THE EFFECT OF BREWING TEMPERATURE AND TIME ON ANTIOXIDANT ACTIVITY AND SENSORY PROFILE OF THE BOTANICAL BEVERAGE FROM CAT'S WHISKERS (*Orthosiphon spicatus*) LEAVES

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ABSTRACT

The Cat's Whiskers plant (*Orthosiphon spicatus*) is renowned for its numerous health benefits. The plant contains various bioactive compounds, including phenolic compounds, and one effective way of utilizing its leaves is by transforming them into a functional beverage. This study aimed to determine the effects of brewing time and temperature on the antioxidant activity and sensory profile of the botanical beverage from Cat's Whiskers leaves. The treatments included two levels of brewing time (3 and 5 minutes) and three levels of brewing temperature (70 ºC, 85 ºC, and 100 ºC). The results indicated that brewing temperature and time significantly affected ($p<0.05$) total phenolic content (TPC), antioxidant activity, and panelist preference levels for the botanical beverage from Cat's Whiskers leaves. Brewing at 100 ºC for 5 minutes yielded the highest TPC (7.05 mg GAE/g) and antioxidant activity (88.97%), with an overall liking score of 5.43. The beverage exhibited attributes of brightness, a grassy, woody, fresh aroma, a grassy, earthy, bitter flavour, and a grassy, bitter, astringent aftertaste. Principal component analysis revealed a positive correlation between TPC, antioxidant activity, and the preference levels of the panelists.

Keywords: antioxidant activity; botanical beverage; cat's whiskers leaves; sensory profile; total phenolic content.

ABSTRAK

Tanaman kumis kucing (*Orthosiphon spicatus*) merupakan salah satu tanaman yang memiliki banyak manfaat kesehatan. Tanaman ini mengandung beberapa senyawa bioaktif seperti senyawa fenolik. Pemanfaatan daun kumis kucing yang cukup mudah adalah dengan mengolahnya menjadi minuman fungsional. Penelitian ini bertujuan untuk menentukan pengaruh waktu dan suhu penyeduhan terhadap aktivitas antioksidan dan profil sensori minuman botanikal daun kumis kucing. Perlakuan yang digunakan terdiri dari dua perlakuan waktu penyeduhan (3 dan 5 menit) dan tiga tingkat suhu penyeduhan (70 ºC, 85 ºC, dan 100 ºC). Hasil penelitian menunjukkan bahwa suhu dan waktu penyeduhan berpengaruh nyata ($p<0,05$) terhadap total senyawa fenolik (TSF), aktivitas antioksidan, dan tingkat kesukaan panelis terhadap minuman botanikal daun kumis kucing. Penyeduhan pada suhu 100ºC selama 5 menit menghasilkan TSF tertinggi (7,05 mg GAE/g) dan aktivitas antioksidan tertinggi (88,97%) dengan tingkat kesukaan secara keseluruhan sebesar 5,43. Minuman botanikal daun kumis kucing memiliki atribut warna cerah; dengan aroma *grassy*, *woody*, *fresh* aroma; *grassy*, *earthy*, *bitter flavor*; *grassy*, *bitter*, *astringent*, serta *aftertaste* pahit. Berdasarkan analisis komponen utama, minuman botanikal daun kumis kucing memiliki korelasi positif antara TSF, aktivitas antioksidan, dan tingkat kesukaan panelis.

Kata kunci: aktivitas antioksidan; daun kumis kucing; minuman botanikal; profil sensori; total senyawa fenolik

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INTRODUCTION

The Cat's Whiskers plant (*Orthosiphon spicatus*), also known as Java tea, is a medicinal plant widely recognized in Indonesia. It is commonly used to treat inflammation, fever, rheumatism, diabetes, and to dissolve kidney stones. Various studies have shown that the active ingredients in this plant include terpenoids, sterols, and phenolics, with phenolic compounds being the most dominant (Aziz et al., 2021). The presence of these active ingredients suggests that Cat's Whiskers leaves have the potential to be processed into functional beverages, including botanical beverages.

Botanical beverages are defined as drinks made from extracts of specific plant parts (such as stems, leaves, roots, or flowers)—as well as teas and herbal beverages—with or without the addition of other food ingredients (BPOM, 2019). The botanical beverages derived from Cat's Whiskers leaves will have good functional properties due to their antioxidant components. The total polyphenol content in the root, stem, and leaf of the Cat's Whiskers leaves have been found to be 266.25 ± 25.26, 82.92 ± 5.42, and 187.08 ± 28.42 µg GAE, respectively (Cai et al., 2018). The antioxidant content of beverages can be influenced by the brewing process, which involves extracting active components from the ingredients using a solvent. Factors such as temperature and time significantly affect the extraction rate during brewing (Ariffin et al., 2011). Generally, higher temperatures and longer extraction times yield higher concentrations of extracts, thereby influencing the sensory, physical, and chemical properties of beverage products.

Previous research has shown that brewing temperature and time significantly affect the sensory characteristics and antioxidant activity of avocado leaf tea beverages (Dewata et al., 2017). The selection of brewing temperature and time is crucial for achieving optimal beverage quality. This study aimed to determine the effects of temperature and brewing time on TPC, antioxidant activity, and sensory profile of the botanical beverage Cat's Whiskers leaves. The findings are expected to provide valuable information regarding the optimal brewing conditions for shorter-brewing Cat's Whiskers leaf-based beverages.

MATERIALS AND METHOD

Materials

The materials used in this study were fresh, young Cat's Whiskers leaves that were grown within the community in Lembang, West Java, Indonesia. The following reagents were utilized: Folin–Ciocalteu reagent (Merck, Darmstadt, Germany), Na2CO3 (Merck, Darmstadt, Germany), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, St. Louis, MO, USA), gallic acid (Merck, Darmstadt, Germany), ethanol, distilled water (aquades), and bottled water.

Experimental Design

The research design employed was a Completely Randomized Design (CRD), and the treatments were as follows:

- Sample 108: Brewing at 70 ºC for 3 minutes.
- Sample 219: Brewing at 70 ºC for 5 minutes.
- Sample 320: Brewing at 85 ºC for 3 minutes.
- Sample 431: Brewing at 85 ºC for 5 minutes.
- Sample 542: Brewing at 100 ºC for 3 minutes.
- Sample 653: Brewing at 100 ºC for 5 minutes.

For sensory evaluation, repetitions were not conducted as the number of panelists was deemed sufficient. However, the total phenolic content (TPC) and antioxidant activity tests were repeated three times and the results presented as mean ± SD.

Preparation of Dried Cat's Whiskers Leaves

The preparation of dried Cat's Whiskers leaves was carried out based on the method described by Cai et al. (2018), although the method was modified in this study. Samples of 100 grams of Cat's Whiskers leaves were collected and washed with running water. The leaves were then subjected to a withering process at room temperature for 24 hours. Afterward, the leaves were cut into small pieces measuring approximately 1 cm and arranged in an aluminum pan. The leaves were dried in a warehouse for five days until completely dry, with an average drying temperature of 28.9 ºC and an average moisture content of 7.76%. Subsequently, the dried leaves were ground using a blender.

Brewing Treatment

The brewing treatment was conducted according to the methods described by Dewata et al. (2017) and Mutmainnah et al., 2018). The mashed Cat's Whiskers leaves were packed into tea bags measuring 6x8 cm, each containing 2 grams of leaves. Each sample was brewed with 200 mL of water, adhering to the specified temperature conditions and brewing times.

Determination of Total Phenolic Content and Antioxidant Activity

The determination of TPC was analyzed using the Folin-Ciocalteu method (Diouf et al., 2009), while the antioxidant activity was assessed using the DPPH method (Saefudin et al., 2013).

Sensory Evaluation

Sensory evaluation was conducted using the Free Choice Profiling (FCP) method (Santos et al., 2015) and an overall hedonic test. The FCP method consisted of two sessions: the first involved describing and generating the attributes detected by the panelists, while the second session assessed the intensity of the attributes selected in the first session. Each panelist received a set of samples and completed a two-part questionnaire. In the first session, panelists freely described the sensory attributes of each sample, including color, aroma, taste, and aftertaste. In the second session, panelists evaluated the intensity of the identified attributes by marking an "X" on a provided line scale ranging from 1 to 9, with higher numbers indicating stronger intensity. The overall hedonic testing utilized a 1–7 scale (1, dislike extremely; 2, dislike very much; 3, dislike moderately; 4, neither like nor dislike; 5, like moderately; 6, like very much; 7, like extremely). Both sensory methods involved 30 untrained panelists, consisting of 17 females and 13 males, based our previous studies (Pandiansyah et al., 2024). Additionally, mineral water and crackers were provided on the testing table to cleanse the palate between samples.

Data Analysis

The resulting data from TPC, antioxidants, and hedonic tests were analyzed using SPSS with a two-way ANOVA test at a significant level of 5%. Where significant differences were found, Duncan's difference test was conducted. R^2 correlation analysis was performed using simple linear regression methods to determine the correlation between TPC and antioxidant activity. The FCP test results were analyzed with XLSTAT 2023 software using the Generalized Procrustes Analysis (GPA) method.

RESULTS AND DISCUSSION

Different Brewing and Time Treatments

The use of different brewing temperatures and times yielded variations in color intensity across samples (Figure 1).

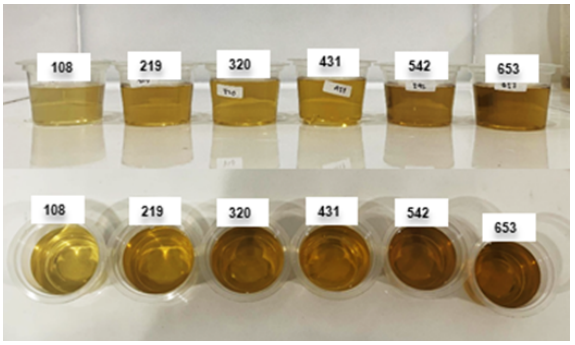

Figure 1. Appearance of sample in various brewing conditions

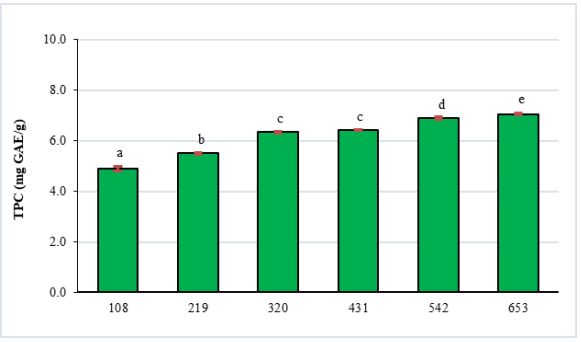

Figure 2. TPC of Cat's Whiskers leaves drink prepared with different time and temperatures. Different lowercase letters indicate significant differences between sample values ($P < 0.05$).

The color intensity of the resulting brew tended to increase with higher temperature and longer brewing times. The brownish-yellow color observed in the drink was attributable to the accumulation of phenolic compounds, particularly flavonoids, from the Cat's Whiskers leaves dissolved in water. The color intensity of the brew increases with the concentration of flavonoids extracted (Cai et al., 2018).

In the present study, higher temperatures were found to enhance the ability of the solvent to extract phenolic compounds, leading to increased accumulation in the brew. This phenomenon is similar to that observed with *Muntingia calabura* L leaves when heated at 90 ºC (Fikri et al., 2021). Additionally, longer brewing times allow for extended contact between phenolic compounds and the solvent (water), thus optimizing the extraction process and increasing the color intensity to a certain extent. A similar effect was noted in white tea (*Camellia assamica*) brewed at 95 ºC (Putra et al., 2020).

Total Phenolic Content

The total phenolic content (TPC) data in this study ranged from 4.91 to 7.05 mg GAE/g (Figure 2). Statistical analysis revealed that differences in temperature and brewing time significantly affected ($p<0.05$) the TPC of the botanical beverage from Cat's Whiskers leaves.

Sample 653 (brewing at 100ºC for 5 minutes) exhibited the highest TPC value of 7.05 mg GAE/g, while the lowest TPC value was recorded in sample 108 (brewing at 70 ºC for 3 minutes) at 4.91 mg GAE/g.

In this study, brewing temperature and time were found to influence the extraction rate of phenolic compounds in the beverage. For instance, the high temperature conditions (100ºC) in sample 653 accelerated cell wall breakdown, thus optimizing the extraction of phenolic compounds. Longer brewing time also enhances the contact between phenolic compounds and water, allowing for better dissolution and accumulation, as observed in white tea (*Camellia sinensis* [L.] Kuntze) brewed at 90 ºC (Rohdiana et al., 2013). Conversely, the lower temperature and time in sample 108 (brewing at 70 ºC for 3 minutes) inhibited the extraction process, as these conditions limited cell wall breakdown as well as the solubility of compounds. This phenomenon is similar to that in Galangal brewed at 60 ºC (Tambun et al., 2016).

Antioxidant Activity

The antioxidant activity of the samples ranged from 65.74% to 88.97% (Figure 3). Statistical analysis showed that variations in temperature and brewing time significantly affected the antioxidant activity of the Cat's Whiskers leaf botanical beverage ($p<0.05$).

The highest percentage of inhibition was observed in sample 653 (brewing at 100 ºC for 5 minutes), while the lowest percentage was recorded in Sample 108 (brewing at 70 ºC for 3 minutes).

Antioxidant activity is considered high if the percentage of inhibition exceeds 50%, medium if it falls between 20% and 50%, and low if it is below 20% (Saefudin et al., 2013). The botanical beverage in this study demonstrated high antioxidant activity, as the percentage of inhibition exceeded 50%.

Correlation analysis using simple linear regression illustrated the relationship between TPC and antioxidant activity (Figure 4). The analysis revealed a positive correlation between TPC and antioxidant activity in the botanical beverage, with a regression coefficient (R^2) of 0.965, which indicates that 96.5% of the antioxidant activity in the beverage was influenced by the presence of phenolic compounds. A study by Sumarno et al. (2021) also demonstrated a strong positive correlation between TPC and antioxidants in black tea drinks, with a correlation coefficient, R^2, of 0.903. Thus, a higher phenolic compound content correlates with greater antioxidant capacity in the inhibition of free radicals.

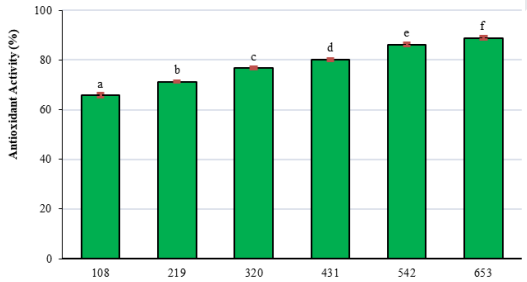

Figure 3. Antioxidant activity of Cat's Whiskers leaves drink prepared with different time and temperatures. Different lowercase letters indicate significant differences between sample values ($P < 0.05$).

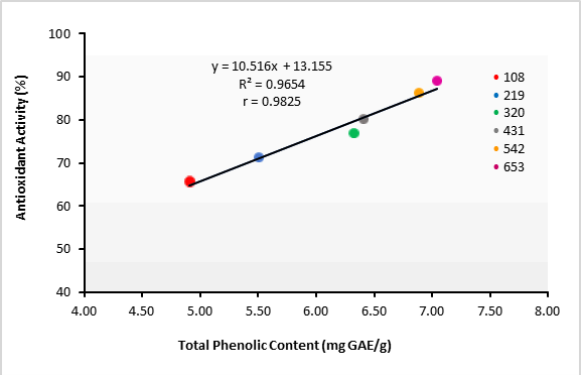

Figure 4. Correlation between TPC and antioxidant activity of Cat's Whiskers leaves beverage prepared with different time and temperatures.

Sensory Profiles

The sensory profile biplot for each sample is presented in Figure 5. The use of different brewing temperatures and times resulted in distinct dominant attributes for each sample, with each sample occupying a different quadrant. Sample 108 exhibited dominant attributes of brightness, along with fresh and woody aromas. The brightness in Sample 108 was likely due to the temperature and brewing time, resulting in a lighter brew color compared to that in the other samples. The woody aroma may be attributed to the presence of β-caryophyllene compound (A'yuni et al., 2022). Sample 431 displayed a dominant earthy flavor and grassy aftertaste, likely due to the presence of 1-octen-3-ol compounds that imparted earthy-fungal type flavor (The Good Scents Company, 2024). Samples 542 and 653 were located in the same quadrant, and exhibited dominant grassy aromas, grassy and bitter flavors as well as bitter and astringent aftertastes. This shows that panelists found it challenging to distinguish between the two samples, likely due to the similar brewing temperature of 100 ºC, which may have reached the optimal limit for releasing sensory components. The grassy attributes in samples 542 and 653 may be attributed to the presence of cis-3-hexen-ol and hexan-1-ol compounds, which provide grassy-green aromas and flavors (The Good Scents Company, 2024).

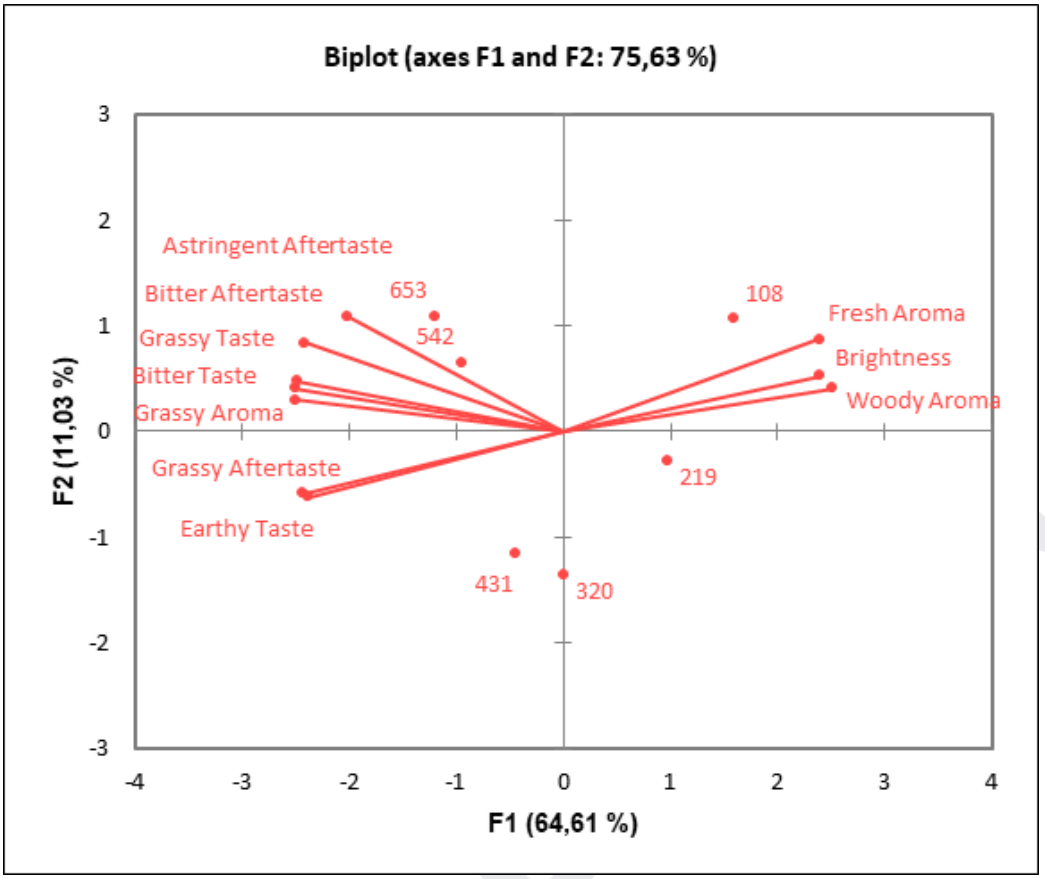

Figure 5. Biplot sensory attribute of samples

Furthermore, samples 219 and 320 did not seem to have any dominant attributes, possibly because panelists could not perceive distinct characteristics when tasting these samples.

The overall hedonic testing assessed the preferences of panelists for each sample (Permadi et al., 2019). The results of the overall hedonic test are shown in Figure 6, with scores ranging from 3.73 to 5.43. Analysis of variance indicated that temperature and brewing time significantly affected panelists' level of liking ($p<0.05$). Overall, the panelists tended to prefer beverages brewed at higher temperatures and longer times. This preference may be attributed to the optimal extraction of sensory components in the ingredients when brewed under these conditions, resulting in a more distinctive taste compared to lower temperatures and shorter brewing times (Dewata et al., 2017). The Principal Component Analysis (PCA) was conducted to examine the relationship among TPC, antioxidant activity, and hedonic test results (Figure 7). The PCA analysis biplot indicated a positive correlation among the three parameters. The TPC and antioxidant parameters were closely positioned with a slight angle, while the hedonic parameter displayed a larger angle but did not form an orthogonal angle (90º) with the TPC and antioxidant parameters. Despite not being a strong correlation, the data suggests a positive relationship between hedonic parameters and TPC and antioxidant values.

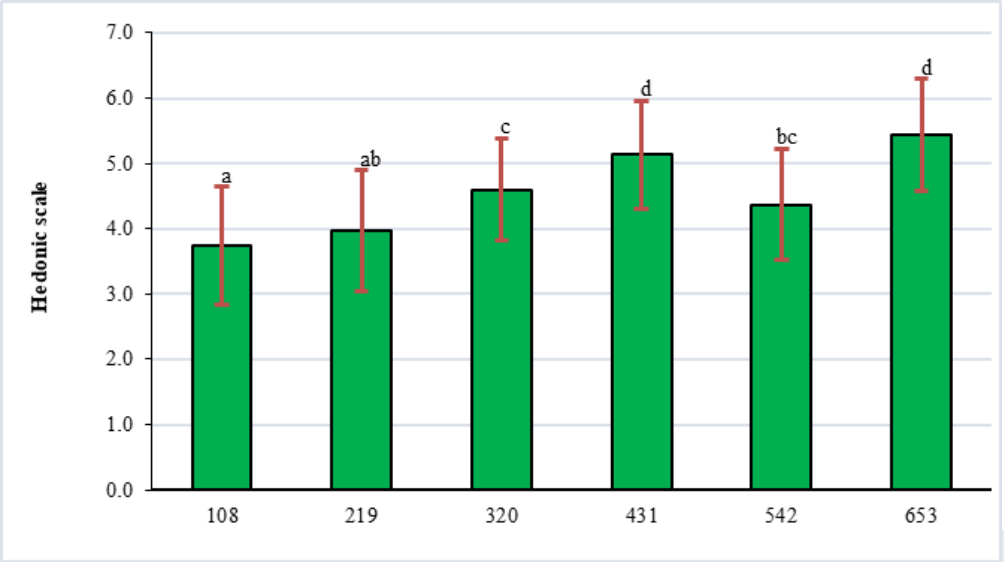

Figure 6. Overall acceptability of Cat's Whiskers leaves beverage prepared with different time and temperatures. Different lowercase letters indicate significant differences between sample values ($P < 0.05$).

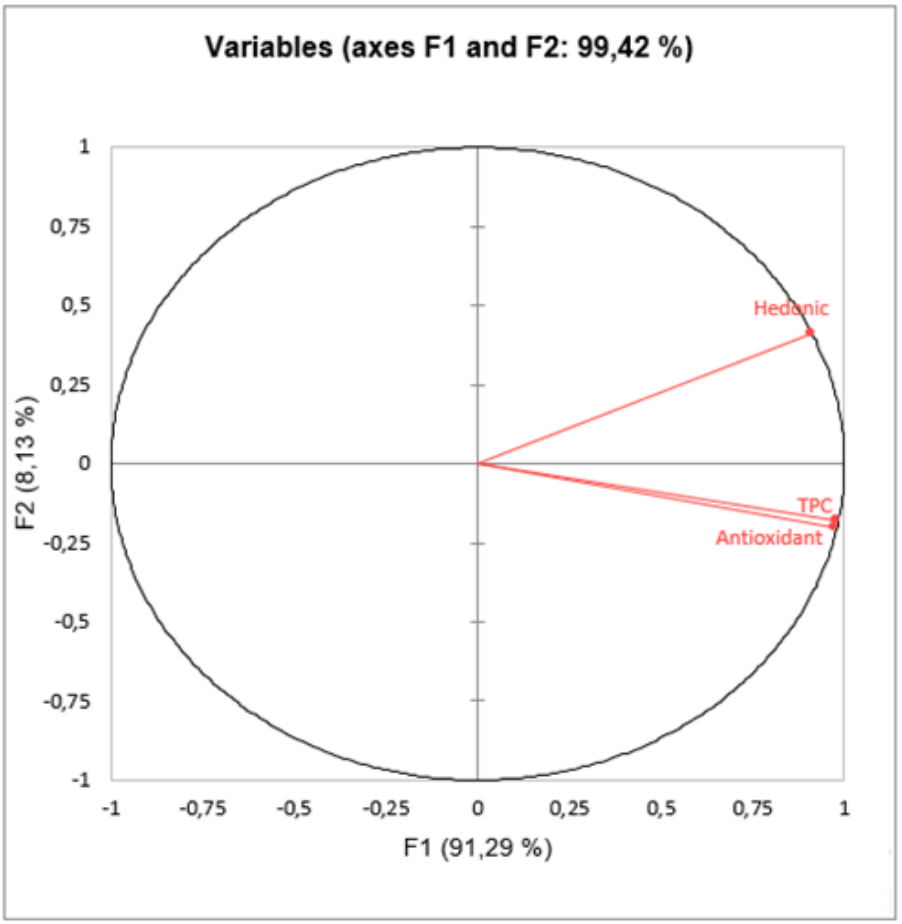

Figure 7. Biplot correlation between TPC, antioxidant activity, and hedonic test

CONCLUSION

The use of temperature and brewing time for botanical beverage from Cat's Whiskers' leaves significantly affected TPC, antioxidant activity, and overall liking scores ($p<0.05$). The optimal treatment was achieved with samples brewed at 100 ºC for 5 minutes, resulting in the highest TPC and antioxidant activity. Variations in brewing temperature and time also influenced the dominant attributes of each sample. The botanical beverage in this study exhibited brightness, grassy, woody, fresh aromas; grassy, earthy, and bitter flavors; and grassy, bitter, astringent aftertastes. This study's limitations focused primarily on TPC as the main bioactive compounds in the samples. Therefore, future research should analyze other compounds, such as total flavonoids. The findings from the present study support the innovation of functional beverages derived from antioxidant-rich food ingredients.

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