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Effect of brewing conditions on flavonoid, phenolics, anthocyanins and antioxidant contents in hibiscus tea infusion

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ABSTRACT

The hibiscus flowers are cherished for its high aesthetic value and in India it is one of the popular flower cultivated in almost every households due to its religious significance. Besides its hardy nature and wider adaptability in differ agro-climatic conditions. The hibiscus flower has been historically used in Ayurveda and Unani system to cure various ailments. Presently, the hibiscus flowers are being used in various value added products. Therefore, in the present investigation efforts to study variations in secondary metabolites from hibiscus petal tea infusions caused due to drying temperature (50º, 60º and 70ºC), quantity used (1.0, 1.5 and 2.0 g) and dipping duration (90, 120 and 180 sec). The treatment combination of 60ºC drying temperature, 2 g quantity used and 120 s dipping duration obtained as optimized combination of selected independent variables which gave infused tea with high amount of bioactive compounds like total phenols content (75.65 mg GAE/g), total flavonoids content (64.73 mg/g catechin), total anthocyanins content (91.54 mg/l), antioxidant activity (88.38% and 0.37 µmol TE/g) along with superior aroma, taste and good colour retention up to 90 days of storage duration.

Key words: Antioxidants, hibiscus tea, phenols, flavonoids, anthocyanins.

INTRODUCTION

Hibiscus rosa-sinensis, a perennial shrub extensively cultivated in tropical regions around the world. In India, its popular household shrub and various plant parts, including leaves, flowers and roots, have been used due to their medicinal attributes, encompassing aphrodisiac, hemorrhagic and laxative properties (Yashaswini *et al.*, 23). Notably, Hibiscus has been incorporated into diverse herbal blends and beverages. The infusions of Hibiscus petals yield a rich repository of bioactive compounds, encompassing phenolics, flavonoids, antioxidants and anthocyanin pigments (Maganha *et al.*, 12). This infusion has demonstrated the potential to mitigate reactive oxygen species and cholesterol levels (Sanadheera *et al.*, 17). The pivotal role of antioxidants in stabilizing free radicals, thereby preventing cellular damage, underscores their paramount importance in maintaining optimal cellular health and overall well-being. Moreover, Hibiscus and its constituent chemicals have been associated with an array of therapeutic properties, including anti-tumor, antifertility, anti-inflammatory, analgesic, anti-estrogenic, antipyretic, anti-spasmodic, antiviral, antifungal, antibacterial, CNS (Central Nervous System) depressant, hypotensive and juvenoid activity (Jadhav *et al.*, 6). Given these multifaceted benefits, the present study endeavors to establish a standardized protocol for the preparation of Hibiscus petal tea infusions. It also investigates the influence of drying temperature, quantity of petals employed and dipping duration on bioactive compounds over the course of storage duration.

MATERIAL AND METHODS

The experiment was conducted at ICAR-DFR, Pune (Maharashtra, India, N 18° 32' 17.27", E 73° 50' 37.21"). The average annual rainfall in this area is 650-750 mm and is normally distributed during June to October. The maximum temperature ranges between 34º to 40ºC in summer but on the onset of monsoon, it drops down to 27ºC. The minimum temperature ranges from 12º to 24ºC prevails in the winter season from November to the middle of February. The average maximum and minimum temperature recorded during the period of experiment was 39º and 23ºC, respectively. The relative humidity during the lab experiment ranged between 55 to 97 per cent. Fully opened hibiscus flowers were harvested from the hibiscus shrubs garden located in the campus of College of Agriculture, Pune (Maharashtra, India, N 18º 32' 17.27", E 73º50' 37.21"). The freshly harvested flowers were cleaned, petals were separated from other floral parts and collected in cleaned trays, allowed for further drying in hot air oven at selected experimental conditions (Fig. 1).

Petals were dried in hot-air oven till the moisture reaches at its equilibrium moisture content (EMC).

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Fig. 1. Fresh hibiscus petals separated from flowers.

The initial moisture content of the hibiscus petals was 86 %; while after drying it reaches up to 2 with 12% of remained dry matter. The samples were stabilized for constant weight for three days and then utilized for tea preparation and further analysis.

The experimental plan with treatment details is presented below:

Factor			
Factor A (Temperature for drying of petals in ºC for 24 h)	a_1=50 ºC	a_2= 60 ºC	a_3= 70 ºC
Factor B (Quantity required for tea preparation in g)	b_1= 1.0 g	b_2= 1.5 g	b_3= 2.0 g
Factor C (Duration in s)	c_1=90 sec	c_2=120 sec	c_3= 180 sec

Total phenolic content of hibiscus petal tea was determined using folin-ciocalteau method (Singleton *et al.*, 19). Total phenol content was calculated using formula and expressed as milligram of gallic acid equivalent per gram of fresh weight (mg GAE g^{-1}).

$$\text{Total phenolics content} = \frac{\text{Conc.} \times \text{Vol. of sample solution} \times \text{Dilution factor of the sample solution}}{\text{Sample weight}}$$

The aluminium chloride method was used for estimation of flavonoids (Zhishen *et al.*, 24). Quantification was based on the standard curve of catechin. Total flavonoid content (mg/g catechin) was calculated using formula

$$\text{Total flavonoids content} = \frac{\text{Conc.} \times \text{Vol. of sample solution} \times \text{Dilution factor of the sample solution}}{\text{Sample weight}}$$

The pH-differential method using two buffer systems- potassium chloride buffer (pH1.0) and sodium acetate buffer (pH 4.5) was used for determination of total anthocyanins content (Wrolstad *et al.*, 21) in hibiscus petal tea.

$A = (A_{520nm} - A_{700nm})\ pH\ 1.0 - (A_{520nm} - A_{700nm})\ pH\ 4.5$

Total anthocyanins (mg/L) = A x MW x DF x 10^3/ε x L

MW: Molecular weight of predominant anthocyanin, ε: molar extinction coefficient, DF: Dilution factor, L: path length of cuvette, A: Absorbance

The DPPH radical scavenging assay method was used to determine antioxidant capacity (Brand-Williams *et al.*, 2).

$$\text{Inhibition \%} = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100$$

Where, $A_{control}$=Absorbance of DPPH, A_{sample}= Absorbance of sample

The antioxidant capacity was determined by Trolox equivalent antioxidant capacity (TEAC) method (Re *et al.*, 15).

$$\text{Inhibition \%} = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100$$

where, $A_{control}$=Absorbance of ABTS, A_{sample}= Absorbance of sample

$$\text{Trolox (µmol TE/g)} = \frac{\text{Trolox (µmol/L)}}{\text{Sample (g/L)}}$$

Acceptance tests were conducted on seven sample infusions using ten panelists by using nine-point Hedonic Scale (nine-point hedonic scale ranging from 1 = Dislike extremely and 9 = Like extremely). Sample infusions were three-digit coded and served randomly to the panelists to avoid the biasness. About 30 ml of each infusion was served in transparent glass. One sample was served at a time. The nine point hedonic scale was used for organoleptic analysis of colour, taste, aroma and overall acceptability of hibiscus petal tea. The data was recorded and analyzed by the technique of analysis of variance and the test of significance was carried out with statistical model of factorial completely randomized design (Panse and Sukhatme, 13). Stastical analysis was done using OPSTAT software.

RESULTS AND DISCUSSION

The significant effect of drying temperature, petal quantity used and dipping duration were found on bioactive compounds and organoleptic observations of hibiscus petal tea with respect to the storage. The experimental data recorded during the investigation is presented in table 1.

The total phenol content (TPC) pf hibiscus petal tea was examined over a span of 0, 45, and 90 days of storage, and the finding revealed variations based on the geographical origin of the parent plant. The TPC of hibiscus tea on 0, 45 and 90 days of storage ranged from 64.04-112.20 mg GAE/g (0 day), 61.17-95.94 mg GAE/g (45 days), 44.37-92.03 mg GAE/g (90 days). The increase in the total phenol content was likely influenced by the optimum temperature,

Table 1. Effect of drying temperature, petal quantity used and dipping duration on TPC, TFC, total anthocyanins and antioxidant activity of hibiscus petal tea infusions.

Treatment	TPC (mg GAE/g)			TFC (mg/g catechin)			Total anthocyanins (mg/l)			Antioxidant activity (DPPH)			Antioxidant activity (ABTS)		
	0 day	45 days	90 days	0 day	45 days	90 days	0 day	45 days	90 days	0 day	45 days	90 days	0 day	45 days	90 days
a_1 (50ºC)	78.47^{a}	60.77^{a}	50.59^{a}	65.38^{a}	63.34^{a}	57.99^{a}	91.52^{a}	80.28^{a}	53.33^{a}	86.60^{a}	84.03^{a}	82.50^{a}	0.39^{a}	0.32^{a}	0.32^{a}
a_2 (60ºC)	88.41^{b}	81.07^{b}	73.42^{b}	71.45^{b}	70.26^{b}	60.31^{b}	92.48^{a}	80.62^{a}	69.59^{b}	89.1^{b}	86.35^{b}	85.23^{b}	0.39^{a}	0.32^{a}	0.32^{a}
a_3 (70ºC)	94.45^{c}	82.64^{b}	73.76^{b}	76.36^{c}	73.17^{c}	63.74^{c}	97.80^{b}	90.88^{b}	69.69^{b}	89.36^{b}	87.29^{c}	85.76^{b}	0.41^{b}	0.33^{b}	0.32^{a}
SE (m) ±	0.54	0.67	0.59	0.52	0.64	0.07	0.31	0.17	0.23	0.36	0.18	0.29	0.002	0.001	0.000
CD (at 1%)	2.01	2.56	2.26	2.01	2.45	0.26	1.19	0.66	0.89	1.39	0.70	1.11	0.008	0.002	0.002
b_1 (1.0 g)	84.94^{a}	75.30^{a}	65.33^{a}	67.30^{a}	63.59^{a}	54.35^{a}	62.04^{a}	54.70^{a}	45.33^{a}	87.54^{a}	84.32^{a}	81.42^{a}	0.28^{a}	0.22^{a}	0.22^{a}
b_2 (1.5 g)	86.53^{a}	71.04^{b}	64.53^{a}	71.49^{b}	70.17^{b}	63.22^{b}	102.48^{b}	96.41^{b}	66.43^{b}	88.59^{a}	86.08^{b}	85.82^{b}	0.37^{b}	0.30^{b}	0.30^{b}
b_3 (2.0g)	89.87^{b}	78.14^{c}	67.91^{b}	74.40^{c}	73.01^{c}	64.47^{c}	117.28^{c}	100.65^{c}	80.87^{c}	88.93^{a}	87.26^{c}	86.26^{c}	0.53^{c}	0.45^{c}	0.45^{c}
SE (m) ±	0.54	0.67	0.59	0.52	0.64	0.07	0.31	0.17	0.23	0.36	0.18	0.29	0.002	0.001	0.000
CD (at 1%)	2.01	2.56	2.26	2.01	2.45	0.26	1.19	0.66	0.89	1.39	0.70	1.11	0.008	0.002	0.002
c_1 (90 Sec.)	84.27^{a}	68.41^{a}	57.42^{a}	70.11^{a}	67.75^{a}	59.89^{a}	86.30^{a}	79.17^{a}	59.19^{a}	87.12^{a}	84.68^{a}	81.98^{a}	0.38^{a}	0.32^{a}	0.33^{a}
c_2 (120 Sec.)	87.88^{b}	74.14^{b}	65.58^{b}	70.58^{ab}	68.93^{a}	60.89^{b}	95.40^{b}	86.26^{b}	60.84^{b}	89.09^{b}	86.94^{c}	86.66^{c}	0.37^{a}	0.32^{a}	0.32^{a}
c_3 (180 Sec.)	89.19^{c}	81.93^{c}	74.78^{c}	72.50^{b}	70.08^{a}	61.26^{c}	100.11^{c}	86.34^{b}	72.59^{c}	88.85^{b}	86.04^{b}	84.85^{b}	0.43^{b}	0.33^{b}	0.33^{b}
SE (m) ±	0.54	0.67	0.59	0.52	0.64	0.07	0.31	0.17	0.23	0.36	0.18	0.29	0.002	0.001	0.000
CD (at 1%)	2.01	2.56	2.26	2.01	2.45	0.26	1.19	0.66	0.89	1.39	0.70	1.11	0.008	0.002	0.002

Note: Treatment having common super script are statistically non-significant otherwise significant.

which enhanced cell wall permeability and exposed the cellular matrix of petals in solvent, particularly evident at 70°C (94.45 mg GAE/g) on day 0 as reported in rose (Selvi *et al*., 18). Similar results were observed at 60°C on days 45 and 90, possibly due to the breakdown of the cellular matrix and the release of more phenolics compounds at higher drying temperature, a phenomenon observed in *Thymus vulgaris* (Vergara-Salinas *et al*., 20) and in Rose (Baibuch *et al*., 01). The quantity of petals used also had a significant impact, with 2.0 g yielding the highest TPC at all storage durations (89.87 mg GAE/g on day 0, 78.14 mg GAE/g on day 45 and 67.91 mg GAE/g on day 90). This indicated a direct relationship between the amount of petals used in tea preparation and TPC, as reported by Vergara-Salinas *et al*. (24) in Thymus. Additionally, the duration of dipping petals in the solvent played a role in TPC, with a 180 s dip showing superior results over 90 and 120 s. This could be attributed to the stimulant properties of tea, which are most potent steeped for two to five minutes (Safdar *et al*., 16). Overall, these results highlight the intricate interplay of temperature, quantity, and dipping duration in influencing TPC in hibiscus petal tea, consistent with established principles of phenolic compound solubility and bioactivity.

Total flavonoids content (TFC) holds pivotal significance due to its functional attributes, encompassing the regulation of immune functions, inhibition of cancer cell proliferation, and reduction of blood triglycerides (Koley *et al*., 9). Pertinently, the drying temperature applied during tea processing exhibited a pronounced influence on TFC across various storage periods. The apex of TFC, quantified in milligrams per gram of catechin, was attained at 70ºC, significantly surpassing the outcomes achieved at 60º and 50ºC. Specifically, TFC measured at 76.36 mg/g catechin (day 0), 73.17 mg/g catechin (day 45), 63.74 mg/g catechin (day 90) testified to this temperature-dependent phenomenon. Notably, an optimal drying temperature of 68ºC was discerned as instrumental in maximizing flavonoids content in rose tea (Pashazadeh *et al*., 14). Furthermore, the quantity of raw material employed, specifically 2 g yielded the most substantial TFC outcomes registering values of 74.40 mg/g catechin (day 0), 73.01 mg/g catechin (day 45), 64.47 mg/g catechin (day 90). This magnitude of TFC was statistically superior to quantities of 1.5 and 1 g over the respective storage duration, thus validating a direct correlation between quantity utilized and the augmentation of flavonoid content, as corroborated in roselle tea (Joseph and Adogbo, 7). Concurrently, the duration of immersion in the solvent played a pivotal role in TFC, with an immersion period of 180 seconds exhibiting superiority over 120 and 90 seconds specifically on day 90 of storage, while displaying parity with 120 second immersion on days 0 and 45. Consequently, it manifested the highest

TFC values of 72.50 mg/g catechin (day 0), 70.08 mg/g catechin (day 45), 61.26 mg/g catechin (day 90) respectively. This observation corresponds with the phenomenon observed in green tea, where increased TFC correlated with extended steeping durations (Pashazadeh *et al.*, 14).

The intricate interplay of drying temperature, quantity used and dipping duration yielded multifaceted results. Notably, at zero (80.27 mg/g catechin) and 45 (76.59 mg/g catechin) days, the interactive effect of these variables was not statistically significant. The zenith of flavonoid content was achieved through the interaction of 70ºC drying temperature, 2.0 g quantity used and 180 seconds dipping duration, while the nadir was witnessed in the interaction involving 50ºC drying temperature, 1.0 g quantity and 90 s dipping duration registering 56.57 mg/g catechin (day 0) and 55.20 mg/g catechin (day 45). However, at day 90, a significant impact was discerned regarding the interaction between drying temperature, quantity employed and dipping duration. This is evident in Table 2, where the

Table 2. Interaction effect of temperature, petal quantity used and dipping duration on TPC, TFC and total anthocyanins of hibiscus petal tea infusions.

Int. A × B × C (Temp. × Qty. × Duration)	TPC (mg GAE/g)			TFC (mg/g catechin)			Total anthocyanins (mg/l)		
	Mean			Mean			Mean		
	Zero days	45 days	90 days	Zero days	45 days	90 days	Zero days	45 days	90 days
$a_1b_1c_1$	64.04^{a}	61.17^{bc}	44.37^{b}	56.57	55.20	50.18^{a}	42.40^{a}	37.58^{b}	23.75^{a}
$a_1b_1c_2$	75.23^{b}	55.49^{b}	36.18^{a}	58.79	57.38	53.23^{d}	47.91^{b}	40.33^{c}	29.59^{b}
$a_1b_1c_3$	87.33^{fghijk}	60.32^{bc}	45.59^{b}	61.25	59.45	52.28^{bc}	62.01^{d}	48.25^{d}	46.87^{d}
$a_1b_2c_1$	84.41^{defghi}	47.71^{a}	47.30^{bc}	64.97	63.18	59.79^{h}	96.70^{h}	96.22^{jk}	53.62^{e}
$a_1b_2c_2$	62.29^{a}	48.64^{a}	47.30^{bc}	65.53	63.33	60.90^{i}	105.37^{j}	96.43^{jk}	62.08^{f}
$a_1b_2c_3$	77.33^{bc}	65.14^{cd}	64.41^{efgh}	66.04	65.10	60.78^{i}	107.64^{jk}	94.71^{j}	60.02^{f}
$a_1b_3c_1$	78.71^{bcd}	60.75^{bc}	43.94^{b}	71.20	68.06	61.40^{ij}	116.32^{mn}	101.31^{no}	66.28^{gh}
$a_1b_3c_2$	85.95^{efghij}	70.95^{def}	58.64^{de}	71.82	68.81	61.84^{jk}	119.42^{n}	112.26^{p}	64.84^{g}
$a_1b_3c_3$	90.94^{jkl}	76.76^{fghi}	67.59^{ghij}	72.21	69.58	61.52^{ij}	125.88^{o}	95.40^{j}	72.96^{jk}
$a_2b_1c_1$	83.17^{cdefg}	75.54^{fgh}	53.04^{cd}	62.00	61.51	51.80^{b}	47.22^{b}	28.29^{a}	25.95^{a}
$a_2b_1c_2$	95.70^{lmno}	84.09^{ij}	82.86^{n}	65.89	62.10	53.04^{cd}	58.23^{c}	48.04^{d}	37.85^{c}
$a_2b_1c_3$	97.53^{mno}	95.94^{k}	92.03^{o}	70.85	65.99	54.23^{e}	61.67^{cd}	57.54^{e}	51.07^{e}
$a_2b_2c_1$	100.79^{op}	67.06^{cde}	66.33^{fghi}	73.48	73.41	62.47^{kl}	92.09^{g}	91.34^{i}	73.44^{jk}
$a_2b_2c_2$	76.02^{b}	73.01^{efg}	61.11^{efg}	73.30	73.26	63.03^{l}	101.59^{i}	98.70^{l}	75.58^{k}
$a_2b_2c_3$	81.07^{bcdef}	80.91^{hi}	80.87^{mn}	74.30	73.53	63.90^{m}	105.45^{j}	99.60^{lmn}	86.72^{l}
$a_2b_3c_1$	80.18^{bcde}	77.40^{fghi}	64.65^{efgh}	74.95	74.13	64.28^{mn}	114.80^{lm}	98.70^{l}	89.40^{lm}
$a_2b_3c_2$	85.71^{efghij}	84.45^{ij}	75.65^{klm}	75.76	74.32	64.73^{no}	126.10^{o}	101.47^{o}	91.54^{m}
$a_2b_3c_3$	95.52^{lmno}	91.27^{jk}	84.21^{n}	72.55	74.09	65.27^{op}	125.20^{o}	101.87^{o}	94.78^{n}
$a_3b_1c_1$	83.48^{cdefgh}	75.53^{fgh}	71.56^{ijk}	74.19	69.05	56.61^{f}	72.20^{e}	69.17^{f}	60.36^{f}
$a_3b_1c_2$	88.37^{ghijk}	79.20^{ghi}	78.83^{lmn}	76.57	70.32	58.59^{g}	83.63^{f}	80.32^{g}	65.59^{g}
$a_3b_1c_3$	89.59^{hijkl}	90.38^{jk}	83.48^{n}	79.58	71.31	59.15^{gh}	83.08^{f}	82.80^{h}	66.90^{gh}
$a_3b_2c_1$	112.20^{q}	72.76^{defg}	60.66^{ef}	75.21	69.46	65.96^{pq}	97.25^{h}	95.05^{j}	68.76^{hi}
$a_3b_2c_2$	91.26^{jklm}	91.10^{jk}	78.83^{lmn}	75.15	75.10	65.70^{pq}	104.96^{ij}	97.94^{kl}	46.39^{d}
$a_3b_2c_3$	93.38^{klmn}	93.05^{k}	73.98^{jkl}	75.47	75.12	66.44^{qr}	111.22^{kl}	97.74^{kl}	71.24^{ij}
$a_3b_3c_1$	103.92^{p}	77.79^{fghi}	64.90^{efghi}	78.38	75.77	66.48^{qr}	97.67^{h}	94.85^{j}	71.17^{ij}
$a_3b_3c_2$	97.89^{nop}	80.30^{ghi}	70.77^{hijk}	72.43	75.77	66.93^{r}	111.30^{l}	100.83^{mno}	74.06^{k}
$a_3b_3c_3$	89.99^{ijkl}	83.60^{ij}	80.85^{mn}	80.27	76.59	67.75^{s}	118.87^{n}	99.18^{lm}	102.76^{o}
SE (m) ±	1.63	2.00	1.76	1.57	1.91	0.21	0.93	0.52	0.70
CD at 1%	6.27	7.69	6.78	NS	NS	0.79	3.58	1.99	2.68

treatment combining of 70ºC drying temperature, 2 g quantity used and 180 s dipping duration yielded the highest flavonoid content at 67.75 mg/g catechin, significantly outperforming all other treatments. Conversely, the treatment comprising 50ºC drying temperature, 1.0 g quantity used and 90 s dipping duration exhibited the lowest flavonoid content at 50.18 mg/g catechin. This pattern mirrors findings in edible flower tea (Yang and Lee, 22).

The drying temperature significantly impacted the total anthocyanin content in hibiscus petal tea at different storage intervals. At day 0 and 45, 70°C drying temperature yielded the highest anthocyanin content, while at day 90, it equaled the performance of 60°C. Consistent with the findings in Kenyan tea, this temperature effect is noted for optimal secondary metabolite extraction (Kilel *et al.*, 8). More over the quantity of petals used exhibited a direct relationship with anthocyanin content, with 2 g leading to the highest levels. Longer dipping durations, particularly 180 s, consistently resulted in more anthocyanins. However, anthocyanin levels decreased with prolong brewing, potentially due to degradation (Fig. 2).

At zero days storage the treatment combinations favoring 60ºC drying temperature, 2.0 g quantity used and 120 s dipping duration were optimal at day 0, whereas (Table 2) treatment combination of 50°C drying temperature, 2.0 g quantity used and 180 s dipping duration excelled at day 45. At day 90, treatment combination of 70ºC drying temperature, 2.0 g quantity used and dipping duration of 180 s led while treatment combination of 50ºC drying temperature, 1.0 g quantity used and dipping duration of 90 s lagged in anthocyanin content. In low temperature the anthocynins are thermostable therefore at 50ºC temperature anthocyanin content found was less, the results are aligning with similar findings in Kenyan tea, blended tea, hibiscus and green tea (Kilel *et al.*, 8; Yang and Lee, 22).

Fig. 2. Storage of tea bags.

The radical scavenging activity is used to evaluate the capacity of tissue to scavenge free radicals, which mainly functions of bioactive compounds present in the tissue. The drying temperature of 70ºC recorded highest antioxidant activity during storage (89.36, 87.29, 85.76%) this can be attributed to increase in total phenols content (Hajiaghaalipour *et al.*, 4). High antioxidant activity was recorded during storage (88.93%) for 2.0 g of petal quantity used. Similar findings were reported in roselle tea (Joseph and Adogbo, 7). The antioxidant activity of hibiscus tea with dipping duration of 120 s (89.09%) equaled with 180 s at zero day storage, whereas it was significantly superior over the rest of treatments at 45 (86.94%) and 90 (86.66%) days of storage. This might be due to the optimum time exposure at 120 s.

The total antioxidant activity (DPPH) ranged from 69.90 - 90.53% during storage. According to the findings, there is high capacity to neutralize radicals when sample was dried at 60ºC (Hihat *et al.,* 5) reported thus the treatment combination of 60ºC drying temperature, 1.5 g quantity used, 2.0 g quantity used and 90 s of dipping duration recorded high antioxidant activity (90.53 and 89.44%) at zero and 45 days of storage. Similarly, for 90 days storage the treatment combination of 50ºC drying temperature, 1.5 g quantity used and 120 s of dipping duration was superior over other treatments and maintained high antioxidant activity (89.03%) which can be attributed to synergistic effect of drying temperature, quantity used and dipping duration.

Among various methods used for analyzing antioxidant activity, the most popular method is ABTS assay. Different drying temperatures showed variations in total antioxidant activity of tea during storage (Table 1). Drying temperature of 70ºC was found significantly superior at zero and 45 days of storage with maximum antioxidant activity (0.41 µmol TE/g, 0.33 µmol TE/g) respectively as increase in extraction of polyphenols at high temperature, led to increase in antioxidant activity. The 2.0 g quantity used recorded maximum antioxidant activity (0.53 µmol TE/g) and it was significantly superior over 1.5 g and 1.0 g during storage up to 90 days. Similar trend was observed in roselle tea (Joseph and Adogbo, 7). The higher brewing temperature and longer brewing time increase the antioxidant activity of green tea infusions as solubility of phenolic compounds increases with an increase in time thus 180 s of dipping duration recorded maximum antioxidant activity (0.43 µmol TE/g) and was significantly superior over 120 and 90 s during storage.

The positive correlation between increased extraction time and temperature on antioxidant activity was reported (Kosińska and Andlauer, 10). The

results in Table 3 indicate that treatment combination of 70ºC drying temperature, 2.0 g quantity used and 180 s dipping duration was superior over other interactions and recorded maximum antioxidant activity (0.46 µmol TE/g). The interaction effect of drying temperature, quantity used and dipping duration on total antioxidant activity (ABTS) of tea at 45 and 90 days was found significant. The treatment combination of 50º, 60º, 70ºC drying temperature, 2.0 g quantity used and 180, 120, 90 s dipping duration (0.37 and 0.36 µmol TE/g) were statistically at par with each other. It was found that large number of the polyphenols were released within 5 min of immersion of tea bags with respect to chamomile tea which contributed to high antioxidant activity (Kosińska and Andlauer, 10).

The colour scores were found in decreasing trend with respect to the increase in storage time (Table 4). The highest sensory score for colour (8.2) of tea infusions was recorded by panel to the treatment combination of 70ºC drying temperature, 2.0 g quantity used and 180 s dipping duration.

Table 3. Interaction effect of temperature, petal quantity used and dipping duration on antioxidant activity (using DPPH and ABTS) of hibiscus petal tea infusions.

Int. A × B × C (Temp. × Qty. × Duration)	Antioxidant activity (DPPH)			Antioxidant activity (ABTS)		
	Mean			Mean		
	Zero days	45 days	90 days	Zero days	45 days	90 days
$a_1b_1c_1$	79.75^{a}	78.39^{a}	69.90^{a}	0.35^{a}	0.29^{a}	0.29^{a}
$a_1b_1c_2$	85.74^{ab}	80.40^{ab}	80.17^{c}	0.35^{a}	0.29^{a}	0.29^{a}
$a_1b_1c_3$	87.94^{c}	84.90^{de}	82.05^{cd}	0.37^{a}	0.29^{a}	0.29^{a}
$a_1b_2c_1$	82.32^{ab}	81.99^{bc}	80.22^{c}	0.38^{a}	0.31^{b}	0.29^{a}
$a_1b_2c_2$	89.37^{c}	85.79^{efgh}	89.03^{j}	0.38^{a}	0.31^{b}	0.29^{a}
$a_1b_2c_3$	87.94^{c}	85.24^{ef}	84.90^{defg}	0.40^{b}	0.32^{b}	0.29^{a}
$a_1b_3c_1$	89.82^{c}	84.79^{de}	84.90^{defg}	0.43^{c}	0.36^{c}	0.37^{c}
$a_1b_3c_2$	88.45^{c}	87.58^{ghijk}	84.62^{defg}	0.43^{c}	0.36^{c}	0.36^{c}
$a_1b_3c_3$	88.09^{c}	87.18^{fghij}	86.74^{fghij}	0.45^{d}	0.37^{c}	0.37^{c}
$a_2b_1c_1$	87.02^{c}	84.79^{de}	75.12^{b}	0.35^{a}	0.29^{a}	0.29^{a}
$a_2b_1c_2$	88.88^{c}	87.58^{ghijk}	87.08^{fghij}	0.35^{a}	0.29^{a}	0.29^{a}
$a_2b_1c_3$	89.77^{c}	86.54^{efghi}	84.09^{def}	0.37^{a}	0.29^{a}	0.29^{a}
$a_2b_2c_1$	90.53^{d}	86.79^{efghij}	86.59^{fghij}	0.38^{a}	0.31^{b}	0.32^{c}
$a_2b_2c_2$	90.23^{d}	88.89^{jk}	88.65^{ij}	0.38^{ab}	0.31^{b}	0.31^{b}
$a_2b_2c_3$	89.00^{c}	86.35^{efgh}	84.90^{defg}	0.40^{bc}	0.32^{b}	0.32^{b}
$a_2b_3c_1$	89.47^{c}	89.44^{k}	86.95^{fghij}	0.43^{c}	0.36^{c}	0.37^{c}
$a_2b_3c_2$	90.26^{d}	88.61^{ijk}	88.38^{hij}	0.43^{cd}	0.36^{c}	0.37^{c}
$a_2b_3c_3$	89.07^{c}	86.61^{efghi}	85.31^{defgh}	0.45^{d}	0.37^{c}	0.37^{c}
$a_3b_1c_1$	88.42^{c}	82.79^{cd}	82.38^{cde}	0.36^{a}	0.29^{a}	0.29^{a}
$a_3b_1c_2$	89.84^{cd}	87.42^{ghijk}	86.91^{fghij}	0.36^{a}	0.29^{a}	0.29^{a}
$a_3b_1c_3$	90.50^{d}	86.12^{efgh}	85.07^{defgh}	0.37^{a}	0.29^{a}	0.29^{a}
$a_3b_2c_1$	89.47^{c}	85.99^{efgh}	85.37^{defghi}	0.39^{b}	0.32^{b}	0.32^{b}
$a_3b_2c_2$	89.70^{c}	87.70^{hijk}	87.18^{fghij}	0.39^{b}	0.32^{b}	0.31^{b}
$a_3b_2c_3$	88.75^{c}	85.96^{efgh}	85.51^{efghi}	0.40^{b}	0.32^{b}	0.32^{b}
$a_3b_3c_1$	87.30^{c}	87.15^{fghij}	86.41^{fghij}	0.44^{d}	0.37^{c}	0.37^{c}
$a_3b_3c_2$	89.31^{c}	88.49^{ijk}	87.93^{ghij}	0.44^{d}	0.37^{c}	0.36^{c}
$a_3b_3c_3$	88.60^{c}	85.50^{efg}	85.07^{defgh}	0.46^{d}	0.37^{c}	0.37^{c}
SE (m) ±	1.09	0.55	0.70	0.007	0.001	0.001
CD at 1%	4.18	2.11	2.68	0.027	0.008	0.005

Table 4. Organoleptic analysis of tea infusions for colour, taste, aroma and overall acceptability.

Treatment	Colour			Taste			Aroma			Overall acceptability		
	0 days	45 days	90 days	0 days	45 days	90 days	0 days	45 days	90 days	0 days	45 days	90 days
T_5 ($a_1b_2c_2$)	7	6.7	6.5	7	6.8	6.6	6.5	6.2	6	6.9	6.8	6.5
T_8($a_1b_3c_2$)	7.6	7.1	7	6.9	6.3	6.1	7.1	6.9	6.9	6.6	6.3	6.0
T_{16}($a_2b_3c_1$)	7.5	7.2	7.1	6.7	6.1	6	7.1	6.8	6.8	6.7	6.1	6.0
T_{17}($a_2b_3c_1$)	7.4	7.2	7.1	8	7.8	7.8	7.4	7	7	7.6	7.4	7.0
T_{12}($a_2b_1c_3$)	7.9	7.4	7.2	6.6	6.1	6	7.2	7.1	7	6.7	6.5	6.3
T_{22}($a_3b_2c_1$)	7	6.8	6.8	6.7	6.3	6.1	7.2	7	7	6.2	6.1	6.0
T_{27}($a_3b_3c_3$)	8.2	8	8	6.4	6.2	6.1	7.2	7	7	6.5	6.3	6.1

The color intensity was strongly correlated with the infusing period because samples infused at a lower temperature but for a longer period showed a higher colour in both roselle and green tea (De-Heer, 3; Lee *et al.*, 11).

The taste of hibiscus petal tea infusions during storage showed the decreasing trend from 0 to 90 of days storage period (Table 4). The treatment combination of 60ºC drying temperature, 2.0 g quantity used and 120 s dipping duration (T_{17}) recorded highest sensory score for taste of tea infusions (8). The green tea samples dried at higher temperatures led to dehydration of leaves producing strong bitter taste (Lee *et al.*, 11). The treatment combination of 60ºC drying temperature, 2.0 g quantity used and 120 s dipping duration recorded highest sensory score for aroma of tea infusions (7.4). The optimum temperature might have led to release of more aroma in the infusion. Aroma of tea infusions declined with increase in storage duration from 0 to 90 days storage. The drying temperature, quantity used and dipping duration produced a synergistic effect thus resulting in high aroma in herb tea from *Moringa oleifera*, *Hibiscus sabdariffa* and *Cymbopogon citratus* (De-Heer, 3). The overall acceptability of tea infusions was significantly influenced by colour, taste and aroma. The organoleptic analysis of tea infusions for overall acceptability recorded the highest score to the treatment combination (T_{17}) of 60ºC drying temperature, 2 g quantity and 120 s dipping duration which also contain high total amount of phenols content (75.65 mg GAE/g), flavonoids content (64.73 mg/g catechin), total anthocyanins (101.47 mg/l) and found superior over rest of the treatment.

The hibiscus petal tea infusion conditions affects the biochemical and organoleptic properties of tea, thus optimum conditions are very much essential for ideal tea infusion, therefore the treatment combination of 60ºC drying temperature, 2.0 g quantity used and 120 s of dipping duration were found to be most ideal for better taste, flavour, and colour, with more stability of total phenols, total flavonoids and anthocyanins.

AUTHORS' CONTRIBUTION

Conceptualization of research (GBK, SMJ); Designing of the experiments (SMJ, GBK); Contribution of experimental materials (SMJ, GBK); Execution of field/lab experiments and data collection (SGM, NAG); Analysis of data and interpretation (AAB, MBS); Preparation of the manuscript (SMJ, GBK SGM).

DECLARATION

The authors declare that they do not have any conflict of interest.

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