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Optimisation of corn silk tea production, and its antioxidant profile

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Abstract

Following COVID-19 pandemic, functional foods and beverages continue to develop, especially those with high antioxidant activity. The functional beverage from corn silk can be processed into brewed tea. The critical points in the making process of corn silk tea are the drying equipment, drying temperature, and drying time. With efficient method, corn silk tea can produce high antioxidant activity. The present work optimised the temperature and drying method based on antioxidant activity. The results of the present work are expected to be used in micro/small/medium enterprises (MSMEs) to produce corn silk herbal tea. The present work used Randomised Block Design (RBD) with two factors; drying temperatures (45, 55, and 65°C) and drying equipment (cabinet and oven) for 5 h. The analysis carried out was antioxidant activity and profiling using LC-HRMS (liquid chromatography high-resolution mass spectrometry) that ran in a positive (+) mode. The processing of corn silk tea using cabinet drying at 65°C for 5 h produced antioxidant activity of 82.00 ± 0.75%. This was higher than commercial black tea (81.71 ± 0.15%) and commercial green tea (78.37 ± 0.43%). The analysis using LC-HRMS showed that the corn silk tea contained betaine (N,N,N-trimethylglycine) (RT 0.928), 6-methylquinoline (RT 4.741), hesperidin (3’,5,7-trihydroxy-4’-methoxyflavanone) (RT 302.07817), luvangetin (10-methoxy-2,2-dimethylpyrano[3,2-g]chromen-8-one) (RT 258.08837), embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone) (RT 13.074), and eucalyptol (1,8-cineole) (RT 13.325).

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Introduction

The tea beverage, or simply tea, originally comes from the tea (*Camellia sinensis* (L.) Kuntze) leaves (Bag *et al*., 2022). Still, along with the development of ethnobotany, people often now use the term ‘tea’ for any brewed beverages derived from any dried plant parts (Cao, 2013; Oliveira, 2022). The drying method is a critical point in the tea-making process. It is related to the bioactive compounds contained therein, in which the stability is related to the drying temperature used (Kongsoontornkijkul *et al*., 2006; Zheng *et al*., 2015; Samoticha *et al*., 2016). Functional activities in tea are mostly antioxidants. Antioxidants obtained from tea is associated with prevention of various diseases (Sindhi *et al*., 2013).

Antioxidants can be used for health effects such as anti-cancerous agents (Kostova, 2005; Hamid *et al*., 2010; Jain *et al*., 2013), hepaprotective agents (Singal *et al*., 2011), nervous systems (Imosemi and Imosemi, 2013), interaction with red blood cells (Li, 2009; Agarwal and Sekhon, 2010; Gautam *et al*., 2012; Aggarwal *et al*., 2013), and therapeutic uses (Sindhi *et al*., 2013). Beverages with antioxidant activities can strengthen the immune system, becoming more popular during the COVID-19 pandemic. Compounds with antioxidant activities are known to reduce oxidative stress and inflammation (Kashiouris *et al*., 2020), minimise cardiovascular risk, improve immune system, and accelerate patient recovery process (Rosa and Santos, 2020; De Luca *et al*., 2012).

Some herbal tea from dried plants are made from medicinal plants (Van Wyk and Wink, 2018). Corn silk is one of the agricultural wastes that have high potential as herbal tea. A previous study claimed that corn silk has several health benefits such as antioxidant, antihyperglycaemic, antidepressant,

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antifatigue, and diuretic agent. Almost all activities that are related with flavonoid and terpenoid are within (Hasanudin *et al*., 2012; Sarepoua *et al*., 2013). In addition to flavonoids and terpenoids, anthocyanins and proanthocyanidins in corn silk also contribute to antioxidant activities (Sarepoua *et al*., 2013; Žilić *et al*., 2016).

Previous studies have shown the presence of flavonoids and terpenoids in corn silk. Some flavonoid compounds in corn silk are 3'-methoxymaysin, 2"-O-α-L-rhamnosyl-6-C-quinovosylluteolin, 6,4'-dihydroxy-3'-methoxyflavone-7-O-glucosides, 7,4'-dihidroxy-3'-methoxyflavone-2"-O-α-Lrhamnosyl-6-C-fucoside, and isoorientin-2-2"-O-α-L-rhamnoside (Ren *et al*., 2009; Hasanudin *et al*., 2012). Meanwhile, the terpenoid compounds in corn silk are α-terpineol, citronellol, 6,11-oxidoacor-4-ene, *trans*-pinocamphone, eugenol, neo-iso-3-thujanol, and *cis*-sabinene hydrate (Velazquez *et al*., 2005; Hasanudin *et al*., 2012). The utilisation of corn silk into herbal tea can reduce the waste produced annually. This idea can also contribute to good management of corn silk waste.

Tea derived from dried plants, commonly referred to as herbal teas, have a critical temperature point (Chong and Lim, 2012; Thamkaew *et al*., 2021). Some large industries use effective temperature selection based on the desired phytochemical characteristics or activities (Gupta and Dey, 2010). The use of temperature affects the content of secondary metabolites contained in herbal teas. The drying process used in the present work were oven and cabinet drying. The difference in the dryer and in temperature used can yield different antioxidant activities and water contents of the corn silk waste samples. Cabinet drying machine provides drying results with secondary metabolites that are better maintained due to the air flow in the system, whereas oven drying tends to damage the stability of the compound due to no air circulation (Kongsoontornkijkul *et al*., 2006; Chan *et al*., 2009). The best results obtained from the drying processes can determine their influence on corn silk waste processing.

Therefore, the present work aimed at choosing the temperature and drying method based on the antioxidant activity. The results of the present work are expected to be applied in the micro/small/medium enterprises (MSMEs) to produce corn silk herbal tea. The present work also compared the antioxidant activity of corn silk herbal tea from the most effective drying temperature and equipment with commercial black tea and commercial green tea.

Materials and methods

Research method

The present work was conducted with "Sragi" Regional Owned Enterprises (Sragi Village, Blitar, East Java, Indonesia) using Randomised Block Design (RBD) with two factors namely drying temperatures (45, 55, and 65°C) and drying equipment (cabinet and oven). Each treatment was tested for antioxidant activity. The data were statistically analysed using One-way analysis of variance (ANOVA). All results were calculated at a significance level of α of 0.10, presented as means, and means followed by similar lowercase superscripts were not significantly different at 0.1% level of probability based on Tukey's test. The treatment which gave the highest antioxidant activity was then analysed by LC-MS to obtain its phytochemistry profile. For comparison, the present work also analysed the antioxidant activity of commercial black tea and commercial green tea.

Sample cultivation and collection

The raw material used was corn silk from bonanza corn (sweet corn hybrid; code: F1; registration number in Indonesian Agrotechnology Ministry: 2071/KPTS/SR.120/5/2009; weight: 300 - 400 g/unit with corn silk 3 g/corn unit) from Sragi Village, Blitar, East Java, Indonesia. The corn samples were harvested after 80 d.

Corn silk tea making process

The corn silk from the corn plant was washed to remove dust and dirt. After being cleaned, it was dried under the sun for 2 d to remove the remaining water from the washing process. The corn silk was then subjected to drying process at 45, 55, and 65°C using a cabinet dryer (Maksindo, KD-35AS) and oven (Romand, binder GmbH Im Mittleren Osch 5). The temperatures were chosen based on a preliminary test. Based on the preliminary test results, the duration for the drying process was determined to be 5 h. The dried corn silk was then chopped and packaged into tea bags (each bag contained 2 g of chopped corn silk).

Antioxidant test

The samples were analysed for their antioxidant activity using the decoction method (solvent: diluted water; reagent: DPPH, 2,2-diphenyl-1-picrylhydrazyl; Sigma Aldrich). DPPH powder was prepared by mixing 2.5 mg of DPPH reagent and 25 mL of ethanol in a measuring flask. For 2 g of corn silk, the tea was dissolved in 250 mL diluted water until it reached 100°C. That corn silk decoction liquid was tested for antioxidant activity (%) with a DPPH reagent (3 mL of DPPH for 100 mL decoction liquid). The DPPH solution was covered with aluminium foil and kept in the dark. After 30 min, the tube's absorbance was measured using a UV-Vis spectrophotometer (Shimadzu UV 18000) at 517 nm. The standard was Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2- carboxylic acid) (Sigma-Aldrich). The antioxidant activity was calculated using Eq. 1:

$$\text{\% antioxidant activity} = \frac{(\text{Abs.blank} - \text{Abs.sample})}{(\text{Abs.blank})} \times 100 \quad \text{(Eq. 1)}$$

Profiling by LC-HRMS

The corn silk sample was tested with LC-HRMS (Thermo Fisher Scientific, positive ionic mode) at Hayati Laboratory Brawijaya University, Malang, East Java, Indonesia. The corn silk tea was extracted with acetone (according to the LC-HRMS solvent requirement). Before the sample was injected to the column, it was checked by looking at the density of the sample with a final volume of 1,300 µL. Next, the sample was homogenised by vortex (2,000 rpm, 2 min). Then, the sample was separated by centrifugation (6,000 rpm, 2 min). The supernatant was taken using a 0.22 µm syringe filter, and put into a vial bottle. The sample was put into an autosampler and injected into the LC-HRMS. Then, the analysis was done using full scan at 70.000 resolution data-dependent MS2 at 17.500. The resolution utilised a run time of 30 min. Processing data software used compound discoverer with *mz* Cloud MS/MS Library.

Results and discussion

Antioxidant activity

The corn silk tea (Figure 1) which was made by each treatment was analysed for their antioxidant activity. The treatment represented the critical processing to keep the secondary metabolite in stable condition. The highest activity was chosen as the best treatment in terms of drying temperature and drying equipment. The results of the antioxidant activity of corn silk tea samples are shown in Table 1. It can be seen that cabinet dryer provided better antioxidant activity results than oven. The use of cabinets in the making of brewed tea has been reported by several previous studies such as black tea (Perera *et al.*, 2015), green tea (Shomali and Abbasi Souraki, 2019), and Cascara Arabica coffee tea (Nafisah and Widyaningsih, 2018). Cabinet dryer has airflow which prevents damage to the sample's bioactive compound. This renders cabinet an effective tool for making brewed tea. The results in Table 1 show that drying in a cabinet at 65°C for 5 h yielded 82.00 ± 0.75% antioxidant activity of corn silk tea which indicated efficient utilisation of cabinet dryer.

Figure 1. Corn silk tea product.

Table 1. Antioxidant activities of corn silk processed using different dryers and drying temperatures.

Dryer	Temperature (°C)	Antioxidant activity (%)
Oven	45	46.00 ± 0.03^c
	55	40.96 ± 0.27^b
	65	58.23 ± 0.80^d
Cabinet	45	60.09 ± 0.26^e
	55	37.90 ± 0.71^a
	65	82.00 ± 0.75^f

Means followed by similar lowercase superscripts are not significantly different based on Duncan's test at α = 1%

The present work also compared the antioxidant activity of corn silk tea with commercial black tea and commercial green tea. The results are presented in Table 2. The antioxidant activity of corn silk tea made through cabinet drying (65°C, 5 h) had higher activity than commercial black tea and commercial green tea. Corn silk tea had antioxidant activity of 82.00 ± 0.75%, while commercial black tea had activity of 81.71 ± 0.15%, and commercial green tea of 78.37 ± 0.43%. Black tea is made of *Camellia sinensis* L. leaves which turn brown upon oxidation during drying (Jolvis Pou, 2016). Meanwhile, green tea is heated after picking to prevent oxidation (Xu and Chen, 2002). Corn silk's antioxidant activity, which was higher than commercial black tea and commercial green tea, indicated high potential of corn silk tea as a functional beverage.

Table 2. Antioxidant activities of corn silk, black, and green teas.

Tea	Antioxidant activity (%)
Corn silk tea (cabinet-drying, 65°C, 5 h)	82.00 ± 0.75
Commercial black tea	81.71 ± 0.15
Commercial green tea	78.37 ± 0.43

Corn silk phytochemicals

Phytochemical screening of corn silk tea using LC-HRMS was to strengthen its status as a functional beverage. The results can be seen in Table 3. Based on the analysis, it was found that corn silk tea contained betaine, 6-methylquinoline, hesperidin (3',5,7-trihydroxy-4'-methoxyflavanone), luvangetin (10-methoxy-2,2-dimethylpyrano[3,2-g]chromen-8-one), embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone), and eucalyptol (1,8-cineole). Betaine is an amino acid derived from glycine, 6-methylquinoline is an alkaloid, hesperidin is a flavonoid, luvangetine is a chromene, embelin is a benzoquinone, and eucalyptol is a monoterpenoid.

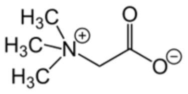
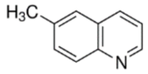
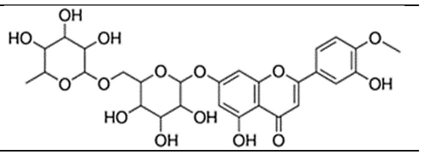
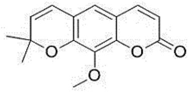
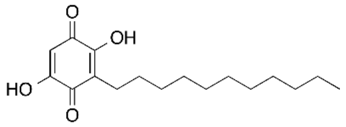
Figure 2 shows the peak of betaine. Betaine had a retention time of 0.928 (*m/z* 117.07876; $C_5H_{11}NO_2$). Betaine has been studied and used as a nutraceutical supplement. Betaine functions to protect internal organs, and decrease vascular risk, and can also prevent various chronic diseases (Craig, 2004). Betaine works with choline (vitamin B_4) to strengthen brain, heart, and liver functions (Ueland, 2011). Betaine is known to increase non-enzymatic antioxidants, S-adenosylmethionine (SAM), and methionine through regulation of the methionine-omocysteine cycle. Betaine can also serve as a methyl donor ($-CH_3$) to prevent the induction of oxidative stress from ROS formation and cell damage (Zhang *et al*., 2016). In addition to corn silk, betaines are also found in shellfish, beets, spinach, citrus fruits, alfalfa sprouts, coffee, peas, and wheat (De Zwart *et al*., 2003).

Figure 3 shows the peak of 6-methylquinoline which had a retention time of 4.751 (*m/z* 143.07315; $C_{10}H_9N$). Its antioxidant activity has been investigated, and is known to have been isolated and synthesised for health purposes as quinoline derivates (Orhan Puskullu *et al*., 2013; Abu-Hashem *et al*., 2017; Chioua and Marco-Contelles, 2021). Besides its antioxidant activity, 6-methylquinoline has several other activities such as antimicrobial, antimalarial, antiviral, antidepressant, anticancer, antihypertensive, and anti-inflammatory (Narwal *et al*., 2017).

Figure 4 shows the peak of hesperidin (3',5,7-trihydroxy-4'-methoxyflavanone) which had a retention time of 11.072 (*m/z* 302.07817; $C_{16}H_{14}O_6$). Hesperidin is a flavanone from flavonoid group usually found in lemon or sweet orange (Wilmsen *et al*., 2005; Kanaze *et al*., 2009; Kamel *et al*., 2014; Tejada *et al*., 2018). Hesperidin has functions such as antioxidant, antiallergenic, antimicrobial, anticarcinogenic, antihypotensive, and vasodilator (Garg *et al*., 2001; Tejada *et al*., 2018). The antioxidant activity of hesperidin has been explored and developed as a cancer chemoprotective agent (Ahmadi and Shadboorestan, 2016) through reduction of oxidative stress (Parhiz *et al*., 2015).

Table 3. Phytochemicals of corn silk tea, their chemical formula, and their chemical structures.

No.	**RT**	***m/z***	**Predicted compound**	**Chemical formula**
1	0.928	117.07876	Betaine (N,N,N-trimethylglycine)	$C_5H_{11}NO_2$
2	4.741	143.07315	6-methylquinoline	$C_{10}H_9N$
3	11.072	302.07817	Hesperidin (3',5,7-trihydroxy-4'-methoxyflavanone)	$C_{16}H_{14}O_6$
4	12.519	258.08837	Luvangetin (10-methoxy-2,2-dimethylpyrano[3,2-g]chromen-8-one)	$C_{15}H_{14}O_4$
5	13.074	294.1822	Embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone)	$C_{17}H_{26}O_4$
6	13.325	136.12482	Eucalyptol (1,8-cineole)	$C_{10}H_{18}O$

		
Betaine	6-Methylquinoline	Hesperidin
		
Luvangetin	Eucalyptol	Embelin

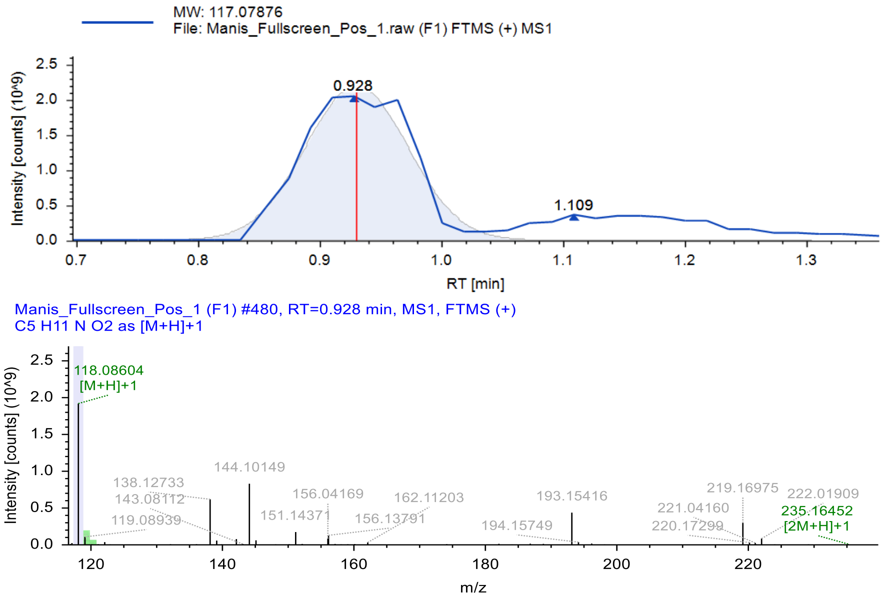


Figure 2. LC-HRMS peak of betaine in corn silk tea.

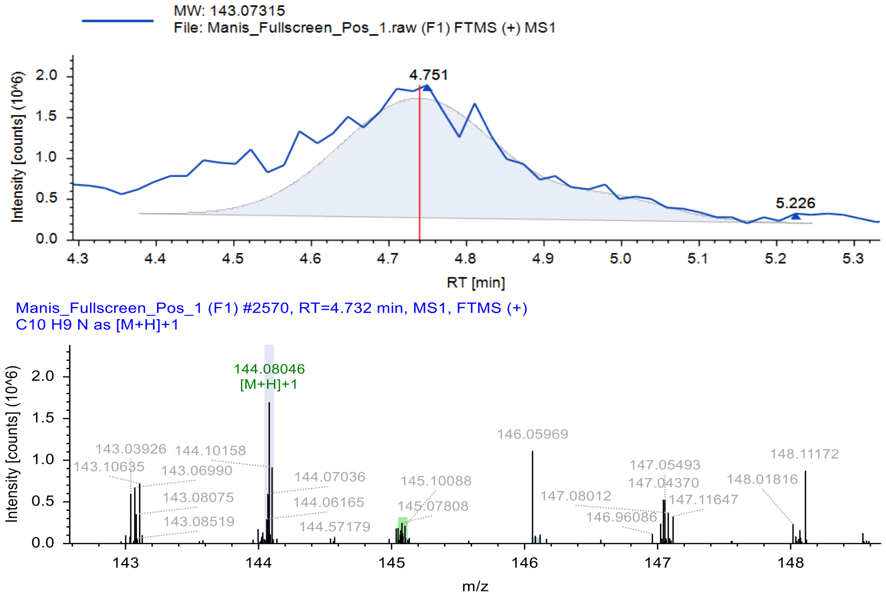


Figure 3. LC-HRMS peak of 6-methylquinoline in corn silk tea.

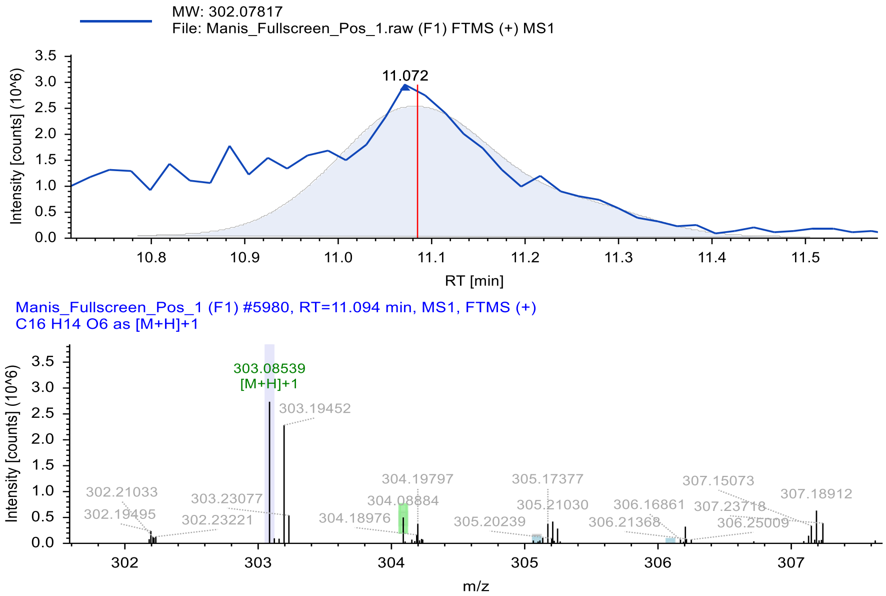


Figure 4. LC-HRMS peak of hesperidin in corn silk tea.

Figure 5 shows the peak of luvangetin which had a retention time of 12.519 (*m/z* 258.08837; $C_{15}H_{14}O_4$). Besides being found in corn silk, luvangetin is also found in mojo or bael fruit *(Aegle marmelo)* (Gupta *et al*., 2011; 2019; Panda *et al*., 2014; Yadav *et al*., 2020). It has several activities such as antioxidant, anti-inflammatory (Gupta *et al*., 2019), antitumor (Cao *et al*., 2013), and as protective agent against COVID-19 (Yadav *et al*., 2020).

Figure 6 shows the peak of embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone) which had a retention time of 13.728 (*m/z* 294.1822; $C_{17}H_{26}O_4$). It is usually found in false black pepper (*Embelia ribes*) (Othman *et al*., 2020). The antioxidant activity of embelin has been investigated and applied to scavenge DPPH radical (Joshi *et al*., 2007; Caruso *et al*., 2020), superoxide radical through the cell membrane (Caruso *et al*., 2020), inhibit hydroxyl radical-induced deoxyribose degradation (Joshi *et al*., 2007), overcome the prevention of lipid peroxidation (Sreepriya and Bali, 2006), hepatic glutathione antioxidant defence (Sreepriya and Bali, 2006), and protect pancreatic β-cells (Sreepriya and Bali, 2006).

Figure 7 shows the peak of eucalyptol which had a retention time of 13.325 (*m/z* 136.12482; $C_{10}H_{18}O$). Eucalyptol was originally found in eucalyptus plants. After the COVID-19 pandemic, interest in eucalyptol and its antioxidant activity rose

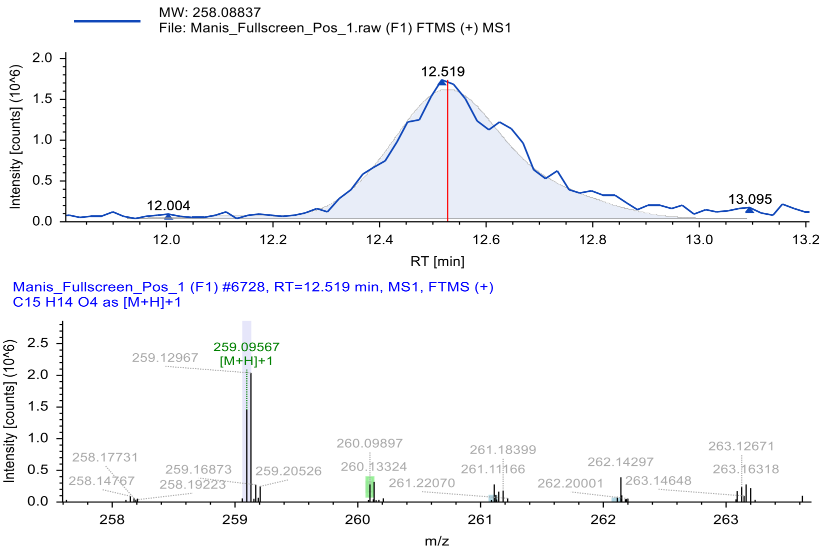


Figure 5. LC-HRMS peak of luvangetin in corn silk tea.

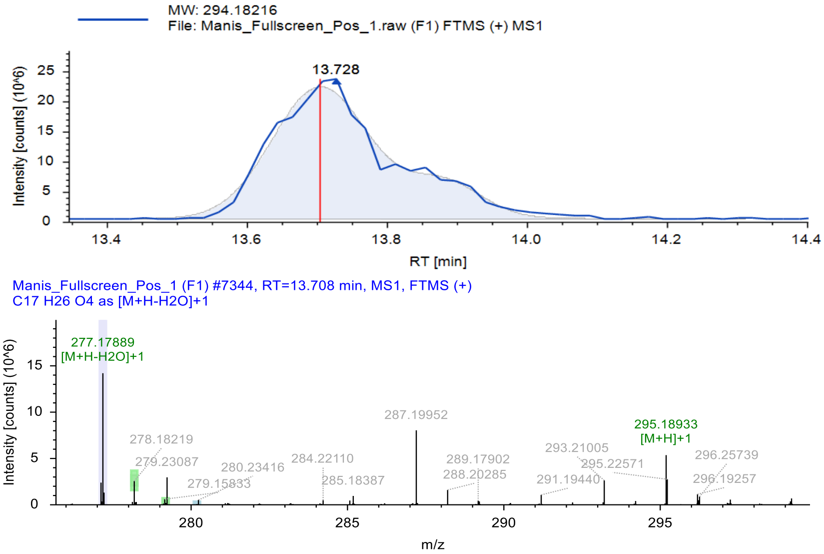


Figure 6. LC-HRMS peak of embelin in corn silk tea.

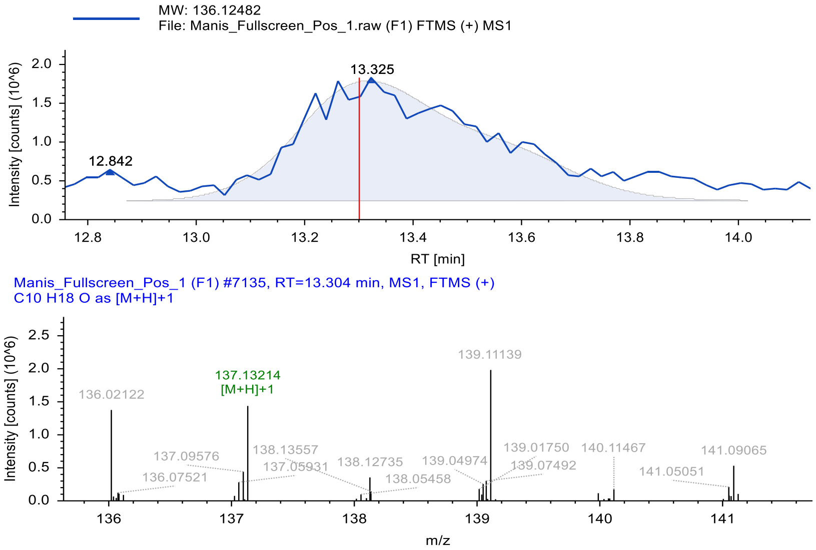


Figure 7. LC-HRMS peak of eucalyptol in corn silk tea.

(Sharma, 2020; Sharma and Inderjeet, 2020; Campos and Berteina-Raboin, 2022). Eucalyptol acts as an antioxidant by reducing oxidative stress by regulating signalling pathways and radical scavenging. In addition to antioxidants, eucalyptol also acts as an anti-inflammatory (Seol and Kim, 2016; Juergens *et al.*, 2018).

Conclusion

Following the COVID-19 pandemic, the development of functional foods and drinks flourished, particularly in the area of foods with high antioxidant activity. Beverage made from corn silk such as brewed tea is such drinks. In producing corn silk tea, the drying equipment, temperature, and length of use are crucial factors. Corn silk tea can yield strong antioxidant activity when prepared through efficient approach. The temperature and drying technique selection, based on antioxidant activity, was the main emphasis of the present work. It is anticipated that micro-, small-, and medium-sized companies (MSMEs) will employ the findings of the present work to produce corn silk tea. The processing of corn silk tea using cabinet drying at 65°C for five hours produced antioxidant activity of 82.00 ± 0.75%. This was higher than commercial black tea (81.71 ± 0.15%) and commercial green tea (78.37 ± 0.43%). The analysis using LC-HRMS showed that the corn silk tea contained betaine (N,N,N-trimethylglycine) (RT 0.928), 6-methyl quinoline (RT 4.741), hesperidin (3',5,7-trihydroxy-4'-methoxyflavanone) (RT 302.07817), luvangetin (10-methoxy-2,2-dimethylpyrano[3,2-g]chromen-8-one) (RT 258.08837), embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone) (RT 13.074), and eucalyptol (1,8-cineole) (RT 13.325).

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