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Physicochemical Analysis and Sensory Evaluation of Barley Tea Drink supplemented with Lemon and Honey

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Abstract

Infusion tea such as Barley Tea addition with other ingredient like lemon and honey, addresses the rising global demand for a healthful barley-based beverage. Therefore, the main goals of this study are to investigate the physicochemical effects and organoleptic properties of barley tea supplemented with lemon and honey. Five formulations represent variations in tea supplementation, involving roasting at 240°C for 15 minutes, steeping in water at 100°C for 10 minutes, and incorporating either lemon or honey or both after chilling to 31°C. Formulation 2, comprising 94.25% of barley tea and 5.75% of honey, emerged as the preferred choice, earning the second- highest average total attribute score of 6.84±1.56 among 50 untrained panelists. Physicochemical analyses revealed that Formulation 2, featuring honey, exhibited commendable characteristics, including low turbidity (222.00±2.00), high total soluble solid (7.80±0.02 TSS Brix), and a beverage-safe pH (4.64±0.0). GC-MS analyses of volatile compounds indicated that Formulation 2 displayed a more complex profile, particularly with abundant esters and furanones contributing to unique flavour and odour profiles. ATR-FTIR analysis also revealed a commonality across all formulations with amines and alcohol as prevalent organic compounds. This study underscores the positive impact of supplementing barley tea with honey on both physicochemical and sensory qualities, providing valuable insights into the potential nutritional and marketable appeal of the beverage. These findings have significant implications for the beverage industry, introducing an innovative approach to enhance the attractiveness of barley tea through the addition of honey.

1. Introduction

Tea, originating from the *Camellia Sinensis* plant in southwest China, holds a distinguished place as a globally cherished non-alcoholic beverage [1]. China, a leader in tea leaf production, boasts five main tea types, each with unique flavors influenced by diverse drying and fermenting processes [1]. Tea processing involves key steps outlined by previous study [2] including withering, fixation, rolling, fermentation, post-fermentation, drying, and roasting, contributing to its rich cultural significance.

In the realm of alternative infusions, barley tea, known as mugicha, has gained popularity in East Asian nations such as Japan, Korea, and China. Brewed by steeping roasted barley grains, this unique tea offers a delightful nutty and slightly sweet flavor [3]. Beyond taste, barley tea is esteemed for its rich concentrations of antioxidants, dietary fiber, and essential minerals, contributing to its perceived health benefits. Research [4] highlights potential anticancer effects, with antioxidant compounds such as vanillic and chlorogenic acids enhancing fat-burning capacity for weight management [5].

Lemon and honey are natural flavor enhancers that can significantly improve the sensory qualities of beverages, including tea. According to [6], adding honey and citrus limonum (lemon) to tea brew are likely used to change the grassy, vegetal, and astringent flavour characteristics as well as to boost the tea's health benefits. Lemon is well known for containing high levels of citric acid which helps in balancing the sweetness in sodas, teas, juices, and other beverages by imparting a sour flavour to foods and providing a somewhat tangy, refreshing flavour. It also contains vitamins, minerals, and antioxidants that contribute to its health benefits, such as supporting digestion, reducing inflammation, and promoting overall well-being [6]. Besides that, the use of honey can be suggested to sweeten tea as a healthier way of tea consumption with the preferred sweet taste. It is rich in antioxidants, vitamins, and minerals, including vitamin C, which can boost the immune system and promote overall health [6].

While barley-based beverages are highly regarded, their exploration beyond East Asia remains limited, creating a research opportunity, particularly in regions like Malaysia. This scarcity of investigation has prompted an empirical study into the sensory qualities and physicochemical properties of barley tea, especially when infused with flavors such as lemon or honey. The goal is to develop a flavorful and marketable barley tea product that resonates with global consumer preferences [7]. This study involves conducting experiments to assess both sensory and physicochemical aspects, going beyond a mere examination. It centers on understanding the potential of barley tea as a healthful and appealing beverage, with a focus on its sensory attributes and physicochemical characteristics.

2. Methodology

2.1 Materials

The white pearls barley was purchased from the online Affordable Organic brand which originated from Holland. The supplementing ingredient in tea, consist of pure honey by CED brand and lemons from Eureka species were purchased from local supermarket.

2.2 Method

2.2.1 Preparation of Barley Tea (Supplemented with lemon and honey)

A 400-gram batch of barley grains underwent thorough cleaning to remove visible particles. The grains were then roasted in an oven (Butterfly BEO -5229 -Electric Oven), at 240°C for approximately 15 minutes until they achieved a brownish hue [8]. The roasted barley was introduced to the boiling water (1000ml) and allowed to brewing for a minimum of 10 minutes. The resulting barley tea was filtered through muslin cloth to eliminate solid residues, and the filtrate cooled to the ambient room temperature of 31°C before supplement addition, as per the procedure by previous study [6]. The barley tea extract was divided into five cups, in which 1 cup was leaved as control. Following the study, honey and lemon were added according to the sequence outlined in Table 2.1 according to the previous study [6].

Table 2.1 *Formulation of barley tea drink*

Formulation	Barley Tea (%)	Lemon (%)	Honey (%)
Control	100	-	-
Formulation 1	94.25	5.75	-
Formulation 2	86.96	-	13.04
Formulation 3	75	13.04	11.96
Formulation 4	75	11.96	13.04

2.2.2 Physicochemical Analysis

2.2.2.1Determination of colour analysis

The barley tea's color attributes, including L* (brightness or whiteness), a* (redness to greenness), and b* (yellowness to blueness), were assessed [10]. The samples were contained in a transparent vessel, and the average result of triplicate analyses for each unique barley tea formulation was reported using the colorimeter (HunterLab MiniScan EZ 4500S).

2.2.2.2Determination of pH analysis

10 ml of each sample was placed in a beaker, and the pH levels of the samples were determined using a pH meter [21]. The pH measurements for all samples were conducted in triplicate, and the results were obtained using a pH meter (Sanxin SX751) model from China.

2.2.2.3Determination of Total Soluble Solid(TSS)

Two drops from each sample, dispensed using a dropper, were placed onto a portable refractometer (Atago Digital Refractometer RX-9000a) to assess the total soluble solids. In this instance, triplicate analyses were conducted, and the outcomes were reported in °Brix [20].

2.2.2.4Determination of Turbidity

Approximately 10 ml of each sample was carefully transferred into a clean and transparent container. Turbidity measurements were conducted using a turbidity meter (Eutech ECTN100IR model) [19]. Turbidity, reflecting the cloudiness or haziness caused by individual suspended particles in the fluid, was quantified in Nephelometric Turbidity Units (NTUs) for each sample, with triplicate measurements taken to ensure precision.

2.2.2.5Determination of Organic Compound

Freshly prepared barley tea samples were subjected to FTIR analysis to ensure the accuracy of the results. ATR-FTIR analysis was carried out using an Agilent Cary 630 FTIR Spectrometer, featuring a resolution of 4 cm-1 and a wavelength range spanning from 4000 to 400 cm-1 [9]. Data processing was executed using LabSolutions IR software, and reference spectra were sourced from existing research.

2.2.2.6Determination of Volatile Compound

Using the GC-MS method, an Agilent 7890B GC system, coupled with an Agilent 5977A MSD mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) was used to identify volatile compounds in all samples. Helium was used as the carrier gas in a capillary column (Agilent HP-5MS, 30 m × 0.25 mm internal diameter × 0.25 m film thickness) with a flow rate of 1 mL/min. In splitless mode, the injector temperature was kept at 250 °C. A 3-minute hold at 180 °C, a 5-degree increase to 150 °C, a 15-degree ramp to 230 °C, and a final 2-minute hold at 230 °C comprised the temperature program for the GC oven. The initial setting was set at 50 °C with a ramp rate of 3 °C/min [12]. Following the GC-MS analysis of the barley tea, the obtained results were meticulously analyzed to discern individual chemical components. This involved associating the identified compounds with specific sensory characteristics observed during the analysis. To achieve this connection, Gas Chromatography-Olfactometry (GC-O) data were consulted, enabling a comprehensive understanding of how volatile compounds contribute to the sensory profile of the barley tea. The integration of GC-O data allowed for a more nuanced interpretation of the results by linking chemical information with sensory attributes, providing valuable insights into the aromatic complexity and flavor characteristics of the beverage.

2.2.3 Sensory Evaluation

Acceptance test which using 9-Hedonic Scale were used to conduct the sensory analysis [18] and about 50 untrained panellists from UTHM Pagoh's Faculty of Applied Sciences (FAST) were selected. Three-digit random numbers were used to identify each sample, and the sensory rating form included 9-point hedonic scales. The participants evaluated the tea's colour, flavor (sweetness and sourness), aroma, astringency, and overall attributes.

2.2.4 Statistical Analysis

Every parameter underwent triplicate analyses, and the results were presented as mean and standard deviation (SD). To identify statistically significant differences among the five formulations, including the control, an analysis of variance (ANOVA) was performed using SPSS software. If the p-value from ANOVA was ≤ 0.05, post-hoc tests were employed for pairwise comparisons to determine specific groups or conditions with significant differences.

3. Results and Discussions

3.1 Physicochemical Analysis of Barley Tea Drink

The physicochemical analysis conducted on barley tea, post-supplementation with lemon and honey in five different formulations, including a control, aimed to provide a comprehensive understanding of the impact on various key parameters. The analysis covered crucial aspects such as pH, colour, total soluble solids (TSS), turbidity as shown in Table 3.1 as well as the presence of volatile and organic compounds.

Table 3.1 *Summary results of physicochemical analysis between samples*

Sample	Colour			pH	TSS (˚Brix)	Turbidity (NTU)
	L*	A*	B*			
Control	62.17±0.98^{a}	-0.69 ± 0.07^{ab}	6.92±0.03^{c}	4.86±0.02^{a}	0.73 ± 0.01^{e}	558.33± 7.02^{a}
Formulation 1	54.67±0.11^{c}	-0.74±0.01^{ab}	7.14±0.08^{c}	2.33 ±0.01^{e}	0.82±0.01^{d}	502.00 ± 7.81^{b}
Formulation 2	62.56±1.11^{a}	-0.48±0.09^{a}	15.17±0.14^{a}	4.64± 0.0^{b}	7.80±0.02^{b}	222.00± 2.00^{e}
Formulation 3	52.58±0.69^{c}	-1.28±0.19^{c}	4.78±0.04^{d}	2.46±0.01^{d}	6.98±0.03^{c}	405.33± 7.23^{d}
Formulation 4	57.96±1.08^{b}	-0.84±0.16^{b}	9.04±0.38^{b}	2.50±0.01^{c}	8.01±0.03^{a}	422.00± 3.61^{c}

*Results were expressed in mean ± standard deviation
**The letters in the same row indicate significant difference (a,b,c,d,e)

3.1.1 Colour Analysis

According to Ly et al. (2020)[10], the lightness, red colour intensity, yellow colour intensity, and relative colour difference index were characterized by the L*, a*, and b* values. The results of triplicate analyses for each distinct barley tea formulation were averaged using the colorimetric technique. The light colour observed in Formulations 1 and 3 can be attributed to the inclusion of lemon juice, imparting a yellowish hue to the barley tea. Despite Formulation 1 exhibiting a generally darker colour with a lower L* value of 54.67, the presence of a noticeable yellowish tone remains evident, as reflected in the comparable b* value of 7.14. This contributes to the formulation's overall vibrancy. The negative a* value of -0.74 indicates the green components present in the formulation.

On the other hand, Formulation 3, with a lower L* value of 52.58 (darker) and a decreased b* value of 4.78, suggests a deeper colour with potential shifts towards blue or green. The negative a* value of -1.28 indicates the presence of green hues. In both cases, the presence of lemon juice contributes to the lighter coloration, as yellowish tones from the lemon juice affect the overall colour characteristics of the barley tea.

In contrast, Formulation 2 and Formulation 4, which have a darker colour, are associated with the presence of honey. Formulation 2, with a higher L* value of 62.56 (lighter) and a notably higher b* value of 15.17 (pronounced yellow hue), suggests a shift towards a more intense yellow colour. The positive a* value of -0.4800 indicates a slight red component. Formulation 4 falls between the control and Formulation 1 in terms of L* and b* values, with a moderate color intensity and a slight yellowish hue (L* = 57.96, b* = 9.04). The a* value of -0.84 is close to zero, signifying a balanced red-green axis. The presence of honey in these formulations contributes to the darker color and the yellowish hue, contrasting with the lighter color imparted by lemon juice in Formulation 1 and Formulation 3.

These indicate that lemon adds a vivid yellow colour to barley tea due to its flavonoid compounds and antioxidants. The acidic nature of lemon may influence the chemical structure of flavonoids, resulting in color variations from orange-yellow to greenish hues [22]. Meanwhile honey colour is positively related to pigments and antioxidant potential, with darker honey having higher total phenolic content [23] and significantly affect the visual of barley tea. Overall, colour analysis shows significant differences among samples, indicating that not only lemon and honey affect the colour, but also the roasting process can influence the tea's color [24].

3.1.2 pH Analysis

The study explores the impact of pH on colour and taste in various barley tea formulations. The base teas' pH of 5.5 is considered a versatile canvas for various flavour profiles [11]. The study found that the pH of plain barley tea was 4.86±0.01, while Formulation 1 combining barley tea with lemon exhibited a lower pH of 2.3300±0.010, resulting in a lighter colour. Formulation 2, incorporating honey, displayed a pH close to the control (4.64±0.0), remaining within a safe range despite a gradual decrease following honey addition. Formulation 3, blending lemon and honey, yielded a pH of 2.46±0.01, influenced by lemon acidity but resulting in a lighter colour. Formulation 4, with adjusted amounts of lemon and honey, maintained a pH of 2.50±0.01, leaning towards honey dominance while presenting noticeable tartness and enriching the tea's colour with a lush hue.

Unexpected lightening of colour in Formulation 1, Formulation 3, and Formulation 4 challenges conventional expectations despite lower pH values, attributed to the acidity of added lemon with pH ranging from 2-3 and honey with pH ranging from 3.2-4.5 [26]. Citric acid in lemon influences pH and imparts tartness [25], while honey's gluconic acid and phytochemical components contribute to its acidity and potential therapeutic properties [26]. Formulation 4's low pH and tartness align with honey's acidity, enriching colour with a lush hue. Concerning dental health, the study notes pH-dependent dental erosion. Both the control and Formulation 2 exhibit neutral pH, indicating no erosive potential on teeth. This aligns with Stephan's report, suggesting Formulation 2's pH levels may mitigate erosive impact on dental enamel, supporting its safety for consumption [27].

3.1.3 Total Soluble Solid (TSS) Analysis

The investigation into total soluble solids (TSS) in barley tea enriched with lemon and honey reveals profound insights into the impact of diverse formulations on sensory attributes and nutritional composition. TSS values, expressed in Brix percentage, serve as a quantitative measure of soluble ingredients, encompassing sugars, organic acids, proteins, and water-soluble vitamins. Higher Brix values correspond to sweeter tastes.

The analysis of TSS in barley tea formulations demonstrates a significant range of values, from 0.73% to 8.01% Brix. Formulation 4 stands out with a notably higher TSS value of 8.01% Brix, attributed to its higher honey content and lower lemon content. Formulation 2 also shows a substantial increase in TSS (7.80 ± 0.02 Brix), emphasizing the influence of honey. Formulation 1, enriched with lemon, presents a modest increase in TSS (0.82 ± 0.01° Brix), overshadowed by the effects observed in Formulations 2 and 4. The control has the lowest TSS value (0.73%).

The impact of added ingredients, particularly honey and lemon, on TSS becomes evident when considering dissolved substances in the tea. Honey, rich in sugars, significantly contributes to TSS, as seen in Formulation 4. In contrast, lemon minimally affects TSS due to its sour taste. Formulation 3, with a higher lemon proportion, exhibits a lower TSS value (6.98%) compared to Formulation 4, highlighting the discernible influence of distinct ingredients on TSS composition.

Lemon doesn't significantly affect Total Solids (TSS) on its own, but it dilutes the original barley tea due to the increase in volume and constant dissolved solids. This dilution can explain why some lemon-infused formulations have TSS values close to the control [25]. The synergistic effect of high honey content and low lemon content in Formulation 4 leads to the highest observed TSS value. The primary reason for elevated TSS in Formulations 2 and 4 is their higher honey content, which mainly consists of fructose and glucose, which contribute significantly to dissolved solids in the tea.

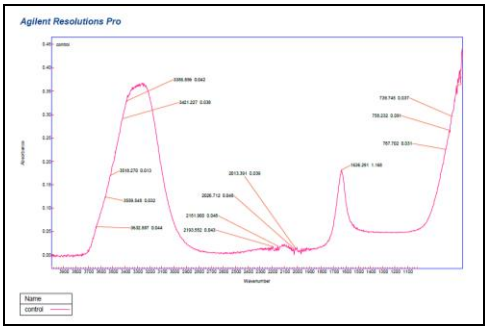
3.1.4 Turbidity Analysis

The examination of turbidity in barley tea enriched with lemon and honey reveals a complex interplay of ingredients and external factors influencing the visual and sensory aspects of the beverage. Turbidity, measured in Nephelometric Turbidity Units (NTU), serves as a crucial indicator of undissolved particles, significantly impacting the perceived smoothness and flavour intensity of the tea. The control group, representing traditional barley tea, exhibited the highest baseline turbidity at 558.33±7.02 NTU, while Formulation 1, incorporating lemon, showed reduced turbidity at 502.00±7.81 NTU. The results indicate a reduction in turbidity in Formulation 1 compared to the control, which can be attributed to the presence of certain mineral compounds in barley tea [28]. These compounds, while slightly soluble in water, tend to become unstable and precipitate when acidic lemon juice is introduced. The precipitation process effectively removes these light-scattering particles from the solution, resulting in an overall decrease in turbidity.

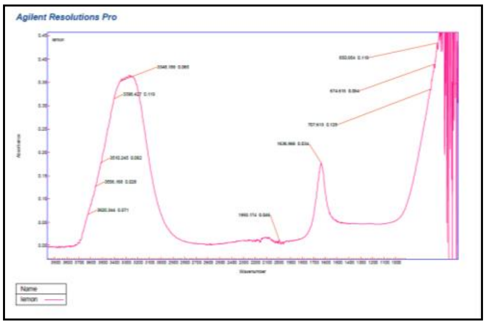
Conversely, Formulation 2, enriched with honey, displayed significantly lower turbidity at 222.00±2.00 NTU, attributed to honey's higher solubility influencing a clearer appearance. Honey contains sucrose, fructose, and fructosyl sugars, all of which have the ability to bind or modify viscosity, which in turn encourages precipitation, gel formation, or size changes for suspended particles. This lowers the remaining particles' concentration and ability to scatter, which results in decreased turbidity [29]. Formulations 3 and 4, blending lemon and honey in varying ratios, showcased a nuanced balance between clarifying and clouding effects, with distinct turbidity levels of 405.33±7.23 NTU and 422.00±3.61 NTU, respectively. These variations highlight the unique impact of each formulation on particle suspension, and external factors like temperature and storage conditions introduce a dynamic dimension to turbidity, influencing the visual and sensory qualities of brewed barley tea over time.

3.1.5 Organic Compound Analysis

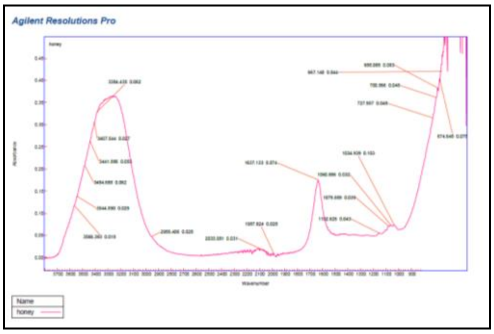
ATR-FTIR analysis was utilized to identify molecular compounds in barley tea formulations. This analytical technique involves measuring the absorbance of infrared radiation by the sample, generating spectra that reveal the functional groups present in the compounds. The resulting spectra, depicting intensity on the x-axis and the amount of absorbed or transmitted infrared light on the y-axis, were instrumental in identifying and characterizing the molecular composition of each sample. The analysis, starting from the high-frequency end, aided in discerning the functional groups within the compounds, providing valuable insights into the organic composition of the barley tea formulations as indicated in Fig. 3.1 below.



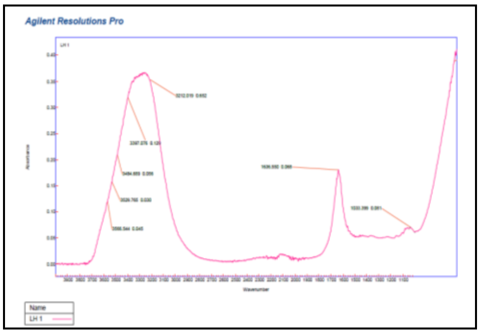
(a) Control



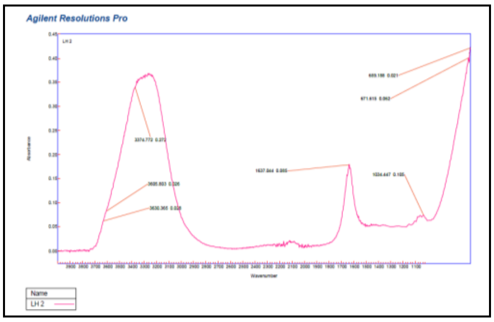
(b) Formulation 1



(c) Formulation 2



(d) Formulation 3



(e) Formulation 4

Fig. 3.1 *Organic Compound founded by ATR-FTIR analysis in samples (a)control (b) Formulation 1 (c)Formulation 2 (d)Formulation 3 and (e)Formulation 4*

The ATR-FTIR analysis of the control sample revealed a complex molecular composition characterized by distinct peaks at various wavenumbers, indicative of specific functional groups [13]. A strong and broad O-H stretch at 3632 cm^{-1} signifies the presence of phenolic compounds, contributing potential antioxidant properties. The FTIR spectra of barley tea samples show multiple peaks in the alcohol region (3559 cm^{-1}, 3518 cm^{-1}, 3421 cm^{-1}), which are consistent with the presence of alcohol compounds in the tea. These peaks align with the roasted aspect of barley tea, as suggested by previous research and the majority of samples analysed. The present of amines at 3385 cm^{-1} add complexity to the flavour profile due to amines are nitrogen-containing compounds that can contribute to the overall flavour and aroma of foods and beverages. They can have a variety of flavours, ranging from fishy to nutty, and can interact with other flavour compounds to create a more complex flavour profile [30]. Alkyne stretches at 2193 cm^{-1} and 2151 cm^{-1}, and strong C-Cl stretches at 787 cm^{-1}, 758 cm^{-1}, and 739 cm^{-1} indicate the presence of alkyl halides, potentially influenced by environmental factors or processing.

In Formulation 1, distinctive peaks revealed specific functional groups, including a robust O-H stretch at 3620 cm^{-1} signifying phenolic compounds. Strong and broad hydrogen-bonded O-H stretches at 3556 cm^{-1} and 3510 cm^{-1} highlighted various alcohol groups, while medium-intensity N-H stretches at 3398 cm^{-1} and 3348 cm^{-1} indicated amines. Strong C-Cl stretches at 707 cm^{-1}, 674 cm^{-1}, and 650 cm^{-1} suggested the presence of alkyl halides, which may result from contamination by surrounding. The presence of alkyl halides in all of your barley tea formulations may be due to contamination during the production process or from the raw materials used. Alkyl halides are a family of organic compounds that contain a halogen atom (fluorine, chlorine, bromine, or iodine) attached to a carbon atom. They are not typically found in barley or its derived products, but can be introduced through environmental factors or processing. For example, halogenated pesticides or cleaning agents used in the production facility may contaminate the raw materials or the final product [31].

In Formulation 2, prominent peaks at 3566 cm^{-1} and 3544 cm^{-1} indicated strong O-H stretching vibrations found in alcohols and phenols, aligning with honey's hydroxyl functional groups. Medium-intensity peaks at 3484 cm^{-1}, 3441 cm^{-1}, 3407 cm^{-1}, and 3384 cm^{-1} suggested N-H stretching vibrations, indicating the presence of amines. Peaks at 2925 cm^{-1} and 1637 cm^{-1} suggested hydrocarbon compounds and aldehyde stretches, respectively, contributing to the complexity of the molecular profile.

Formulation 3 exhibited strong and broad peaks at 3566 cm^{-1} and 3529 cm^{-1}, indicating hydrogen-bonded OH stretching vibrations in alcohols. Medium-intensity peaks at 3484 cm^{-1}, 3397 cm^{-1}, and 3212 cm^{-1} suggested N-H stretching vibrations in amines, and a peak at 1636 cm^{-1} corresponded to the C=O stretching vibration in aldehydes. The presence of ester functionalities at 1033 cm^{-1} added another layer to the molecular complexity.

In Formulation 4, strong and broad peaks at 3630 cm^{-1} and 3605 cm^{-1} indicated OH stretching vibrations characteristic of phenolic compounds. Medium-intensity peaks at 3374 cm^{-1} suggested NH stretching vibrations in amines, and peaks at 1637 cm^{-1} and 1034 cm^{-1} indicated aldehydes and esters, respectively. Strong peaks at 671 cm^{-1} and 659 cm^{-1} revealed alkyl halides, showcasing there are contamination occurred in the formulation.

Comparing these results, certain compounds are consistently present across samples, such as phenolic compounds, alcohol groups, amines, and alkyl halides. The mixed honey and lemon formulation exhibited fewer peaks compared to barley tea with honey only and barley tea with lemon only, suggesting interactions or masking effects between honey and lemon components in the mixture. The intricate relationships among these compounds contribute to the diverse molecular composition of the barley tea formulations, impacting both physicochemical and sensory attributes.

3.1.6 Volatile Compound Analysis

The study investigates the volatile compounds present in different formulations of barley tea, focusing on variations induced by the addition of honey and lemon. Gas Chromatography-Mass Spectrometry (GC-MS) was employed to identify and quantify the volatile compounds in each formulation. The individual chemicals were identified and connected them to the distinct sensory traits noticed during analysis by consulting the GC-O data. The control sample consisted of barley tea alone, and formulations 2, 3, and 4 included additions of lemon and honey in different proportions. In the comparative analysis of the five barley tea formulations, a discernible pattern emerges with respect to the detection of specific compounds, shedding light on potential shared flavour and odour contributors [14].

Table 3.2 *Result of Volatile Compound by GC-MS analysis*

Compound	RT	CAS	Odour Attributes	Flavour Attributes
Control				
- 4-Iodo-2-methoxyphenol	3.167	203861-62-5	smoky, sweet	-
- 4-methyl-2,4-bis (p-hydroxyphenyl)pent-1-ene, 2TMS derivative	4.645	1000283-56-8	-	-
- Benzyl alcohol, TBDMS derivative	6.270	53172-91-1	Sweet, flower	-
- Ethyl bromide	7.122	74-96-4	Ether	burnt taste
- 1-pentene,4,4-dimethyl-1,3-diphenyl-1-(trimethylsilyloxy)	10.023	1000162-32-3	-	-
- Pentanedioic acid, 2-oxo-,dimethyl ester	11.694	13192-04-6	-	-
- Cyclopentasiloxane,decamethyl-	16.752	541-02-6	-	-

-	Cycloctasiloxane, hexadecamethyl-	37.300	556-68-3	-	-
-	Cyclonasiloxane,octadecamethyl-	43.403	556-78-8	-	-
Formulation 1					
-	4-methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, 2TMS derivative	4.669	1000283-56-8	-	-
-	1H-pyrrole,2,4-dimethyl-	4.952	625-82-1	-	-
-	5-hydroxy-7-methoxy-2-methyl-3-phenyl-4-chromenone	10.033	55927-39-4	-	-
Formulation 2					
-	Trifluoromethyltrimethylsilane	3.459	81290-20-2	-	-
-	2-propenoic acid, ethenyl ester	4.076	2177-18-6	-	-
-	4-methyl-2,4-bis (p-hydroxyphenyl)pent-1-ene,2TMS derivative	4.669	1000283-56-8	-	-
-	Carbonocyanidic acid, ethyl ester	4.782	623-49-4	-	-
-	4-methyl-2H-pyran	5.103	1000432-32-7	-	-
-	Methylenecyclopropanecarboxylic acid	6.002	62266-36-8	Fruit, ester	-
-	Ethyl bromide	7.174	74-96-4	Ether	-
-	2(5H)- Furanone	7.598	497-23-4	Caramel	-
-	Proline, 2-methyl-5-oxo-,methyl ester	7.890	56145-24-5	-	-
-	2-Furancarboxaldehyde,5-methyl-	8.912	620-02-0	Caramellic	Spicy-sweet
-	2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	9.576	10230-62-3	-	-
-	Butanedioic acid,dimethyl ester	11.676	106-65-0	-	-
-	1H-pyrazole, 4, 5-dihydro-3-methyl-1-propyl-	20.891	26964-49-8		
Formulation 3					
-	Tert-butyldimethylsilylamine	3.242	41879-37-2	-	-
-	Propanoic acid	3.468	79-09-4	Pungent	-
-	3,5-dihydroxybenzamide	3.916	3147-62-4	-	-
-	2-propenoic acid, ethenyl ester	4.090	2177-18-6	Pungent	-
-	Carbonocyanidic acid, ethyl ester	4.782	623-49-4	-	-
-	3-Furaldehyde	5.098	498-60-2	Almond	Sweet
-	Methylenecyclopropanecarboxylic acid	6.002	62266-36-8	Fruit, ester	-
-	1-pentanone, 1-(2-furanyl)	7.151	3194-17-0	-	-
-	2(5H)- Furanone	7.603	497-23-4	Caramel	-
-	Proline, 2-methyl-5-oxo-, methyl ester	7.894	56145-24-5	-	-
-	2-furancarboxaldehyde, 5-methyl-	8.912	620-02-0	Caramellic	-
-	2,4-dihyroxy-2,5-dimethyl-3(2H)-furan-3-one	9.580	10230-62-3	-	-
-	5-hydroxy-7-methoxy-2-methyl-3-phenyl-4-chromenone	10.028	55927-39-4	-	-
-	1-methyl-5-florouracil	16.544	1000427-92-0	-	-
-	1H-pyrazole,4,5-dihydro-3-methyl-1-propyl	20.872	26964-49-8	-	-
Formulation 4					
-	2 amino-2-methyl-1,3-propanediol	3.403	115-69-5	-	-
-	Propanoic acid	3.624	79-09-4	Pungent	Sour

-	2-propaneoic acid	3.690	79-10-7	Pungent	-
-	2-propaneoic acid, ethenyl ester	4.227	2177-18-6	-	-
-	Pyrrolidine,2-decyl-1-methyl-	4.919	3447-07-2	-	-
-	3-Furaldehyde	5.107	498-60-2	Almond	sweet
-	Methylenecyclopropanecarboxylic acid	6.101	62266-36-8	Fruit, ester	-
-	Ethyl bromide	7.170	74-96-4	Ether	-
-	2(5H)-furanone	7.688	497-23-4	Caramel	-
-	Proline, 2-methyl-5-oxo-methyl ester	7.980	56145-24-5	-	-
-	2-furancarboxaldehyde,5-methyl-	8.917	620-02-0	Caramellic	Spicy-sweet
-	Butanedioic acid, dimethyl ester	11.728	106-65-0	Fruity, winey	Burny
-	5-hydroxymethylfurfural	20.811	67-17-0	Chamomile flower	Butter,caramel,musty

*RT : Retention Time
**CAS : Chemical Abstracts Services
***a: From Flavornet database(http://www.flavornet.org; accessed October 2009) or PubChem (https://pubchem.ncbi.nlm.nih.gov/).

As shown in Table 3.2, compound 2(5H) furanone, 2-furancarboxaldehyde, and Methylenecyclopropanecarboxylic were consistently identified in formulations 2, 4, and 3, suggesting a common aromatic profile possibly influenced by the presence of honey in these formulations. These substances predominate both in proportion and absolute amount in the browning reactions of carbohydrates, which may be the cause of their predominance in formulations 2, 4, and 3 in which the roasted of barley react with the present of honey (high sugar content) compared to formulation 2 and control. According to the past study, Methylenecyclopropanecarboxylic in food give fruit and ester-like odour which may appear with the present of sugar content.

Formulations 3 and 4 contain 3 furaldehyde and propionic acid, giving them distinct almond and sweet odours, and pungent to rancid odours with a sour and mildly cheese-like flavour, respectively [15]. The combination of honey and lemon, known to inhibit microbial growth, could influence propionic acid concentration, adding a unique touch to barley tea. The presence of 3 furaldehyde from honey and other sources creates a nuanced sensory experience in both formulations, emphasizing the intricate relationships among flavour compounds in complex mixtures like tea. Notably, ethyl bromide, identified in most samples, serves as both a potential flavour-contributing compound and a historical fumigant, highlighting the diverse roles of volatile compounds [17].

The presence of 4-methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, 2TMS derivative in the control, formulation 1, and formulation 2 is notable. This discovery underscores the significance of considering packaging materials as potential sources of compounds that may influence the composition of barley tea [16]. The intricate mixture of lemon and honey in formulations 3 and 4 may either overshadow the odour of 4-methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene or potentially interact with it, resulting in its omission from the volatile profile of these formulations.

3.2 Sensory Evaluation of Barley Tea

A comprehensive sensory evaluation test utilizing a 9-point hedonic rating involved 50 panelists for each of the five barley tea sample formulations. The hedonic scale, designed to measure liking degrees for a product, ranged from "like extremely" to "dislike extremely." The panelists rated each sample based on sensory characteristics, including colour, aroma, astringency, sweetness, sourness, and overall attributes. The ANOVA test was conducted to identify the optimal formulation, and the results of the Tukey HSD and Post Hoc tests were employed to compare each formulation and determine significant differences in sensory attributes. Table 3.3 provides detailed insights into the panelists' ratings, facilitating a comprehensive understanding of the sensory preferences and distinctions among the different barley tea formulations.

Table 3.3 *Summary of sensory evaluation results for 50 panelits*

	Colour	Aroma	Astringency	Sweetness	Sourness	Overall Attributes
Control	6.52 ± 1.199	5.62 ± 1.354	4.64 ± 1.804	3.38 ± 1.576^b	4.00 ± 1.818^b	4.48 ± 1.632

	ab	b	c			b
Formulation 1	6.12 ± 1.288^{b}	5.38 ± 1.537^{b}	4.88 ± 1.976^{bc}	3.60 ± 1.690^{b}	4.36 ± 2.211^{b}	4.52 ± 1.821^{b}
Formulation 2	6.89 ± 1.100^{a}	6.74 ± 1.259^{a}	6.12 ± 1.409^{a}	6.58 ± 1.739^{a}	5.60 ± 1.738^{a}	6.74 ± 1.322^{a}
Formulation 3	6.36 ± 1.225^{ab}	6.08 ± 1.426^{ab}	5.70 ± 1.741^{ab}	5.74 ± 1.988^{a}	6.16 ± 2.024^{a}	6.34 ± 1.710^{a}
Formulation 4	6.76 ± 1.287^{ab}	6.50 ± 1.669^{a}	6.14 ± 1.750^{a}	6.50 ± 1.821^{a}	6.60 ± 1.773^{a}	6.84 ± 1.557^{a}

*Results were expressed in mean $\pm$ standard deviation
**The letters in the same row indicate significant difference (a,b,c,d,e)

3.2.1 Colour attributes

The sensory evaluation of five barley tea formulations reveals distinct preferences among consumers. Notably, the colour attribute, crucial for consumer acceptance, exhibits significant variations across formulations. Formulation 2, which incorporates honey, achieved the highest mean colour score at 6.89 ± 1.100, demonstrating a significant difference from Formulation 1 which having the lowest mean value at 6.12 ± 1.288. The higher score suggests that Formulation 2's visual appeal is clearly preferred. This preference is probably related to the honey's golden hue, which enhances the beverage's overall attractiveness. The panelists appear to prefer Formulation 2's aesthetic features over others. On the other hand, the results indicate that there is no significant difference between panelists' color preferences in the control, Formulation 3, and Formulation 4. This suggests that panelists may like all colours, or they may have difficulty differentiating between colours, making it challenging for them to choose a preference. Additionally, factors such as the effects of sensory room lighting or perceptual nuances captured by human senses could contribute to the observed outcomes.

3.2.2 Aroma attributes

In terms of aroma, Formulation 2 once again stood out, achieving the highest mean score (6.74 ± 1.259), indicating a distinct and preferred aroma profile. In contrast, the control sample recorded a lower mean aroma score of 5.62 ± 1.354. The presence of honey in Formulation 2 likely contributed to the heightened olfactory appeal, and the significant difference observed between this sample and the control, as well as Formulation 1, underscores a specific preference for the aroma profile introduced by honey. Moreover, the results reveal that there is no significant difference in aroma between Formulation 4 and 3, possibly due to the combination of the roasted barley tea's aroma with the sweet aroma of honey and the citrus aroma from lemon.

3.2.3 Astringency attributes

There were noticeable variations in each formulation's astringency, which is the sensation a little dry or puckering. Formulation 4 had the highest mean astringency score (6.14 ± 1.750) than the control sample (4.64 ± 1.804), indicating that panelists were more likely to perceive astringency in Formulation 4 than in the other samples. Given that honey and lemon were present in these samples, the results also show that there was no significance difference between Formulations 2, 3, and 4. The reduction of astringency could be attributed to the formation of complexes between the tannins in barley tea and the citric acid in lemons. Moreover, honey's sugars may coat tongue receptors, momentarily reducing sensitivity to tannins and perceived astringency, both of which affect panelists acceptability.

3.2.4 Sweetness

Sweetness, a critical aspect of sensory perception, identified Formulation 2 as the most favourable, achieving the highest mean sweetness score (6.58 ± 1.739). This underscores the formulation's success in attaining a desirable level of sweetness, a characteristic shared with Formulations 3 and 4, possibly due to the synergistic effects of honey. Surprisingly, the amount of lemon added to Formulations 3 and 4 didn't affect the panelists' preferences, possibly because the combination of honey and lemon resulted in a unique sweetness that appealed to their taste. With the lowest mean value of 3.38 ± 1.576 in the control, the data show no significant difference with

Formulation 1, even though lemon was added to the latter. This indicates that panelists did not desire the sweetness level in both control (plain roasted barley tea) and Formulation 1 (citrus like flavour).

3.2.5 Sourness

Sourness, an attribute contributing to overall taste balance, was most prominent in Formulation 4, securing the highest mean sourness score (6.60 ± 1.773). As shown in Figure 4.9, the well-balanced sour note achieved in this formulation likely resonated well with the sensory panel, leading to its preference. Formulation 2 (5.60 ± 1.738) and Formulation 3 (6.16 ± 1.024) exhibited intermediary levels of sourness, with no significant difference between them, indicating that many panelists enjoyed this particular level of sourness. Conversely, the control sample recorded a lower mean sourness score of 4.00 ± 1.818, followed by Formulation 1 (4.36 ± 2.211). Although it is clearly that lemon was added in Formulation 1, providing a better taste in terms of sourness, it is still evident that panelists cannot tolerate the taste of citrus alone, which may overshadow the taste of barley tea itself. This is also the reason why Formulation 4 was chosen, as the high amount of honey mixed with a small amount of lemon provided a perfect balance in terms of sourness.

3.2.6 Overall Attributes

The comprehensive assessment of overall attributes revealed distinct preferences, with Formulation 4 obtaining the highest mean score (6.84 ± 1.557), while the control sample recorded a lower mean for overall attributes score of 4.48 ± 1.632. The substantial difference between Formulation 4, the control, and Formulation 1 underscores its superior overall sensory profile, possibly influenced by the harmonious blend of lemon and honey. This outcome strongly suggests that a majority of panelists participating in the sensory test preferred the sweet taste of barley tea over plain barley tea and barley tea with added lemon. In examining specific attributes, Formulation 4 excelled in colour, aroma, astringency, level of sweetness, and sourness, contributing to its high overall score. The meticulous combination of low lemon and high honey levels in Formulation 4 resulted in an optimal balance, providing a visually appealing golden hue, a distinctive aroma, and a well-rounded taste that resonated with the panelists. Conversely, the control, being plain barley tea, received a lower score across these attributes, indicating a lower acceptability among the panelists. The addition of lemon in Formulation 1 seemed to influence its overall score positively, but the predominant citrus flavour might have overshadowed the barley tea taste, impacting its overall acceptance.

4. Conclusion

In summary, this study aimed to enhance Barley Tea, a popular infusion beverage, by supplementing it with lemon and honey, addressing the growing global demand for healthful barley-based drinks. Formulation 2, consisting of 94.25% barley tea and 5.75% honey, emerged as the preferred choice across all sensory attributes. It is also showcased commendable physicochemical analysis and a complex volatile profile, contributing to a superior aroma and flavour profile. The positive impact of honey supplementation on both sensory and physicochemical qualities underscores the potential market appeal of this innovative approach.

For future research, optimization of roasting parameters is recommended to further enhance physicochemical characteristics without compromising nutritional content or taste. Exploring diverse supplement ingredients and incorporating herbs could broaden health benefits and flavour profiles. Variation in the amounts of lemon and honey should be considered, allowing for a more nuanced understanding of optimal formulations through sensory evaluation. These future avenues of exploration aim to refine formulations and elevate the overall quality of healthful and marketable barley-based beverages, meeting diverse consumer preferences and industry demands.

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The authors confirm their contributions to the paper as follows: **study conception and design**: Shahirah Amirah Mohd Nor, Shakila Abdullah; **data collection**: Shahirah Amirah Mohd Nor**; analysis and interpretation**

of results: Shahirah Amirah Mohd Nor; **draft manuscript preparation**: Shahirah Amirah Mohd Nor, Shakila Abdullah. Both authors reviewed the results and approved the final version of the manuscript.

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