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Prebiotic kelulut float beads beverage: an emerging health benefiting functional beverage

Abstract

Kelulut honey is renowned for its unique therapeutic and organoleptic properties. A novel initiative has been introduced to produce fermented kelulut honey using pure starter cultures, aimed at creating a consistent and high-quality kelulut honey beverage. The presence of organic acids, particularly acetic acid (0.8–1.0%), in fermented kelulut honey imparts a pleasantly moderate sour taste and exhibits significant antimicrobial activity against selected foodborne pathogens. Innovative products such as kelulut float beads and prebiotic kelulut float beads beverages have been developed from fermented kelulut honey to enhance uniqueness, novelty, and a chewy texture, making them more appealing to consumers. The addition of inulin to the prebiotic kelulut float beads beverage further enriches its prebiotic properties, promoting digestive health. Gut microbiota analysis using a rat model revealed that consuming both beverages led to distinct profiles of beneficial bacteria, which contributed to improved immunity and gut health. This was accompanied by a significant increase in short-chain fatty acids (SCFAs). Notably, the prebiotic kelulut float beads beverage demonstrated a twofold increase in butyric acid levels compared to the kelulut float beads beverage and the control group, highlighting its superior health benefits. A 5-point hedonic sensory survey on consumer acceptance rated the prebiotic kelulut float beads beverage at 4.28, significantly higher than the 2.88 score for diluted kelulut honey at the same concentration. These findings underscore the potential of prebiotic kelulut float beads as an innovative functional drink that supports digestive health by enhancing gut microbiome diversity.

Keywords: dietary fibre, digestive system, kelulut honey, microbiota, prebiotic

Volume 13 Issue 1 - 2025

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Received: February 8, 2025 | **Published:** April 21, 2025

Introduction

Since the ancient times, honey has been traditionally used as a remedy for improving health and/or natural medicine for treating various ailments. Stingless bee, belong to the tribe Meliponini, closely related to honey bee (*Apis mellifera*) but different with the presence of very short sting which is not targeting for defence. Approximately 500 species of stingless bee have been recognized worldwide and plays their ecological role as main pollinators for wild and cultivated plants.[1] Honey produced by stingless bees, commonly referred to as kelulut honey, contains trehalulose and demonstrates anti-inflammatory properties. It is rich in antioxidants, including phenolic and flavonoid compounds, which aid in promoting wound healing by enhancing epithelialization and granulation tissue formation.[2]

In Malaysia, stingless bee generally can be classified into two main genera: *Melipona* dan *Trigona*. Majority of the commercial kelulut honey are collected from *Heterotrigona itama* and *Geniotrigona thoracica* species. Distinguished from other honey bees, the taste of kelulut honey is very different in terms of its organoleptic properties. This is primarily because the taste of kelulut honey varies significantly across regions. This variation is largely attributed to the complex interactions among the surrounding microorganisms in the hive, the species of stingless bees, and the variety of flowers, resulting in non-uniform organoleptic properties of kelulut honey. The kelulut honey quality also varies throughout the year, depending on the climate condition. The unique taste of kelulut honey is influenced by nectar and types of flowers within their rearing environment and also partially derived from plants resin those bees collected from nearly surrounding area to build their hives. Generally, the acidic taste of kelulut honey is contributed by the presence of multiple organic acids as a consequence of symbiotic microbial activity from their hives environment.

Recently, there is an emerging interest trend using both indigenous knowledge and modern science that offers alternative preventive or curative approach to strengthen immune system for treating various ailments/diseases with minimum side effects. The market growth of kelulut honey is expanding in popularity with more and more consumers are favoured to consume kelulut honey for apitherapy treatment. Many literature findings have documented scientific evidence of therapeutic benefits related to kelulut honey such as chemo-preventive properties, antibacterial effect, anti-inflammatory, antioxidants, natural wound healer, treatments for glaucoma and cataracts.[3,4] In Malaysia, very little effort has been done to diversify the kelulut honey products and it has been dominantly sold as original honey, which leads to expensive selling price and limits the selling volume as not many people afford to consume it. In this study, our work has been focused on the development of ready-to-drink prebiotic kelulut honey product with value-added functional effects to attract more people to consume kelulut honey in a more convenience way. Diversifying the kelulut honey downstream product is important to expand the market growth and sustain the development of local stingless bee honey industries to earn more income. A targeted mixed cultures consisting of *Dekkera sp.* & *Komagataiebacter sp* has been selected to ferment diluted kelulut honey to enhance the organoleptic and nutritional qualities, particularly to improve the bland taste of diluted kelulut honey.

Materials and methods

Preparation of prebiotic kelulut float beads beverage

Fermented kelulut honey was produced from the suspension of raw kelulut honey at the concentration of 30% (w/v) and was inoculated with 10% (v/v) of selected Kombucha consortium strains (*Dekkera* sp. & *Komagataiebacter* sp.) with the colony count of at least 1×10^8

colony forming units/mL. The fermentation process was continued for 2 days before subjected to centrifugation at 10,000 rpm for 5 min to remove unwanted biomass residue. Kelulut float beads beverage were prepared from fermented kelulut honey with the floating beads consists of fermented kelulut honey (50%, v/v), stevia (0.3%, w/v), sodium alginate (0.01%, w/v), calcium lactate (7%. w/v) and sodium citrate (0.1%, w/v). Whereas the prebiotic kelulut float beads beverage was prepared from fermented kelulut honey with the presence of inulin (1.2%, w/v) in the drink as prebiotic source.

Agar well diffusion assay: antimicrobial activity of fermented kelulut honey

Three types of foodborne pathogens were selected to evaluate the antimicrobial effect of fermented kelulut honey, namely *Staphylococcus aureus* (ATCC 49775™), *Listeria monocytogenes* (ATCC®51772™) and *Escherichia coli O157: H7* (UPMEC32). *Staphylococcus aureus* and *Listeria monocytogenes* were purchased from American Type Culture Collection (ATCC). *Escherichia coli* O157: H7 (UPMEC32) was isolated from beef meat (local isolate). Agar well diffusion assay procedure was conducted according to Mohd Danial et al.'s (2020) method.[5] A clear zone diameter around the well was measured at two perpendicular directions. Penicillin-Streptomycin (1%) and acetic acid (1%) were used as positive control. All experiments were done with three replicates.

Gut microbiota analysis *via* 16S rRNA gene sequencing

A total 18 immunodeficient strain comparable (ICR) female rats were employed in the gut microbiota study *via* 16S rRNA gene sequencing. These rats were divided into 3 groups and fed with respective diet for 28 days: a) normal diet; b) kelulut float beads beverage (4 mL/kg); c) prebiotic kelulut float beads beverage (4 mL/kg). Rats' faecal samples were collected at the end of experiment day and was evaluated using 16S rRNA metagenomic sequencing method as described in Koh et al. (2023).[6] The sequencing of the 16S rRNA libraries was performed using a lllumina HiSeq 2500 platform with 250 bp paired end reads generated.

Evaluation of short chain fatty acids

Short chain fatty acids (SCFAs) were extracted from the rat's faecal samples and quantified using gas chromatography with the running program as described in Koh et al. (2022)'s procedure.[6] A total of 0.3 g faecal sample (accurately weighted up to 4 decimal points) was added onto lysing matrix type E tube (MP Biomedicals, USA) and subjected to a highly shearing homogenization method in a 3 mL of sterile distilled water to get faecal solution. The pH of faecal solution was then adjusted to pH 2 using 10M HCl, followed by vigorously vortex for 10 mins. The homogenate was centrifuged at 10,000 rpm for 10 mins to separate unwanted residue. The supernatant obtained further filtered with 0.22 µm nylon filter before injected into gas chromatography (Agilent 6890N, USA) equipped with a flame ionization detector to detect the presence of SCFAs. The SCFAs peaks was separated using a Zebron ZB Waxplus capillary column (30 m x 0.25 mm internal diameter x 0.25 µm film thickness) under gradient heating parameter: The initial oven temperature was set for 50°C for 1 min before heating up the column to 200°C at 60°C/min and held for 1 min before further raised to 250°C with the heating rate of 20°C/min and then maintained at 250°C for 3 min. The nitrogen was used as carrier gas and the split ratio was set at 100:1 with the split flow of 99.4 mL/min. The flow rate of hydrogen, air and make up gas nitrogen were maintained at 40, 350 and 30 mL/min, respectively with the inlet and FID temperature set at 250°C. The major SCFAs (i.e., acetic acid, propionic acid, and n-butyric acid) were identified and quantified based on an external standard calibration curve. The concentrations of SCFAs of all samples (before and after the treatments) were analysed in triplicate.

Five-points hedonic scale sensory evaluation of kelulut honey drink with prebiotic kelulut float beads beverage

A total of 50 panellists (kelulut honey consumers) participated in a consumer acceptability study to assess their liking preferences for both the prebiotic kelulut float bead beverage and commercial kelulut honey drink, prepared with the same honey concentration. The liking intensity of the new prebiotic kelulut float bead beverage was evaluated using a 5-point Hedonic scale (5: extremely like; 4: like; 3: neither like nor dislike; 2: dislike; 1: extremely dislike). The evaluation focused on the beverage's color, aroma, viscosity, sweetness, sourness, acidity intensity, and overall acceptability.

Statistical analysis

All data are presented as the mean ± standard deviation of the triplicate samples and were analysed using analysis of variance (ANOVA) using statistical software, IBM SPSS Statistic 22.0 (IMB Corp., USA). The comparisons were performed by Duncan's test with statistical significance set at the $p<0.05$.

Results and discussion

When raw kelulut honey was harvested from hives, it needs to be processed immediately to make concentrated honey to minimize the water residue and alcohol formation in order to extend the shelf life of honey. As a consequence, the taste of kelulut honey became very sweet and sour and some people may not like for direct consumption. In Malaysia, there are limited choices for kelulut honey downstream products available in market as most of the kelulut honey is sold in origin state with expensive price. In addition, the price of kelulut honey often fluctuates due to the inconsistency of their physical qualities and appearances, particularly in term of aroma and taste. In fact, these qualities attributed by a variety of factors such as the type of plant resin collected by stingless bee, flowers species and hive microbe environment.

Antimicrobial activity of fermented kelulut honey

In MARDI (Malaysian Agricultural Research & Development Institute), a new kelulut honey beverage with better consistency in terms of taste & quality has been produced from diluted kelulut honey origin using pure mixed starter culture under controlled fermentation process. Fermented kelulut honey is produced using less than one-third of raw kelulut honey as a substrate, leveraging the benefits of combined starter cultures to transform diluted kelulut honey into a more consistent taste and flavour. In other words, diluted kelulut honey could yield a higher profit margin compared to concentrated original kelulut honey. Following fermentation process, the concentration of organic acids particularly acetic acid (0.8%) has been enhanced, resulting in a moderate sour taste (data not shown) and contributes to significant antimicrobial activity. It was evident that fermented kelulut honey has significant antimicrobial activity against three types of selected foodborne pathogens namely *Staphylococcus aureus, Escherichia coli* and *Listeria monocytogenes*, when compared to no microbial activity of raw diluted kelulut honey (Fig. 1). Therefore, fermented kelulut honey is proven with its potential effect in preventing bacterial food poisoning. In a similar study when acetic acid exhibited bacterial growth inhibition capability against various food spoilage bacteria such as *Streptococcus spp*., *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa* and *Proteus spp*. even after being diluted up to 0.5% concentration.[7] In fact, acetic acid, the predominant organic acid found in vinegar, exhibits a bactericidal effect against enterohemorrhagic *E. coli* O157:H7 (known as Shiga toxin-producing *E. coli*), a food-borne pathogenic bacterium. The study demonstrated that the growth of pathogenic strains can be inhibited with as little as 0.1% acetic acid, confirming its effectiveness as an antibacterial agent.[8]

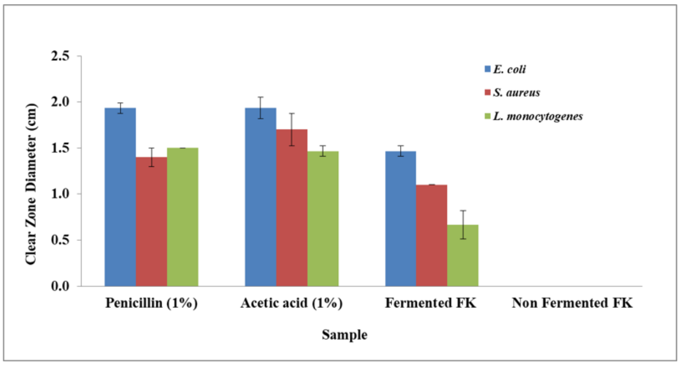

Figure 1 Comparison of anti-microbial activity between fermented kelulut honey (FK) and non-fermented FK against selected foodborne pathogens (*E. coli, S. aureus, L. monocytogenes*).

Gut microbiota analysis

Prebiotic kelulut float beads beverage is a new kelulut honey product made of fermented kelulut honey (main substrate) which has been modified in terms of acidity level. The floating beads were specifically designed to increase the customer mouthfeel sensation and satisfaction, in addition to creating a novelty value of the drink. The presence and appearance of beads floating in the drink are expected to attract customers to try the product. In this study, there were two types of kelulut float beads beverages that have been formulated, namely (a) prebiotic kelulut float beads beverage and (b) kelulut float beads beverage. The prebiotic kelulut float beads beverage has been uniquely formulated with prebiotic ingredients i.e. inulin, known to be a natural dietary fibre that plays a crucial role in inducing metabolic activity of beneficial microorganisms' growth in the gastrointestinal system. Inulin is a type of "non-digestible" compound that cannot be absorbed in the upper part of the gastrointestinal system, but carries prebiotic characteristics such as able to stimulate microbial growth and enhance the diversification microbial composition in the gastrointestinal system. However, it is crucial to make up the beverage formulation with the right amount of inulin to ensure its prebiotic effectiveness upon consumption. An adequate inulin intake (1.2 g/100mL) is also considered during the product formulation to ensure each bottle of product (120 mL) can provide a sufficient prebiotic effect as displayed in Figure 2.

Figure 2 Prebiotic kelulut float beads beverage.

In order to examine the effectiveness of both kelulut float beads and prebiotic kelulut float beads beverages in improving gut microbiome, an *in vivo* rat model study was conducted. All rats were fed with respective diet with a daily intake dose of 4 mL/rat body weight, continuously for 1 month. Throughout the experimental period, the weight of the rats was found to increase consistently each week without any toxic effects were observed. The blood glucose content of rats fed with both kelulut float beads drink showed healthy glucose levels, comparable to control rats (Table 1), thus these data confirmed that both kelulut float beads beverages did not give hyperglycemia effect even though both are sweet and sour drinks. Besides, blood haematology analysis on white blood cell count, red blood cell count, platelet count, hemoglobin and hemocrit profiles in both beverages remain within healthy levels, comparable to control (Table 1). These findings confirmed that both kelulut float beads beverages do not burden the rat body function when continuously consumed at the feed dose of 4 mL/kg body weight for duration period of 1 month.

Table 1 Glucose & blood haematology profile of rats under different diet interventions

Treatment	Glucose (mmol/l)	Red blood cell (10^{12}/l)	White blood cell (10^9/l)	Platelet (10^9/l)	Hemoglobin (g/dl)	Hematocrit (%)
Control	4.97+0.25[a]	11.27+0.19[a]	8.76+0.49[a]	911.60+73.97[a]	21.80+0.55[a]	60.22+1.25[a]
Prebiotic kelulut float beads	4.80+0.23[a]	11.23+0.29[a]	7.87+1.62[a]	901.40+81.75[a]	20.67+0.69[a]	59.12+1.69[a]
Kelulut float beads	4.47+0.25[a]	11.20+0.62[a]	8.05+0.96[a]	898.00+35.63[a]	20.70+0.89[a]	59.23+3.25[a]

Feeding rats with different fermented kelulut beverages resulted in changes to the gut microbiome compared to the control group, which was given water. Notably, the gut microbial composition, particularly the ratios of *Firmicutes* and *Bacteroidetes*, underwent significant alterations depending on the dietary intervention (Figure 3). For instance, consumption of the prebiotic kelulut float beads beverage increased the abundance of *Bacteroidetes* while reducing the *Firmicutes* population compared to the control. Conversely, the kelulut float beads beverage elicited the opposite effect. This plausibly explained by the presence of inulin, of which supplying non-digestible fibre source and thereby stimulating the growth of certain bacteria in the colon, particularly the *Bacteroidetes*. When the bacterial composition and abundances were observed at the genus level from heat map analysis, *Alloprevotella* and *Prevotella* from the phylum of *Bacteroidetes* were greatly enriched in the rat gut treated with prebiotic kelulut float beads beverage (Figure 4). Earlier study has shown that both *Alloprevotella* and *Prevotella* species can ferment soluble fibre such as inulin, to produce short chain fatty acids (SCFAs) like acetate and propionate.[9] In the current study, there were significant increases of acetate and propionic acid levels in both kelulut beverage treatment groups when compared to control (p<0.05), with a higher extend of increment observed in prebiotic kelulut float beads beverage treated rat's faecal samples (Figure 5a, 5b).

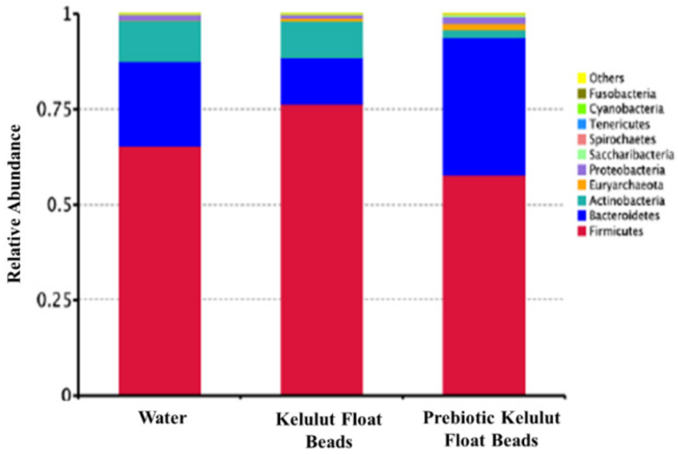

Figure 3 Microbiota composition profile of rats under different diet interventions.

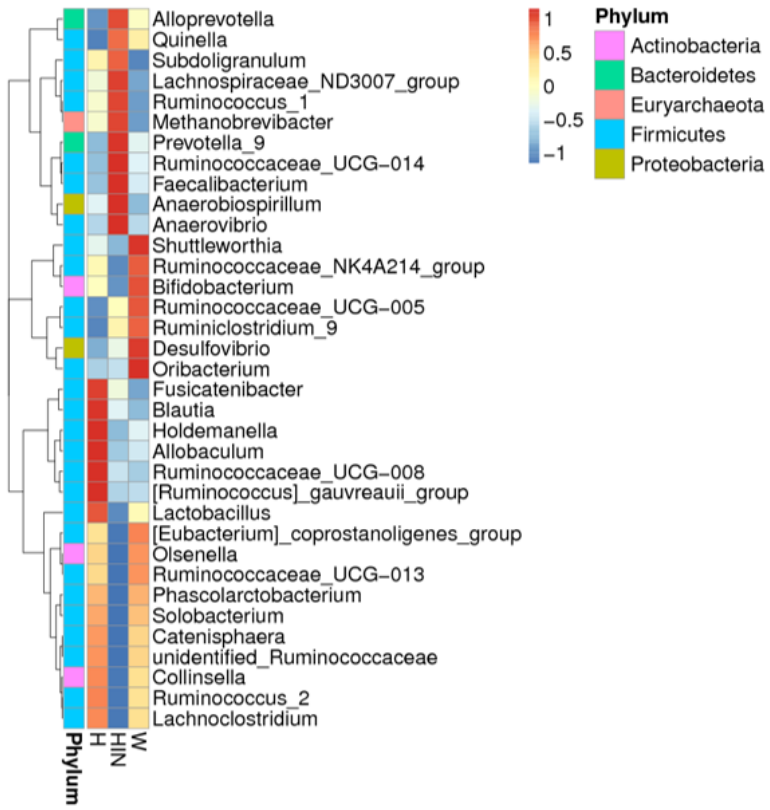

Figure 4 Heat map microbial composition profile of rats under different diet interventions.

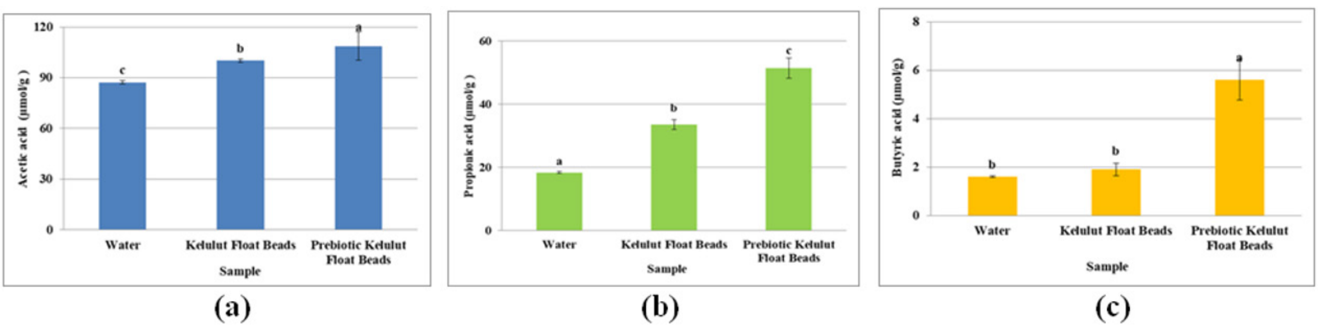

Figure 5 Comparison short-chain fatty acids profile of rats under different diet interventions.

Citation: Koh SP, Sew YS, Nicholas D, et al. Prebiotic kelulut float beads beverage: an emerging health benefiting functional beverage. *MOJ Food Process Technol.* 2025;13(1):17–22. DOI: 10.15406/mojfpt.2025.13.00319

The gut microbiome data revealed that the consumption of kelulut float beads beverage could stimulate the growth of some beneficial microbes i.e. *Fusicatenibacter, Blautia, Holdemanella, Allobaculum, Ruminococcaceae and Lactobacillus* (Figure 4). Majority of these bacteria were identified as having prominent roles in enhancing host energy metabolism, reducing inflammatory responses, and strengthening host immunity either directly or indirectly.[10-13] However, the gut microbiota in the rat colon showed distinct changes when inulin was added to prebiotic kelulut float beads beverage. Bacteria from the phylum of *Firmicutes* i.e. *Quinella, Subdoligranulum, Lachnospiraceae, Ruminococcaceae* and *Faecalibacterium* were significantly enriched in prebiotic kelulut float beads beverage treated rat faecal samples (Figure 4). These bacteria are known to be the butyrate producing bacteria, consistent with the SCFAs analysis results (Figure 5c). For instance, *Quinella* has been shown to positively correlated with butyric acid levels,[14] while majority strains of *Faecalibacterium* are able to produce butyric acid.[15]

It has been well established that the gut microbiome plays a crucial role in the production of SCFAs, which are key metabolites derived from the fermentation of dietary fibre by gut bacteria. The SCFAs, including acetate, propionate, and butyrate, serve as vital energy sources for colonocytes, regulate inflammation, and support gut barrier integrity. They also influence systemic health by modulating immune responses, glucose metabolism, and even neurological functions via the gut-brain axis.[16,17] An imbalance in the gut microbiome, or dysbiosis, can reduce SCFAs production, contributing to conditions such as inflammatory bowel disease, obesity, and metabolic disorders. This highlights the interplay between gut health, diet, and overall well-being. Our data indicated that the SCFAs contents are significantly higher in both kelulut float beads beverage when compared to control, however prebiotic kelulut float beads beverage contains the highest SCFAs among all ($p<0.05$). The most prominent differences between both kelulut float beads beverages, obviously inulin supplemented prebiotic kelulut float beads beverage which could stimulate higher butyric acid content potentially provide promising health benefiting effects. This is due to the fact that butyric acid plays a key role in immune modulation by enhancing anti-inflammatory responses, promoting regulatory T cell differentiation, and suppressing pro-inflammatory cytokine production, which is essential for maintaining immune homeostasis.[18,19] It is noteworthy that consuming both kelulut float beads and prebiotic kelulut float beads beverages demonstrated potential benefits in managing gut dysbiosis by limiting the growth of certain opportunistic pathogens, such as *Desulfovibrio* and *Oribacterium*.[20,21]

Consumer acceptance study

A comparative study was conducted to evaluate consumer acceptance of a prebiotic kelulut float beads beverage versus regular diluted kelulut honey (both were prepared with the same honey concentration), involving 50 kelulut honey loving consumers (Fig. 6.). Overall, the prebiotic kelulut float beads beverage received a significantly higher consumer acceptance score of 4.28±0.62, compared to 2.88±0.96 for the regular kelulut honey drink. Sensory attributes such as colour, aroma, viscosity, sweetness, sourness, and acidity also scored higher for the prebiotic beverage, highlighting its superior organoleptic properties and greater appeal to consumers. In contrast, the regular diluted kelulut honey was perceived as less flavourful and failed to capture consumer interest at the same concentration. These findings underscore the potential of enhancing diluted honey through microbial fermentation to improve both product quality and marketability. The prebiotic kelulut float beads beverage offers a convenient, ready-to-drink option that not only appeals due to its taste but also provides the added benefit of requiring no preservatives, thanks to the natural acetic acid present in fermented kelulut honey. This innovative product showcases significant market potential as a new functional drink, expected to generate additional revenue and contribute to the sustainability of the local kelulut honey industry.

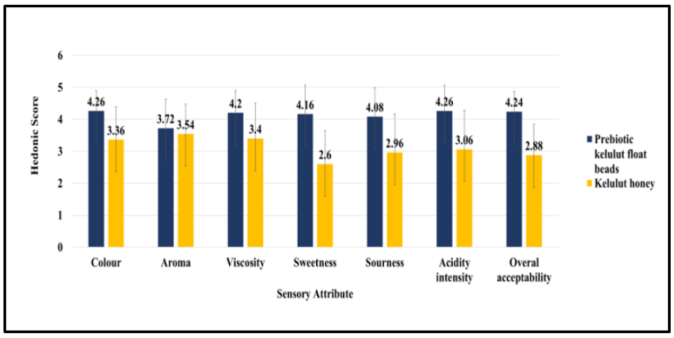

Figure 6 Five-points Hedonic scale of consumer acceptance study on prebiotic kelulut float beads beverage and commercial kelulut honey**.**

Abbreviations: 5 - extremely like; 4 - like; 3 - neither like nor dislike; 2 - dislike; 1- extremely dislike)

Conclusion

The current research on kelulut float beads and prebiotic kelulut float beads beverages has provided valuable insights into their health-promoting functions, particularly in enhancing gut microbiota and increasing short chain fatty acid content, thereby contributing to improved health, immunity and overall well-being. This innovative beverage offers new niche market for kelulut honey industry, presenting a fresh and appealing image to attract more consumers to enjoy this delicious drink. The development of prebiotic kelulut float beads is crucial for supporting the diversification of kelulut honey downstream products and enhancing global competitiveness, ultimately driving increased revenue generation.

Acknowledgements

This study is financially supported by Fund Development of Ministry of Agricultural (MOA) (project code: K-RF175-1401-KSR999).

Conflicts of interest

The authors declare that there are no conflicts of interest.

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