storage. Significant reductions were observed for several B-complex vitamins and vitamin C over the storage period.

oxygen, and fermentative microbial activity (Galani *et al.*, 2017)<sup>[21]</sup>. In this study, although samples were stored under refrigeration, enzymatic activity and residual oxygen may have contributed to the observed losses.

The decline is particularly relevant when considering the functional positioning of honey-fermented garlic as a health-enhancing product. While bioactive phenolics may compensate for antioxidant losses (see upcoming section on TPC and TFC), the diminished vitamin C concentration highlights the need for potential protective measures like vacuum packaging, inert atmosphere storage, or vitamin fortification.

These results underscore the need to balance microbial activity, fermentation kinetics, and nutrient preservation in functional food development. Given the novelty of using stingless bee honey, particularly dammar bee honey, as a fermentation substrate, these findings provide a foundational reference for optimizing vitamin retention in such matrices. Future studies might explore synergistic ingredient combinations (e.g., citrus peel, green herbs) or post-fermentation stabilization strategies to enhance vitamin preservation.

### Mineral content

The mineral composition of the selected honey-fermented garlic sample (T<sub>6</sub>) was evaluated at day 0 and after 90 days of refrigerated storage. The minerals assessed include calcium, phosphorus, potassium, iron, and zinc. The values are presented in Table 6.

**Table 6:** Mineral content (mg/100 g) of honey-fermented garlic (T<sub>6</sub>) at initial and final storage

Parameter	Initial (Day 0)	Final (Day 90)	t-value	p-value
Calcium (mg/100g)	105.32 ± 1.04	102.15 ± 1.08	9.754	0.003 **
Phosphorus (mg/100g)	48.27 ± 0.89	46.83 ± 0.92	5.612	0.001 **
Potassium (mg/100g)	210.54 ± 1.12	208.67 ± 1.05	4.305	0.005 **
Iron (mg/100g)	2.68 ± 0.07	2.45 ± 0.06	8.151	0.007 **
Zinc (mg/100g)	1.53 ± 0.05	1.51 ± 0.04	1.414	NS

Significance levels: p < 0.05 (\*), p < 0.01 (\*\*), NS = Not Significant

The calcium content of honey-fermented garlic significantly declined during 90 days of storage (p < 0.01), which may be attributed to partial leaching or redistribution of minerals within the matrix during storage (Mohite *et al.*, 2013)<sup>[13]</sup>. A similar pattern was observed in phosphorus and potassium levels, showing a modest but statistically significant decrease (p < 0.01 and p < 0.05 respectively). These

findings are consistent with earlier studies that report changes in macro-mineral profiles in stored functional food systems (Kiczorowski *et al.*, 2022)<sup>[30]</sup>.

Iron levels also reduced significantly (p < 0.01), possibly due to oxidation reactions that may alter iron's chemical form and bioavailability during extended storage (Barboudi *et al.*, 2021)<sup>[7]</sup>. In contrast, zinc content did not exhibit

significant change ( $p > 0.05$ ), indicating its relative stability over the storage period. Zinc's resistance to degradation during fermentation and storage has been documented in various plant-based products.

The observed mineral losses, though statistically significant in some parameters, remained within nutritionally acceptable ranges and may not drastically affect the therapeutic potential of the product. Moreover, the high retention of potassium and zinc enhances the functional

profile of honey-fermented garlic, especially considering their established roles in cardiovascular and immune health.

### Bioactive and Functional Properties

The bioactive compounds and functional attributes of the selected honey-fermented garlic sample ( $T_6$ ) were assessed initially and after refrigerated storage. The results are presented in Table 7.

**Table 7:** Bioactive and functional properties of fresh and stored honey-fermented garlic ( $T_6$ )

Parameters	0 Day	90 Days	t-value	p-value
Total Phenolic Content (mg GAE/100 g)	218.36	208.91	2.962	0.021*
Total Flavonoid Content (mg/g dry weight)	14.82	13.21	3.401	0.009**
Total Antioxidant Activity (% inhibition)	71.48	65.32	4.115	0.003**
Total Anti-inflammatory Activity (% inhibition)	69.75	64.28	2.873	0.026*

Significance levels:  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), NS = Not Significant

The total phenolic content (TPC) of the selected honey-fermented garlic treatment ( $T_6$ ) significantly declined ( $p = 0.021$ ) from 218.36 mg GAE/100g at baseline to 208.91 mg GAE/100 g after 90 days of refrigerated storage. This reduction may be attributed to oxidative degradation or enzymatic transformation of phenolic compounds, which are known to be sensitive to storage conditions such as oxygen exposure, pH, and light (Cui *et al.*, 2024) [14]. A comparable trend was observed in the total flavonoid content (TFC), which showed a statistically significant ( $p = 0.009$ ) decline from 14.82 mg QE/g to 13.21 mg QE/g dry weight. This may be due to flavonoid oxidation or structural degradation over time, phenomena well-documented in polyphenol-rich systems like honey and garlic-based formulations (Fu *et al.*, 2021).

Functional assays also reflected this biochemical shift. The antioxidant activity, as measured by total antioxidant activity, decreased significantly ( $p = 0.003$ ) from 71.48% to 65.32%. This reduction correlates with the observed drop in TPC and TFC, as both phenolic and flavonoid compounds

are key contributors to radical scavenging ability (Ait *et al.*, 2019). In line with this, nitric oxide scavenging activity, a marker of anti-inflammatory potential, also declined significantly ( $p = 0.026$ ), falling from 69.75% to 64.28%. This could be due to the diminished concentration of active phytochemicals, including garlic-derived organosulfur compounds and polyphenols, which synergistically contribute to inflammatory modulation (Kumar *et al.*, 2024) [31].

Despite these declines, the product retained appreciable levels of bioactive compounds and functional activity even after 3 months of storage, confirming its therapeutic potential and suitability as a refrigerated, shelf-stable functional food.

### Microbial Enumeration

Microbial enumeration of the selected honey fermented garlic sample ( $T_6$ ) was assessed initially and after 3 months refrigerated storage. The results are presented in Table 8.

**Table 8:** Microbial load of fresh and stored honey-fermented garlic ( $T_6$ )

Microbial Parameter	0 Day (log CFU/g)	90 Days (log CFU/g)	t-value	p-value
Total Bacterial Count	2.04	2.56	2.837	0.028*
Yeast Count	1.86	2.13	2.125	0.049*
Fungal Count	1.92	2.10	1.674	0.114 <sup>NS</sup>

Significance levels:  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), NS = Not Significant

The microbial load of honey-fermented garlic showed a statistically significant ( $p < 0.05$ ) increase in total bacterial count after 90 days of refrigerated storage, rising from 2.04 log CFU/g to 2.56 log CFU/g. This could be attributed to the slow but viable proliferation of acid-tolerant and osmotolerant bacteria, potentially originating from the garlic matrix despite the high osmotic pressure of honey.

Yeast counts also significantly increased ( $p = 0.049$ ), from 1.86 to 2.13 log CFU/g, which suggests that certain yeast strains were able to persist or even mildly proliferate in the honey garlic system, likely due to the availability of fermentable sugars and favorable acidic pH. Similar trends have been reported in other honey- or sugar-based fermentations where selective yeast growth occurs under

refrigeration (Auchtung *et al.*, 2025) [4].

However, fungal counts did not show a statistically significant change ( $p = 0.114$ ), indicating that filamentous fungi either failed to survive or were adequately suppressed during storage. This is likely due to the combined antimicrobial effects of honey (particularly its hydrogen peroxide content and low water activity) and garlic-derived organosulfur compounds (Saad *et al.*, 2013) [39].

Although microbial counts increased slightly during storage, all values remained within acceptable thresholds reported for comparable fermented functional products (FSSAI, 2018). This suggests the product maintained microbiological stability under refrigerated conditions over the 3-month storage period.



## Organoleptic Evaluation

**Table 9:** Sensory scores of honey-fermented garlic (T<sub>6</sub>) during 3 months refrigerated storage

Sensory Parameter	0 Day	30 Days	60 Days	90 Days
Appearance	8.2 ± 0.42	8.1 ± 0.48	7.9 ± 0.50	7.8 ± 0.57
Colour	8.1 ± 0.37	8.0 ± 0.45	7.8 ± 0.49	7.6 ± 0.54
Flavour	8.3 ± 0.48	8.2 ± 0.51	8.0 ± 0.53	7.9 ± 0.52
Taste	8.4 ± 0.40	8.3 ± 0.44	8.0 ± 0.58	7.8 ± 0.61
Texture	8.5 ± 0.33	8.4 ± 0.37	8.3 ± 0.35	8.2 ± 0.39
Overall Acceptability	8.6 ± 0.31	8.4 ± 0.38	8.3 ± 0.42	8.1 ± 0.44

Monthly sensory evaluation revealed a gradual but consistent decline in most sensory parameters over the 90-day storage period. Appearance decreased from 8.2 at day 0 to 7.8 at day 90, while colour dropped from 8.1 to 7.6. These changes indicate minor visual degradation, likely due to natural pigment breakdown or browning reactions during storage. Similarly, taste declined from 8.4 to 7.8, and flavour from 8.3 to 7.9, reflecting subtle shifts in palatability as fermentation progressed. However, the rate of decline was not abrupt, suggesting a slow and stable transformation rather than spoilage.

Texture and overall acceptability remained comparatively stable, with texture decreasing slightly from 8.5 to 8.2 and overall acceptability from 8.6 to 8.1. These values remained well above 8 throughout the storage period, indicating that the product retained strong consumer appeal. The relatively smaller decline in texture suggests structural integrity was preserved during storage, while overall acceptability stayed consistently high, supporting the product's sensory shelf life of at least three months under refrigerated conditions.

## Conclusion

The study successfully standardised the fermentation of garlic using *Tetragonula iridipennis* (dammar bee) honey, resulting in a functionally rich, microbiologically stable, and organoleptically acceptable product. Among the seven formulations, T<sub>6</sub> (60% honey + 40% garlic) demonstrated superior performance across sensory parameters, particularly in appearance, flavour, and overall acceptability, while effectively diminishing the characteristic pungent odour and harsh taste of raw garlic. Comprehensive analyses revealed that the fermented product retained substantial levels of bioactive compounds, exhibited notable antioxidant and anti-inflammatory activities, and showed no microbial spoilage throughout three months of refrigerated storage. These findings affirm the potential of dammar bee honey not only as a natural fermentation substrate but also as a bio-functional enhancer that supports the development of value-added garlic-based products with extended shelf life and improved palatability.

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