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Impact of Storage Temperature on Green Tea Quality: Insights from Sensory Analysis and Chemical Composition

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Abstract: This study investigated the impact of storage temperatures (−20 °C, room temperature, and 40 °C) on the sensory evaluation, metabolites, and volatile compounds of green tea stored for 12 months. The sensory evaluation revealed that tea samples stored at −20 °C retained their emerald green colour, tender aroma, and refreshing taste. Green tea biochemical constituents, including water extracts and total free amino acids, were measured by Chinese National Standard Methods (GB/T 8305-2013 and GB/T 8314-2013). Tea polyphenols and flavonoids were determined using spectrophotometric methods, while phytochemicals were detected using validated HPLC, and volatile compounds were detected using validated gas chromatography coupled with triple quadrupole mass spectrometry. The analysis showed that tea polyphenols were highest at −20 °C, and flavonoids were significantly reduced at higher temperatures. A similar trend was observed for amino acids, soluble sugar content, and water extracts. Tea catechins, including (-)-epigallocatechin-3-gallate (EGCG) and (-)-gallocatechin gallate (GCG), were highest at −20 °C, showing their susceptibility to temperature. A volatile compound analysis revealed distinct profiles with variations in the abundance of compounds, such as di-methyl sulfide, phenyl ethyl alcohol, indole, and benzaldehyde. This study identifies temperature-sensitive compounds, providing insights into the mechanisms underlying tea quality deterioration during storage.

Keywords: green tea; phytochemicals; polyphenols; flavonoids; catechins; volatile compounds

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1. Introduction

One of the most widely consumed drinks in the world is green tea. Green tea's distinct aroma and possible health benefits have drawn attention from the food and nutritional science community [1–3]. According to the Food and Agriculture Organization (FAO) of the United Nations, approximately two-thirds of the world's population consume tea on a regular basis [4].Tea leaves produce natural compounds that may protect plants from invading phytopathogens and are known to have positive effects when included in the human diet [5]. In addition to being one of the most popular drinks in the world, green tea has been linked to several medical and health benefits. Several countries worldwide, such as China, Indonesia, and Japan, cultivate tea plants in regions characterized by high humidity and acidic soil conditions.

Usually, green tea is made from the leaves or buds of the *Camellia. sinensis* (Family *Theaceae*) plant. Tea can be served hot or cold. Tea leaves vary according to how theyare harvested and processed and how much of the polyphenols in the fresh tea leaves have been oxidized [6]. Tea extract is known for having significant levels of polyphenols, particularly flavanols (also known as catechins or flavan-3-ols). The antioxidant properties of tea's polyphenols are assumed to be the source of its positive health effects [7]. Green tea has more polyphenols than black tea on average. Catechins make up approximately 70–80% of the total tea polyphenols and are predominantly found in the buds and leaves of young tea plants [8]. Tea catechins primarily consist of four compounds: (-)-epigallocatechin-3-gallate (EGCG), which accounts forapproximately 59%; (-)-epigallocatechin (EGC), which accounts for approximately 19%; (-)-epicatechin-3-gallate (ECG), which accounts for approximately13.6%; and (-)-epicatechin (EC), which accounts for approximately 6.4% of the total catechin content [9]. These polyphenols can transform into their non-epicatechin epimers during fermentation. Due to their abundance in nature and biological properties, which include antioxidant [10,11], anti-cancer [12], and anti-allergic [13] properties, the polyphenolic contents have attracted considerable attention.

Compared to regular teas, green tea undergoes significant changes in critical ingredients, affecting its colour, aroma, taste, and antioxidant properties. During long-term storage, the concentrations of gallic acid in Jinhua white tea increased while tea polyphenols, catechins, caffeine, and amino acids decreased [14]. Similarly, extended storage of large-leaf black tea resulted in decreases in flavonoids, catechins, and amino acids, accompanied by increases in brownies and thearubigins [15]. Oolong tea experienced rising lipid and organic acid contents over storage years, coupled with declines in tannins, phenolic acids, amino acids, and their derivatives [16]. Notably, compounds such as EC, EGC, EGCG, tea polyphenols, and amino acids decreased with higher expansion temperatures, while GA and C increased, with EGC emerging as significantly affected by temperature variations [17].Conversely, green tea polyphenols are sensitive to oxidation, with higher storage temperatures accelerating this process and leading to reduced levels of polyphenols and antioxidant capacity [18]. During fermentation, catechins can transform into more complex and oxidized forms, such as theaflavins and thearubigins, emphasizing their importance in green tea. Additionally, the primary constituents of green tea, including gallic acid, caffeine, catechins, and flavonols, can vary depending on brewing conditions [19–21].The antioxidant activities, total phenolic contents, and total flavonoid contents of green tea vary depending on brewing temperature and infusion duration, with catechins susceptible to degradation, epimerization, and oligomerization at temperatures above 90 °C [18,22]. Elevated storage temperatures can alter the flavour, aroma, and volatile compounds of green tea, potentially reducing its antioxidant activity, which is linked closely to polyphenol content [23–26]. Storage duration also affects key components, with longer periods correlating with decreased polyphenols and amino acids, while shorter storage periods exhibit stronger antioxidant activity [27]. A volatile compound analysis in green tea over storage periods of 1 to 16 years revealed an increase in total volatiles, with significant correlations between storage years and specific compounds [28]. Steeping temperatures influence the aroma composition of black tea, with higher temperatures positively correlating with the variety and concentration of volatile compounds in the infusion [29]. Additionally, differences in storage resilience between black and green teas underscore the need for tailored storage management practices [30]. Changes in taste and colour are observed during the storage of Meixian green tea, with volatile compounds notably linked to storage time and tea classification [31]. Studies on green tea preservation techniques highlight the efficacy of freeze drying and block freeze concentration in maintaining and enhancing bioactive compounds without compromising quality, while hot-air drying shows promise in improving the quality and functionality of various teas [32–36].

The study aimed to investigate the quality disparities of high-quality green tea stored under varied temperature conditions by sealing 50 g samples in opaque aluminium foil bags and subjecting them to three distinct temperature settings: G1 (low temperature) at −20 °C, G2 (room temperature) at approximately 25 °C, and G3 (high temperature) in an incubator at 40 °C for 12 months. The study offers a comprehensive understanding of the effect of storage temperatures on green tea quality by combining a biochemical and metabolite analysis with sensory evaluation. Tea components and volatile chemicals can be precisely quantified and identified using triple quadrupole mass spectrometry in conjunction with gas chromatography, HPLC, and spectrophotometric techniques. Variations in tea production, storage, and preparation methods can influence both the sensory experience and health advantages it offers. The outcomes of this study could not only offer valuable insights into maintaining quality standards for special grade green teaof Hunan XiangboGreen Tea Technology Co., Ltd., but also necessary to comprehend how volatile chemicals, polyphenols, and tea quality are affected by various storage temperatures.

2. Materials and Methods

2.1. Sampling

Green tea samples were purchased from Hunan Xiangbo Green Tea Technology Co., Ltd. (Changsha, China) The sample grade was special grade green tea, contained 50 g per bag, was sealed and packaged in opaque aluminium foil bags, and was stored at different temperatures for 12 months: G1 was placed in a −20 °C freezer, G2 was placed at room temperature, and G3 was placed in a 40 °C incubator. Similar procedures were used to treat all of the tea samples. The process involved gathering fresh leaves, spreading for 6 h, blanching at 280 °C for 2 min, twisting for 10 min, initially drying at 120 °C for 5 min, shaping for 5 min, and drying for 1 h. Following manufacturing, the green tea samples were kept in a sealed, cool, and dry environment at ambient temperature (20–25 °C). Green tea samples with 3% moisture content were stored for further experiments.

2.2. Materials

Chemicals, including (-)-catechin (C), (-)-epigallocatechin (EGC), (-)-gallocatechin (GC), (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin gallate (EGCG), (-)-gallocatechin gallate (GCG), anhydrous glucose, and gallic acid (GA), were acquired from Shanghai Yuanye Biotechnology Co., Ltd., based in Shanghai, China. Other chemicals, such as sodium chloride, acetonitrile (analytical grade), aluminum chloride, and methanol (analytical grade), were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), while Folin–Ciocalteureagentand quercetin were collected from Sigma Aldrich, MO, USA. All the chemicals that were used in this study were of highest analytical grade.

2.3. Conventional Sensory Assessment

Sensory assessments of green tea organoleptic qualities were carried out in accordance with Chinese standard methods “GB/T 23776-2018” (China, 2018), comprising examinations of the appearance, leaf bottom, aroma, soup colour, and taste. Sensory evaluation was used as the standard in production to judge whether studied green tea had deteriorated. Based on sensory evaluation, this study measured the contents of main flavour substances and volatile substances in tea under different storage temperature conditions, aiming to use chemical methods to determine whether the studied green tea had deteriorated. The difference in component content preliminarily clarifies the chemical components related to the quality deterioration of green tea, providing a basis for further proposing and studying chemical indicators of green tea quality changes. The sensory evaluation was conducted in an inspection room specially used to evaluate tea quality. Its specific requirements comply with the Chinese National Standard Methodsof the People’s Republic of China GB/T 18797-2002 “Basic Conditions for Tea Sensory Evaluation Room”. According to the Chinese national standard methods “Tea Evaluation Regulations” (GB/T 23776-2018), the panellists accurately weighed 3 g of dry tea into the review cup, added 150 mL boiling water, covered the lid, and soaked them for 4 min, then drained tea soup. The five indicators of tea samples, appearance, soup colour, aroma, taste, and leaf base, were evaluated sequentially. The total score was R = appearance × 25% + soup colour × 10% + aroma × 25% + taste × 30% + leaf base. ×10% weighting value for total score calculation.

The test was repeated three times, and the average value was determined. Twenty panellists (all between the ages of 20 and 40) evaluated and scored each green tea sample (all were adequately trained for sensory analysis). Among the panellists, 50% (n = 10) were male, while 50% (n = 10) were female. About 40% (n = 8) of the panellists were between the ages of 20–30 years, while 60% (n = 12) were between 30–40 years of age. Several criteria were used when selecting the panellists. They were required to hold the national professional qualification certificate of "Panellists" and actively engage in related work. Theyalso had to maintain good health with a vision of 5.0 or above and possess a valid "Health Certificate for Food Workers".

Before commencing evaluations, panellists had to change into appropriate work attire, cleanse their hands using odourless hand sanitiser, and ensure ongoing cleanliness throughout the evaluation process. Furthermore, cosmetics and smoking were strictly prohibited during the review proceedings.

2.4. Analysis of Biochemical Constituents

Green tea biochemical constituents, including water extracts and total free amino acids, were measured in accordance with Chinese National Standard Methods (GB/T 8305-2013 and GB/T 8314-2013). The analytical technique in "GB/T 8305-2013" involves determining the water extract content in tea through a specific extraction method. The sample assessment of water-soluble substance content followed the guidelines stipulated in the Chinese National Standard Method "GB/T 8305-2013" [37]. Initially, 2 g of finely crushed sample werecombined with 300 mL of boiling water and subjected to a water bath at 120 °C for 45 min, with intermittent shaking for 10 min. Subsequently, the mixture was filtered while hot and cooled, and it was filtered once more. The water-soluble substance content was determined by calculating the variance in mass between the dry tea residue and the dry matter content of the leaves prior to infusion.

Amino acids are measured in tea leaves to evaluate the tea quality. The Chinese National standard method "GB/T 8314-2013" is applicable to the analysis of free amino acids in tea. An amount of 1 mL of the test solution (TS) was moved into a 25 mL volumetric flask, following which 0.5 mL of phosphate buffer (with a pH of 8.0) and 0.5 mL of a 2% ninhydrin solution were introduced. The flask was then placed in a boiling water bath for 15 min. Following cooling, water was added to the 25 mL mark on the flask, and the absorbance was measured at 570 nm. Research has shown that tea polysaccharides contain a host of health benefits, such as anti-ageing, anti-tumour, antioxidant, and antibacterial qualities. They can also lower the risk of diabetes, boost immunity, and lessen hepatotoxicity. The method utilized to determine the total soluble sugar content in each sample was based on a described procedure [38] with several adjustments. Initially, 80 mL of boiling water was combined with 1 g of the sample and maintained in a water bath for 30 min. The resulting extract was subsequently filtered, cooled, and adjusted to a volume of 500 mL. Following this, 1 mL of the extract was cautiously added drop by drop into a reagent solution containing 8 mL of anthrone, while distilled water served as the control. The mixture was thoroughly shaken and subjected to a boiling water bath for 7 min before being promptly transferred to an ice water bath to cool to room temperature. Finally, the absorbance at 620 nm was measured.

The Chinese National standard method "GB/T 8313-2018" outlines the methodology for determining the total polyphenol and catechin content in tea using ISO 14502-1:2005 and ISO 14502-2:2005 methods. The determination of catechins using isocratic chromatography was as follows: 1.5 g of the tea sample wereweighed and combined with 250 mL of boiling water, undergoing extraction at 100 °C for 45 min. Following extraction, the solution was filtered and diluted to a final volume of 250 mL, then passed through a 0.45 μm microporous filter membrane. A wechrom C18 column (4.6 × 250 mm, 5 μm) was employed for chromatographic separation. The mobile phase consisted of two components: aqueous phase A, comprising deionized water, and organic phase B, composed of N, N dimethylformamide/methanol/acetic acid in a ratio of 39.5:2:1.5. The flow rate was set to 1.0 mL/min, and the column temperature was maintained at 35 °C. The injection volume was 20 μL, with detection conducted at a wavelength of 278 nm. The experiment was performed in triplicate (n = 3), with six repetitions, and the resulting data were subjected to analysis and calculation.

The polyphenol determination utilizes a spectrophotometric method with Folin–Ciocalteu reagent according to "Chinese National Standard Methodsof the People's Republic of China GB/T 8313-2018" [39]. The grounded tea samples were extracted with 70% methanol aqueous solution in a 70 °C water bath, and gallic acid was used as the calibration standard to quantify tea polyphenols. Next step was to prepare gallic acid standard stock solution (1000 µg/mL) and transfer 1.0 mL, 2.0 mL, 3.0 mL, 4.0 mL, and 5.0 mL of gallic acid standard stock solutions into 100 mL volumetric flasks, and they were diluted to volume with water, shaken well, and prepared ata concentration of 10 gallic acid working solutions of µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, and 50 µg/mL. In the next step, 1.0 mL of gallic acid working solution, water (for blank control), and test solution were transferred into a graduated test tube using a pipette. Later, 5.0 mL of Folin–Ciocalteu reagent wereadded to each test tube and shaken well. Within 3 to 8 min of the reaction, 4.0 mL of 7.5% sodium carbonate (Na_2CO_3) solutionwas added, water was addedto bring the volume to the mark, and it was shaken well. The reaction mix was kept at room temperature for 60 min. The absorbance (A, A_0) was measured with a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at a wavelength of 765 nm. Later, a standard curve was generated based on the absorbance (A) of the gallic acid working solution and the gallic acid concentration of each working solution. Finally, the absorbance of the sample and the standard working solution was compared and calculated according to the following formula:

$$C_{TP} = \frac{(A - A_0) \times V \times d \times 100}{SLOPE_{Std} \times w \times 10^6 \times m}$$

Here, C_{TP} = tea polyphenol content;
A = Absorbance of sample test solution;
A_0 = Absorbance of reagent blank solution;
$SLOPE_{Std}$—the slope of the gallic acid standard curve;
m = sample mass, unit is grams (g);
V = volume of sample extraction solution, in millilitres (mL);
d = dilution factor (usually 1 mL is diluted to 100 mL, then the dilution factor is 100);
w = sample dry matter content (mass fraction),%.

Green tea phytochemicals, such as gallic acid, theobromine, and theophylline, were identified through HPLC (LC-2010AHT, Shimadzu Corp). A mixture consisting of 20% methanol in water (volume/volume) with a flow rate of 1.4 mL/min served as the mobile phase for transferring and isolating the analytes. The elution conditions remained constant throughout the process. Prior to usage, the mobile phases underwent vacuum filtration through a 0.45 µm nylon filter and were degassed. UV detection at a wavelength of 274 nm was employed to monitor the chromatograms.Detection of tea metabolites, namely polyphenols and catechins, followed the Chinese National Standard Method GB/T 8313-2018, while quantification of total flavonoid content relied on the aluminium chloride spectrophotometric assay. This assay involves the formation of a complex between flavonoids and aluminium chloride, leading to a measurable colour change. In brief, tea samples underwent grinding, and flavonoids were extracted using methanol. A standard solution containing a known flavonoid, quercetin, was prepared to generate a standard calibration curve. Subsequently, the sample extract and standard solution were combined with aluminium chloride, and the reaction mixture was incubated for 15 min to allow for the formation of the aluminium chloride-flavonoid complex. Finally, the absorbance of the reaction mixture was measured at 415 nm using a UV-Vis spectrophotometer.

The dry matter content of green tea was measured according to the following protocol: about 5 g of green tea samples were measured into a drying dish of known mass and placed into a drying oven at 103 ± 2 °C for 4 h. Then, the samples were cooled down to room temperature using a desiccator, and the sample weight was measured. This process involved placing the samples in the drying oven, heating them for 1 h, and then cooling them using a desiccator. These heating and cooling steps were repeated until the difference between two consecutive measurements did not exceed 0.005 g, indicating a constant weight.The dry matter content of the green tea samples was expressed in mass fraction (%) and was calculated according to the following formula:

$$\text{Dry matter content} = \frac{m_1}{m_0} \times 100\%$$

Here, m_0 indicatedoriginal mass of the sample (g), while m_1 indicated the mass of the sample after drying (g).

2.5. Extraction of Volatile Compounds

Volatile compounds from treated green tea samples were extracted using the headspace solid phase micro-extraction (HS-SPME) method [40]. Briefly, an appropriate amount of sample was measured and placed into a headspace vial, and saturated sodium chloride was added to it. Then, the mixture was placed in a water bath at 80 °C for 20 min to equilibrate. Afterwards, a solid-phase micro-extraction needle was inserted into the headspace to adsorb volatile compounds and incubated in a water bath at 80 °C for a further 30 min. Finally, volatile compounds were analysed at the GC by injecting them at the injection port at 250 °C for 5 min to desorb the volatiles. Each sample treatment was performed in triplicates.

2.6. Analysis of Volatile Compounds

An Agilent 5975B gas chromatography coupled with a triple quadrupole mass spectrometry (Agilent Technologies, Santa Clara, CA, USA) was used to analyse volatile compounds. The volatile compounds were separated using chromatographic column HP-5MS (30 m × 0.25 mm × 0.25 µm) under a carrier gas (helium) flow at 1 mL/min with a splitless mode. The chromatographic programs were as follows: the initial temperature was 50 °C and kept for 2 min, further increased to 180 °C at 5 °C/min and held for 5 min, held at 10 °C/min, and finally ramped to 250 °C and kept for 5 min while the injection port temperature and transfer line temperature were 250 °C and 280 °C, respectively. In mass spectrometry, electron ionization was used in full scan mode with an ion source temperature of 230 °C and quadrupole temperature of 250 °C. Different volatile organic compounds (VOCs) in green tea samples were identified and confirmed using a sensitive and quick gas chromatography approach. Besides retention time, VOCs were further confirmed through previous references. Every sample was examined three times to capture any variations and take the mean value into account to guarantee the approach's accuracy. Testing was performedto ensure linearity, accuracy, and precision in the calibration studies. The signal–noise ratio was computed as the limit of quantification (10:1) and the limit of detection (3:1). The volatile compounds of the green tea samples were identified based on their mass spectrum and retention index.

2.7. Analysis of the Effect of Moisture Content on Green Tea Quality

The effect of moisture content on green tea flavour and quality was analysed further. Green tea samples (5% moisture content) were stored at freezing temperature (G1), while samples with 3%, 5%, and 7% moisture contents were stored at room temperature for 12 months. Later, flavour, tea polyphenols, amino acids, and catechin contents were measured from the treated green tea samples. Total free amino acids, polyphenols, catechins, and flavour were calculated using the previously described protocols.

2.8. Statistical Analysis

The outcomes of all of the experiments were presented as mean standard deviation (SD). One-way ANOVA was used to determine the significant variations between the samples. Significance levels were defined according to the *p*-values (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

3. Results and Discussion

3.1. Impact of Storage Temperatures on the Biochemical Characteristics of Green Tea

This study selected three temperature treatments: −20 °C, room temperature, and 40 °C. Among them, −20 °C is the temperature with a better preservation effect on green tea. Room temperature treatment is a conventional temperature treatment without any preservation measures. The 40 °C treatment is a temperature set to consider the impact of high temperatures and extreme weather in summer on the quality of green tea and to explore the reasons for the accelerated quality deterioration of green tea under high-temperature conditions.

3.1.1. Amino Acids

Amino acids play a crucial role in the stability and quality of green tea. Different storage temperatures did not affect the amino acid content so much. Higher amino acid content (3.18%) was identified at the lower storage temperature (G1), while the amino acid contents were 3.11% and 3.15%, respectively (Figure 1A). The stability of amino acids, which are the building blocks of proteins, can be influenced by storage temperatures. Elevated temperatures can lead to the degradation of amino acids through various chemical reactions, including Maillard reactions and oxidation. This amino acid gives the tea's flavour profile additional depth, which increases consumer attractiveness. The balance of its amino acids largely determines the taste and entire sensory experience of green tea. A study revealed that high altitudes hindered the biosynthesis of catechins, resulting in increased levels of free amino acids and consequently lowering the ratio of polyphenols to amino acids. Furthermore, certain aroma compounds exhibited heightened levels in fresh or damaged leaves due to the up-regulation of essential structural genes [41].

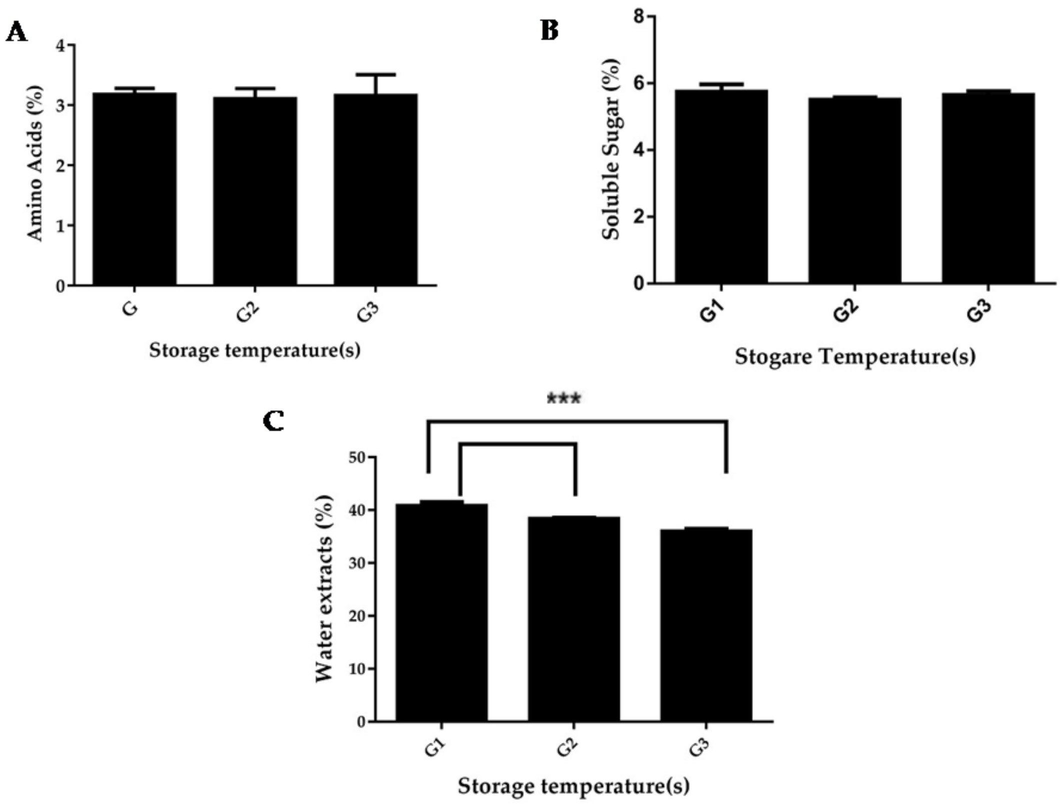

Figure 1. Changes in the biochemical contents of green tea treated at different storage temperatures. (**A**) Amino acid contents; (**B**) soluble sugar contents; (**C**) water extracts; *** $p < 0.001$.

3.1.2. Sugar Content

The storage temperature of green tea can influence the content of soluble sugars, which in turn can affect the flavour and quality of the tea. Storage temperature is a critical factor in determining the rate of sugar degradation in green tea. Changes in sugar content due to improper storage can affect the tea's flavour profile, potentially making it less enjoyable to consumers. In this study, higher sugar content (5.75%) was observed in green tea samples that were stored at lower temperatures, while relatively lower soluble sugar contents were at room temperature (5.51%) and the 40 °C (5.65%) storage temperature (Figure 1B), respectively. Soluble sugars contribute to the sweetness of green tea. As the sugar content decreases due to high-temperature storage, the tea's natural sweetness can diminish. This can negatively impact the overall flavour profile and sensory experience of the tea. Elevated temperatures can promote Maillard reaction reactions, which involve the interaction of reducing sugars (e.g., glucose and fructose) with amino acids. These reactions can result in the formation of new compounds that contribute to the aroma and colour of green tea [42,43]. The Maillard reaction releases flavour and aroma and is employed to make food tasty in practically every aspect of life. The non-enzymatic browning reaction is so named because it occurs without the presence of an enzyme. Maillard reaction products (MRPs) are created when foods are processed or cooked at high temperatures due to a chemical reaction between reducing sugars and amino acids. The Maillard reaction produces various heterocyclic compounds, such as pyrazines, pyrroles, and thiazoles [44], significantly shaping tea stems' sensory characteristics. For instance, the involvement of L-theanine in the Maillard reaction contributes to the development of roasted and caramelized aromas, including pyrazine formation [45,46].

3.1.3. Water Extracts

The water extracts of green tea are a critical factor that can affect the quality and overall characteristics of the tea. Tea's water extracts are essential to the growth and maintenance of the beverage's flavour and fragrance constituents. Moisture levels can affect water-soluble components that give green tea its distinct flavour and scent, such as catechins and volatile organic compounds. Maintaining the intended sensory qualities of the tea requires careful control over water extracts. With various storage temperatures, the water extracts of green tea significantly decreased in this study. The green tea samples that were kept at a lower temperature (G1) had the highest water extracts (40.8%).

In comparison to tea samples held at room temperature (G2), samples had substantially reduced water extracts (38.38%). Compared to the other two conditions of storage, the G3 storage condition's moisture content was much lower (36.04%). According to this study, the water extracts of green tea are strongly influenced by the storage temperature (Figure 1C), which, in turn, can impact the tea's quality and shelf life.

A study found that higher storage temperatures had minimal effect on the amino acids and soluble sugars of green tea. Water extracts were significantly decreased with rising temperatures. Higher storage temperatures hasten the breakdown of some amino acids according to a study [47] that looked into the degradation of amino acids in foods that have been preserved. High temperatures can accelerate the breakdown of soluble sugars, potentially leading to a decrease in sugar content. The loss of sugars can impact the sweetness and overall taste of the tea. A study by Min et al. [48] investigated the impact of storage temperature on the sugar composition of green tea. Another study [49] found that higher storage temperatures (above 25 °C) were associated with a decrease in soluble sugar content in green tea.

3.2. Effect of Storage Temperatures on Green Tea Metabolites

3.2.1. Polyphenols

The storage temperature of green tea can significantly impact the stability of its polyphenols, which are known for their health-promoting properties. The analysis results showed that the highest tea polyphenol contents (28.12 mg GAE/g) were identified in the green tea samples that were stored at −20 °C (G1). However, polyphenol contents were reduced with thehigheststorage temperatures. Polyphenol contents were 26.83 mg GAE/g and 25.58 mg GAE/g at room temperature (G2) and 40 °C (G3), respectively (Figure 2A). Significant differences in polyphenol contents were observed between G1 and G3 storage temperatures.

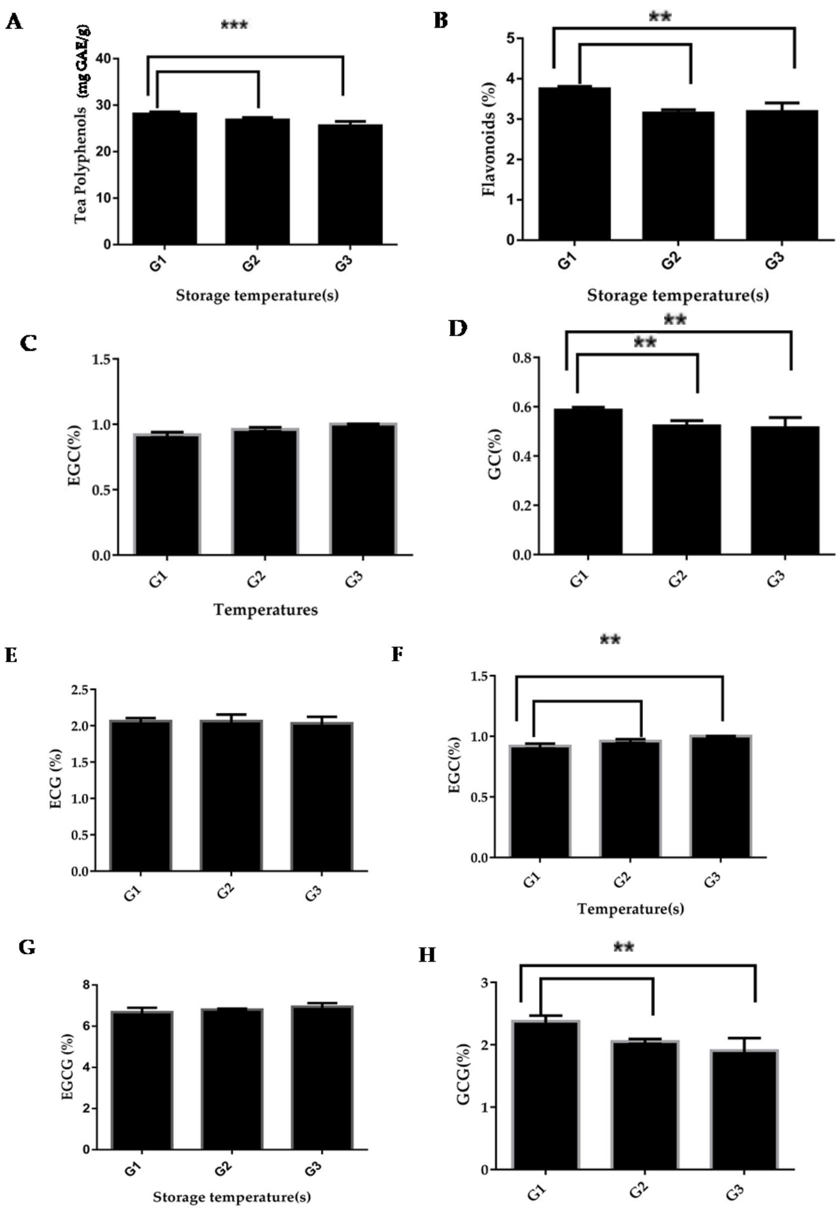

Figure 2. Effect of storage temperatures on green tea metabolites. The metabolites of green tea, such as polyphenols (**A**), flavonoids (**B**), and catechins (**C**–**H**), are significantly impacted by the temperature; ** $p < 0.01$, *** $p < 0.001$.

3.2.2. Flavonoids

The flavonoid content of green tea is a crucial factor that significantly influences its quality. When compared to green tea maintained at −20 °C, the flavonoid concentrations of green tea stored at ambient temperature and 40 °C, respectively, were much lower. The discovered flavonoid contents under G1, G2, and G3 storage conditions were 4.25%, 4.03%, and 4.05%, respectively (Figure 2B). Elevated storage temperatures can accelerate the degradation of flavonoids in green tea. For example, a study [28] demonstrated that higher storage temperatures (above 25 °C) were associated with a significant decrease in the catechin content of green tea. Flavonoids also contribute to the flavour and aroma of green tea.

3.2.3. Catechins

Polyphenolic tea catechins are among the secondary metabolites found in green tea.

Figure 2C–H describes the catechin contents in green tea at different storage conditions. Higher green tea catechin contents (0.59%) were detected in green tea samples, which were stored at a lower temperature, G1 (−20 °C). GC contents were significantly reduced in other storage conditions, such as room temperature (G2) and 40 °C (G3) (Figure 2D). At room temperature, detected GC contents were 0.52%, while at 40 °C, 0.5 (2%) of GC was detected, indicating lower storage temperature can retain the GC contents from reducing than the higher temperatures. EGC and EGCG contents increased in higher temperatures compared to the lower storage temperatures (Figure 2F,G). Detected EGC contents at G1, G2, and G3 storage conditions were 0.92%, 0.96%, and 1.00%, respectively. Similarly, EGCG contents at G1, G2, and G3 storage conditions were 6.68%, 6.81%, and 6.96%, respectively. Other catechins, including EC, ECG, and GCG contents, decreased at higher storage temperatures than at lower storage temperatures (Figure 2C,E,H).

Temperature has a significant effect on green tea metabolites, including polyphenols, flavonoids, and catechins. G1, or green tea stored at −20 °C, had the highest concentration of polyphenols. The polyphenol content varied noticeably between the G1 and G3 storage temperatures. The concentration of flavonoids in green tea was also influenced by the temperature at which it was kept.

Numerous studies have affirmed the influence of various factors on the taste and aroma of tea, particularly temperature. In a study, temperature was detected as the most prominent degradation factor of tea catechins [22]. A study examined the chemical quality changes in green tea powder stored for three months at different temperatures (−20, 4, and 20 °C) and relative humidities (RHs) (23, 69, and 81%). Green tea prepared from the stored powder showed alterations in total phenolic, total flavanol, and ascorbic acid contents. However, storage at −20 or 20 °C with higher RH resulted in significant decreases in chemical compounds [50]. Flavonoids also play a crucial role in shaping the flavour and aroma of green tea. Different types and levels of flavonoids can result in variations in taste, smell, and overall sensory appeal. These characteristics are integral to the quality of the tea [51]. EGCG is the most abundant catechin in green tea, and it is known for its potent antioxidant properties and potential health benefits. It has been widely studied for its role in various health-related aspects [52]. Catechin GCG, also known as epigallocatechin gallate, is a crucial component in maintaining the quality of green tea. It plays a significant role in both the flavour and health benefits associated with this popular beverage.

In this investigation, higher concentrations of tea catechins, specifically GCG and EGCG, were observed at −20 °C, indicating their sensitivity to temperature. GCG, a catechin present in green tea, not only imparts bitter and astringent notes to the beverage but also contributes to its vibrant green hue. The presence of GCG is crucial for maintaining the overall quality of green tea [26]. Additionally, another catechin, EGCG, plays a significant role in the distinctive bitter and astringent flavours of green tea and, like GCG, contributes to the tea's lively green colour. Storage at elevated temperatures can lead to a reduction in the fresh, grassy, and vegetal qualities associated with green tea. Since catechins play a pivotal role in shaping the flavour profile of tea, their degradation due to high temperatures can have adverse effects on the taste and aroma of green tea.

3.3. Temperature-Related Changes in Green Tea Phytochemicals

3.3.1. Gallic Acid

Gallic acid is an essential polyphenolic compound found in green tea, and it plays a significant role in maintaining its quality. Table 1 represents the concentration (%) of theobromine, gallic acid, and theophylline in green tea leaves.In this investigation, the gallic acid content of the green tea samples stored at various temperatures varied very little or not at all. For storage conditions G1, G2, and G3, the amounts of gallic acid were 0.16%, 0.15%, and 0.16%, respectively (Figure 3A).

Table 1. Concentration (%) of theobromine, gallic acid, theophylline (analysed using HPLC), and soluble sugar content at different storage temperatures.

Storage Conditions	G1		G2		G3	
	Average	**SD**	**Average**	**SD**	**Average**	**SD**
Theobromine (%)	0.257	0.008	0.247	0.0016	0.257	0.009
Gallic acid (%)	0.158	0.009	0.148	0.001	0.158	0.0095
Theophylline (%)	0.016	0.0000746	0.0164	0.0001	0.016	0.0000187
Soluble sugar content (%)	5.573	0.030	5.663	0.279	5.67	0.157

Note: The dry matter content of the green tea samples was expressed in mass fraction (%).

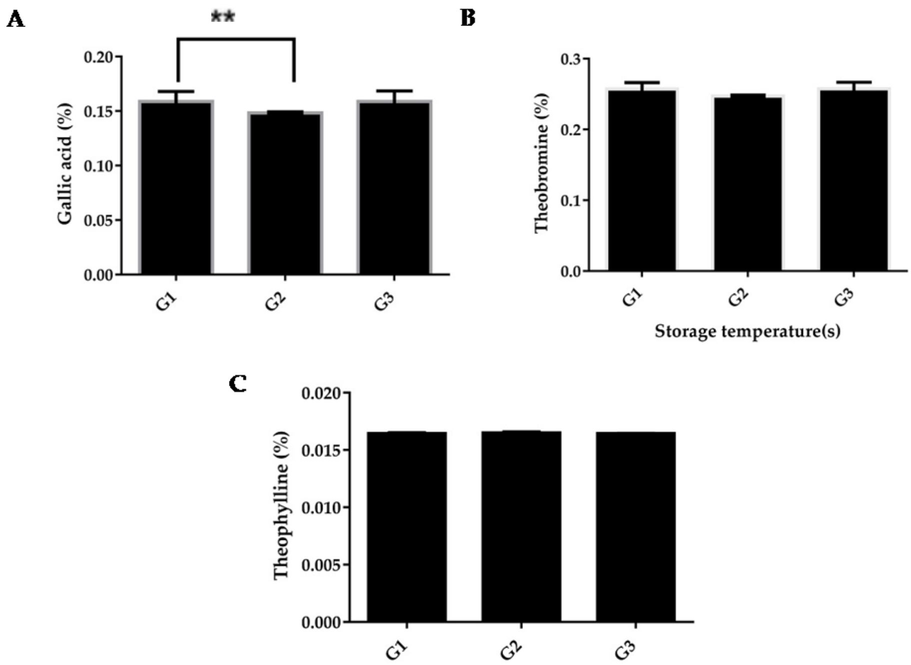

Figure 3. Influence of temperature on the other phytochemical contents in green tea. Storage temperature can have an impact on the phytochemical content of green tea, particularly gallic acid (**A**), theobromine (**B**), and theophylline (**C**); ** $p < 0.01$.

3.3.2. Theophylline

Theophylline is a naturally occurring alkaloid found in green tea, although it is present in smaller quantities compared to caffeine and other compounds. While it may not be as well-known as caffeine, theophylline also plays a role in maintaining the quality of green tea. In this study, no differences in theophylline content were seen across the three distinct storage conditions (G1, G2, and G3) (Figure 3B).

3.3.3. Theobromine

Theobromine is one of the alkaloids found in tea leaves, and various factors, including storage temperature, can influence its content. Theobromine can degrade when exposed to higher temperatures over time. This degradation can lead to a reduction in theobromine content. The rate of degradation may vary depending on the temperature and duration of storage. The average theobromine contents in G1, G2, and G3 storage conditions were 0.26 ± 0.01%, 0.25%, and 0.26 ± 0.01%, respectively (Figure 3C). In this study, not many effects on theobromine content were observed, which indicated that different storage temperatures have a minimal impact on the quality of green tea.

Theobromine is a natural compound found in tea, primarily green tea, and it plays several important roles in maintaining its quality. Theobromine contributes to the characteristic bitter taste of green tea. The presence of theobromine can enhance the aroma and fragrance of green tea. It interacts with other compounds in tea, contributing to the development of the tea's unique aroma and enhancing its overall sensory appeal. Theobromine contributes to the overall flavour profile of green tea, adding complexity and a subtle bitterness.

3.4. Impact of Temperature during Storage on Volatile Substances

As we know, two primary methods are employed to quantify and identify volatile organic compounds (VOCs). The primary approach involves the use of gas chromatography, wherein GC instruments facilitate the separation of gaseous components. When paired with a flame ionization detector (FID), GCs can detect hydrocarbons at deficient concentrations, down to parts per trillion levels. Additionally, GCs equipped with electron capture detectors prove effective in analysing organohalides, such as chloro-carbons. The second major technique for VOC analysis involves mass spectrometry, commonly integrated with GC to form the hyphenated technique known as GC-MS. This combination enhances the capabilities of VOC analysis, providing detailed insights into the composition of volatile organic compounds. The peak area at each storage time over the peak area prior to storage was used to calculate the relative concentration% for each volatile chemical. The volatile components in tea samples held at room temperature and a higher temperature (40 °C) showed minimal variation. However, a longer storage duration caused the volatile profile to change. As is wellknown, the flavour quality of tea is directly proportional to the flavour index value. It was clear that the content had dropped, which suggested that the quality of the tea's aroma had diminished [53].

From this analysis, about 46 volatile compounds were detected in G1 storage conditions, about 43 volatile compounds were detected in G2 storage conditions, and about 49 volatile compounds were detected in G3 storage conditions (Table S1). In G1 storage condition, dimethyl sulphide (11.96 ± 2.31%) was detected as the most abundant volatile compound, which was followed by 1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl-(7.65 ± 1.09%), indole (6.65 ± 1.12%), phenylethyl alcohol (5.86 ± 0.98%), n-Caproic acid vinyl ester (4.49 ± 1.23%), 1,6-Octadien-3-ol, 3,7-dimethyl-(4.45 ± 1.08%), 2-Cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (Z)-(4.44 ± 0.87%), benzyl alcohol (3.84 ± 0.93%), 1-Pentanol (7.32 ± 1.63%), etc., respectively (Table 2).

The phytochemical composition of green tea, especially that of theophylline, gallic acid, and theobromine, can be affected by storage temperature. Since there was a slight variation in the theobromine level, the investigation's findings suggest that variations in storage temperature have little effect on the quality of green tea. Gallic acid, another significant polyphenolic component of green tea, did not significantly change in concentration when kept at various temperatures (G1, G2, and G3). However, there were no differences found in the theophylline concentration in this study between the three storage conditions (G1, G2, and G3). The balance of theobromine with other compounds influences the unique flavour characteristics of different green tea varieties [54].

This study identified storage temperature as a crucial factor in influencing the phytochemical compositions of green tea, with specific attention to theobromine and gallic acid. Theobromine, responsible for the bitterness and astringency in green tea, holds significant importance in upholding the overall taste and quality of the beverage [55]. Conversely, gallic acid serves a protective role by safeguarding the tea against oxidative stress and deterioration, thereby preserving the freshness and overall quality of the tea [56].

Table 2. Comparison of some of the major volatile compounds detected in green tea.

		Contents (%)		
SL No.	**Compound Name**	**G1**	**G2**	**G3**
1	Dimethyl sulphide	11.96 ± 2.31	3.13 ± 1.12	0.36 ± 0.16
2	1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl-	7.65 ± 1.09	5.05 ± 1.27	3.76 ± 0.86
3	Indole	6.65 ± 1.12	2.34 ± 0.91	3.02 ± 0.29
4	Phenylethyl Alcohol	5.86 ± 0.98	17.03 ± 2.93	5.86 ± 1.11
5	n-Caproic acid vinyl ester	4.49 ± 1.23	4.94 ± 1.39	3.49 ± 1.17
6	1,6-Octadien-3-ol, 3,7-dimethyl-	4.45 ± 1.08	5 ± 1.73	3.48 ± 0.65
7	2-Cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (Z)-	4.44 ± 0.87	4.16 ± 1.23	3.75 ± 0.49
8	Benzyl Alcohol	3.84 ± 0.93	13.69 ± 2.18	15.17 ± 2.11
9	Benzaldehyde	0.57 ± 0.24	2.27 ± 0.63	6.7 ± 1.44
10	1-Pentanol	7.32 ± 1.63	5.46 ± 1.14	0.64 ± 0.76

In the G2 storage condition, phenylethyl alcohol (17.03 ± 2.93%) was detected as the most abundant volatile compound. In comparison, the other volatile compounds, including benzyl alcohol (13.69 ± 2.18%), 4-Hexen-1-ol, 5-methyl-2-(1-methyl phenyl)-, (R)-(6.55 ± 1.23%), 1-Pentanol (5.46 ± 2.18%), 1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl-(5.05 ± 1.27%), n-Caproic acid vinyl ester (4.94 ± 1.39%), 2-Cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (Z)-(4.16 ± 1.23%), 3-Hexen-1-ol (3.45 ± 1.12%), etc. were detected in higher amounts.

Benzyl alcohol (15.17 ± 2.11%) was detected as the most abundant volatile compound in G3 storage condition. Other remarkable detected volatile compounds were benzaldehyde (6.7 ± 1.44%), 2-Cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (Z)-(3.75 ± 0.49%), 3, 5-Octadien-2-one (3.73 ± 1.09%), n-Caproic acid vinyl ester (3.49 ± 1.17%), 1,6-Octadien-3-ol, 3,7-dimethyl-(3.48 ± 0.65%), indole (3.02 ± 0.29%), etc., respectively.

The analysis indicated that each storage condition has a different volatile compound profile. The content (%) of most of the volatile compounds reduced as the temperature of the storage conditions rose. However, there were some exceptions, such as phenylethyl alcohol, benzyl alcohol, and benzaldehyde. The content (%) of these compounds increased at higher storage temperatures compared to lower temperatures (Table S1). Volatile compounds play a crucial role in maintaining the quality of green tea by contributing to its aroma and flavour. Volatile compounds are responsible for the complex and diverse aroma and flavour profile of green tea. In this study, an analysis of volatile compounds revealed distinct profiles for each storage condition, with variations in the abundance of compounds such as dimethyl sulphide, phenylethyl alcohol, indole, and benzaldehyde. The combination and concentration of these compounds give each green tea variety its unique scent and taste, making them appealing to consumers [57]. Volatile compounds responsible for the characteristic grassy, floral, and fruity notes of green tea can be compromised, leading to the development of off-flavours [58]. Dimethyl sulphide (DMS) is a volatile organic sulphur compound that can play a role in the quality of green tea. DMS is known for its sulphuric, cabbage-like aroma. This sulphur compound, along with others, can create the characteristic vegetal and grassy notes that are often associated with high-quality green teas. The presence of DMS in green tea can provide a pleasant, slightly pungent aroma that enhances the overall sensory experience. Indole contributes to the characteristic aroma and flavour of the tea. It is responsible for the floral and fruity notes in teas, such as jasmine tea or certain oolong teas. The presence of indole in tea leaves contributes to the complexity and depth of the tea's flavour profile. Benzaldehyde is one of the essential compounds responsible for the characteristic almond-like aroma in tea. The presence of benzaldehyde can distinguish one tea variety from another based on its unique flavour profile [24]. It contributes to the overall fragrance of the tea, making it more appealing to consumers. Benzaldehyde adds complexity to the flavour profile of the tea. It can provide nutty and fruity notes, enhancing the overall taste experience.

3.5. Changes in Appearance, Aroma, Soup Colour, Taste, and Leaf Bottom

In this study, the sensory evaluation of green tea samples was carried out according to the Chinese Standard Methods "GB/T 23776-2018". Twenty panellists (all between the ages of 20 and 40) evaluated and scored each sample of tea. Green tea samples from different storage temperatures were assessed individually. There are several factors that indicate the freshness of green tea samples. Among them, sensory evaluation test results play an important role in determining the quality of green tea samples.The higher scores indicated the freshness of the samples, while lower scores represented the less acceptable samples.

The overall score of green tea samples in terms of appearance, aroma, soup colour, taste, and leaf bottom that were stored at G1, G2, and G3 conditions were 91.5, 76.05, and 65.75, respectively (Table S2). Green tea samples that were stored at −20 °C (G1) were given the highest scores for all of the parameters. The longer time storage at the same temperature slightly altered the colour. Those tea samples also exhibited almost similar fresh and good taste as the fresh samples. Some differences were observed between the tea samples that were stored at −20 °C and room temperature. All of the sensory parameters were different in G2 and G3 storage conditions compared to G1 storage conditions (Figure 4). On the other hand, minimal differences were observed in every parameter between the green tea samples that were stored at room temperature and 40 °C.

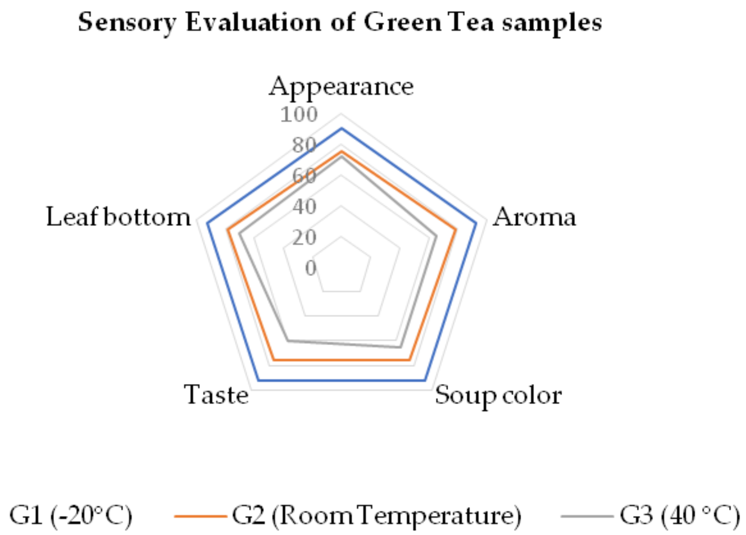

Figure 4. Sensory evaluation scores of green tea samples at different storage conditions (G1, G2, and G3).

The panellists rated the highest scores in the green tea appearance, which was stored at −20 °C. In that condition, the leaves appeared emerald green and oily. The green tea appeared yellowish drier and was stored at room temperature (G2) and 40 °C (G3), respectively. The storage at both room temperature and at 40 °C affected the green tea colour. The tea that was kept at room temperature took on a yellowish drier colour, indicating that its quality had deteriorated over this time of storage (Figure 5a). During preservation, the tea juice and leaves that had been soaked in water almost lost their brightness. At 40 °C, the colour of green tea changed from green to yellowish and brownish. The colour of the tea juice was found to differ from the green tea under other storage conditions (Figure 5b). In contrast, there were fewer changes in the appearance and soup colour of green tea kept at −20 °C (Figure 5c).

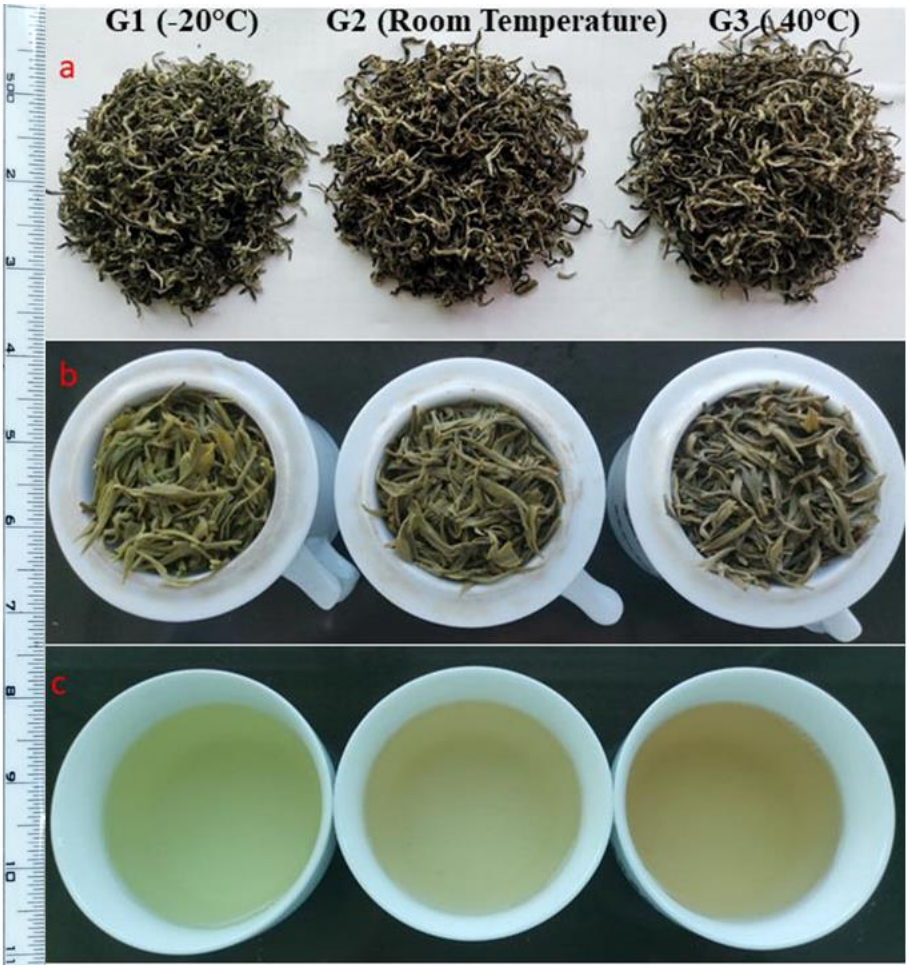

Figure 5. Appearance of green tea and soup colour after being stored at different storage temperature conditions: (**a**) dried tea leaves; (**b**) leaf bottom; and (**c**) soup colour.

A tender and refreshing aroma wasobserved in the green tea of G1 storage condition, while an alcoholic aroma and a smelly, stuffy aroma were observed from the tea stored at G2 and G3 conditions, respectively. In terms of soup colour, green tea from G1, G2, and G3 storage conditions produced transparent yellow-green, light green, and yellowish still-bright soup, respectively. Greener and brighter leaf bottoms were observed from green tea in the G1 condition, while bright yellow and green leaf bottoms were observed in the G2 condition of the leaves, and yellow-green to yellowish leaf bottoms were observed in green tea in the G3 storage condition.

Similarly, the taste of green tea stored at −20 °C achieved the highest score, followed by green tea at room temperature (G2) and 40 °C (G3). The taste of green tea in the G1 condition was fresh and mellow.

Sensory evaluation is a critical aspect of assessing tea quality, and it involves the assessment of various attributes, such as appearance, water extracts, soup colour, leaf bottom, and aroma. Following harvest and processing, green tea leaves are typically dehydrated to eliminate moisture, and the resulting dried leaves are stored at low temperatures to maintain their sensory and nutritional qualities [24]. Xu et al. [59] have noted that storing green tea at −20 °C can significantly preserve its nutritional and sensory attributes by preventing the oxidation of tea polyphenols. Qin et al. [60] have also proposed that Chinese green tea flavours can be effectively stabilized at 4 °C. Additionally, Friedman et al. [61] reported that storing green tea leaves at 4 °C effectively prevents the degradation/oxidation of bioactive compounds. The extremely low-temperature storage of tea at − 70 °C has been shown to preserve tea aroma [62] dramatically. As is well known, chlorophyll is primarily responsible for the colour of tea leaves [63].

The stability of chlorophyll concentration at lower temperatures may be the cause of the identical look of tea before and after storage at −20 °C. According to studies, tea's colour can be preserved by storing green tea at a low temperature [64]. The fresh taste was totally lost due to the long-term storage at 40 °C. The taste was slightly bitter, while an alcoholic taste was observed in tea leaves stored in the G2 condition.

The freshness and flavour of green tea can be maintained by storing it at lower temperatures in a refrigerator [65]. It was observed that teas that were stored at lower temperatures retained taste and aroma for a certain period, but more extended storage can alter both taste and smell. The sensory evaluation analysis suggested we store the tea samples at −20 °C or lower temperatures to preserve the sensory qualities. The current study of sensory evaluation showed almost similar findings in a previous study [66]. High-quality tea leaves should be intact, whole, and uniform in size, which is associated with better flavour and aroma [67]. The moisture content should be balanced to prevent excessive drying or dampness, which can impact the tea's smell and taste [68]. The colour of the tea infusion or soup is a critical sensory attribute that indicates the tea's quality and maturity. A bright and clear soup colour is generally associated with high-quality tea, while a dull or cloudy appearance may suggest lower quality [69].

Overall, green tea samples stored at −20 °C received the highest scores for appearance, aroma, soup colour, taste, and leaf bottom, indicating better freshness compared to samples stored at higher temperatures. Storage at room temperature and 40 °C slightly affected the colour, aroma, and taste of green tea, with samples showing very little colour shifts and slight changes in aroma profiles. Among the biochemical properties, amino acid content was higher at lower storage temperatures, with minimal impact from storage temperature variations. Soluble sugar content decreased with higher storage temperatures, affecting the tea's sweetness. Water extracts are significantly reduced with higher storage temperatures, impacting the tea's flavour and fragrance constituents. Higher storage temperatures led to decreased polyphenol content, affecting the tea's health-promoting properties. Flavonoid concentrations decreased with higher storage temperatures, influencing the tea's flavour and aroma. Catechin content, including EGCG and GCG, decreased at higher storage temperatures, impacting the bitterness and astringency of the tea. Although there is no significant difference in the contents of amino acids and soluble sugar, only some differences are found in polyphenols, flavonoids, and catechins between G1, G2, and G3 storage conditions. Moreover, in the case of polyphenols, flavonoids, and catechins, no significant difference was found between G2 and G3. However, certain compounds, including polyphenols, flavonoids, and catechins, might impact the taste and aroma of green tea, even when there are no significant differences in other compounds (amino acids and soluble sugar) among treatments. This invites reflection on the relationship between chemical composition and sensory attributes, suggesting that while some compounds may not vary significantly, others could still influence the overall sensory experience. For instance, polyphenols contribute to the bitterness and astringency of tea, while flavonoids and catechins can affect its aroma and flavour profile. Therefore, even subtle differences in these compounds could potentially alter the taste and aroma of the tea samples.

Storage temperatures had little effect on gallic acid, theophylline, and theobromine. Volatile compounds in green tea varied with storage temperature, with distinct profiles for each condition. Dimethyl sulphide, phenyl ethyl alcohol, indole, and benzaldehyde were among the detected compounds. Storage temperature influenced the abundance of volatile compounds, affecting the aroma and flavour profile of green tea. Moisture content also influenced green tea quality, with lower temperatures retaining flavour and aroma better than room temperature storage. Overall, storage temperatures play a crucial role in preserving the sensory qualities, biochemical characteristics, metabolites, and volatile compounds of green tea. Lower temperatures help maintain freshness, flavour, and health-promoting properties, while higher temperatures lead to the degradation and alteration of chemical compositions, impacting the overall quality of green tea.

3.6. Effect of Moisture Content on Green Tea Quality

The effect of moisture content (3%, 5%, and 7%) on green tea quality was analysed. Lower storage temperatures retained almost the same flavour as freshly prepared tea. Lower temperatures can preserve volatile compounds responsible for tea fragrance and flavour. Bitterness and astringency were observed in green tea samples stored at room temperature. The breakdown of tea catechins might be the reason for this.

In this investigation, the impact of moisture content on the quality of green tea subsequent to storage at varying temperatures has been examined. Samples with a moisture content of 5% stored under freezing conditions (−20 °C) were juxtaposed with samples stored at ambient temperature with moisture contents of 3%, 5%, and 7%, respectively (Table 3). Substantial alterations in tea polyphenol levels were observed in samples with 5% and 7% moisture content stored at room temperature. Additionally, significant variations in amino acid concentrations were noted in samples with 7% moisture content. Moreover, fluctuations in water extracts were observed across samples with differing moisture levels when compared to the 5% freezing samples. Furthermore, diverse moisture contents at distinct storage temperatures also influenced the levels of catechins, including GC, EGC, EC, EGCG, GCG, and ECG.

Table 3. Effect of moisture content on green tea quality.

Conditions	**Tea Polyphenols (%)**		**Amino Acids (%)**		**Water Extracts (%)**	
	Average	SD	Average	SD	Average	SD
5% MC (Freezing)	23.54	0.29	4.54	0.08	39.25	0.24
3% MC (RT)	23.2	0.67	4.52	0.05	40.95	0.09
5% MC (RT)	22.73	0.35	4.58	0.07	39.99	0.02
7% MC (RT)	23.01	0.03	4.38	0.08	38.72	0.31
	EGCG		**GCG**		**ECG**	
	Average	SD	Average	SD	Average	SD
5% MC (Freezing)	4.41	0.01	2.86	0.01	1.18	0.005774
3% MC (RT)	4.46	0.02	2.59	0.01	1.23	0.01
5% MC (RT)	4.293333	0.015275	2.52	0.01	1.19	0.01
7% MC (RT)	4.26	0.01	2.57	0.01	1.17	0.01

Tea phytochemicals, such ascatechins, undergo changes during storage, particularly when exposed to oxygen, moisture, light, and temperature fluctuations. Catechin levels, notably epigallocatechin gallate, decline significantly within six months of production, emphasizing the importance of consuming green tea as fresh as possible to retain its sensory qualities and potential health benefits. Storing tea in sealed packaging in cool, dark conditions can extend its shelf life. The choice between loose leaf and tea bags affects the taste and phytochemical composition of tea due to differences in oxidation rates and extraction efficiency. While loose leaf tea preserves more aromatic oils, tea bags offer convenience but may hinder optimal extraction and flavour. The freshness of fine green tea is affected differently depending on whether it is frozen, refrigerated, or kept at room temperature,while some teas stored intraditional ways pose problems for tea freshness and effectiveness. Although dry storage is common, it may not preserve the antioxidants in green tea optimally. Temperature plays a crucial role in maintaining freshness. At temperatures exceeding 30 °C (86°F), the green colour of tea leaves starts to fade, even with low light and moisture. Lower temperatures delay oxidation and deterioration, with storage without moisture, light, and oxygen at low temperatures potentially leading to mellowing through ageing. While not all green teas age similarly, low-temperature storage generally offers better preservation compared to room temperature storage.

The volatile compounds of green tea primarily consist of alcohols, aldehydes, ketones, esters, aromatic hydrocarbons, and terpenes [70], which originate from two primary sources within the tea—fresh leaves and substances created during processing. These substances fall into four main categories based on their precursors: those derived from amino acids, which undergo reactions such asthe Strecker and Maillard reactions when exposed to heat and sugars, resulting in the formation of aldehydes, ketones, furans, pyrazines, and other high-boiling compounds [57,71], and compounds released from glycosides, both volatile and non-volatile, which release bound volatile components such aslinalool and geraniol when acted upon by glycosidase or heat [72]. The methods used in processing directly influence the biosynthesis pathways of these volatile compounds and subsequently impact the aroma profile of green tea [73]. Additionally, the different shapes of tea leaves produced by varying processing techniques affect cell breakage and material leaching rates, leading to diverse aroma profiles [74,75].

This study stands out from previous research by delving into the impact of storage temperatures (−20 °C, room temperature, and 40 °C) on green tea over a 12-month period. Unlike many prior works that have explored singular aspects of tea storage, this comprehensive investigation examines sensory, biochemical, and volatile compound changes concurrently. The sensory evaluation not only highlights the visual and gustatory qualities preserved at −20 °C but also sheds light on the nuanced changes in aroma and taste under different storage conditions, providing a holistic understanding of tea quality over time. Moreover, the use of standardized methods, such as Chinese National Standard Methods for biochemical constituent measurement, ensures rigorous and comparable results. The inclusion of various analytical techniques, including spectrophotometry, HPLC, and gas chromatography coupled with triple quadrupole mass spectrometry, allows for a thorough examination of tea components and volatile compounds, providing detailed insights into the underlying mechanisms of quality deterioration. By identifying temperature-sensitive compounds and their variations, this study not only enriches our understanding of green tea storage but also offers valuable guidance for optimizing storage conditions to maintain tea quality.This study sheds light on previously undiscovered aspects of tea preservation by examining the complex interactions between storage temperatures and green tea quality parameters. This study stands out for its novel investigation of the effects of three different temperatures (room temperature, 40 °C, and −20 °C) on volatile compounds, metabolites, and sensory evaluation over a year. It provides priceless insights into the subtleties of maintaining tea quality. The sensory assessment was carried out in accordance with established standards and clarified the discernible variations in appearance, aroma, soup colour, taste, and leaf bottom. It also highlighted the exceptional robustness of green tea kept at −20 °C, which kept its vibrant green colour, delicate aroma, and refreshing flavour. These results establish the groundwork for future research into the ideal storage settings by highlighting the critical role that temperature plays in maintaining the sensory appeal of green tea. Furthermore, the thorough examination of biochemical components revealed dynamics that are temperature-sensitive, with significant differences seen in water extracts, soluble sugar concentration, and amino acids at various storage temperatures. The thermal effects that can be observed on tea polyphenols, flavonoids, and catechins highlight the complex relationship that exists between storage conditions and the retention of vital phytochemicals that are critical to tea quality and health benefits. Additionally, the study of volatile components revealed unique compositions impacted by storage conditions and offered fresh insights into the temperature-dependent variations in aroma profiles. The observed variations in catechins, amino acids, water extracts, and polyphenols in tea highlight the complex process of preserving tea quality and provide insightful advice for maximizing storage conditions to prevent quality deterioration.

4. Conclusions

The findings of this study provide valuable insights into the impact of storage temperatures on the sensory attributes, biochemical characteristics, metabolites, volatile substances, and water content of green tea. The sensory evaluation revealed that green tea samples stored at −20 °C (G1) received the highest overall scores, indicating exceptional freshness, while those stored at higher temperatures (G2 and G3) showed a decline in acceptability, especially in terms of appearance, aroma, taste, and colour. The study also highlighted the importance of maintaining optimal storage conditions to preserve the quality of green tea phytochemicals, such as gallic acid, theophylline, and theobromine. Volatile substances responsible for aroma and flavour were found to be sensitive to storage duration, with variations in the volatile compounds detected at different temperatures. Overall, this comprehensive study underscores the importance of storage temperature in maintaining the quality, freshness, and sensory attributes of green tea. Proper storage conditions, notably lower temperatures, can help preserve the integrity and stability of the chemical compositions of green tea for a more extended period. Lower storage temperatures help slow down the degradation process, preserving the catechins and thus maintaining the tea's antioxidant activity.Similarly, high temperatures can cause volatile compounds to evaporate or degrade, leading to a loss of aroma and flavour. Storing green tea at lower temperatures helps minimize this loss, allowing for the tea to retain its desirable sensory qualities for a longer time. One of the drawbacks is that the study only looks at three storage temperatures, thereby ignoring the impact of intermediate temperatures on the quality of green tea. Although longer-term storage effects may provide additional insights into the stability of green tea constituents over time, the study only looks at the impact of storage for a whole year. The results of this study only apply to the particular green tea variety and storage circumstances that were examined, which restricts how broadly the findings could be used for other tea varieties or storage settings.The novel methodology and thorough results of this study highlight the intricate interactions that occur between moisture content, storage temperatures, and the maintenance of sensory, biochemical, and volatile qualities in green tea. This work sets the path for future research aimed at optimizing storage conditions to maintain the longevity of green tea quality by clarifying temperature-sensitive dynamics and offering insights into preservation processes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/beverages10020035/s1, Table S1: List of volatile compounds detected from green tea leaf samples treated with different storage temperatures (G1, G2 and G3); Table S2: Sensory evaluation scores of green tea samples at different storage conditions (G1, G2 and G3).

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