






Effects of spray and freeze-drying methods on aroma compounds, sensory characteristics, physicochemical composition, antioxidant and antimicrobial properties of instant sage (*Salvia rosifolia* Sm.) tea

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Abstract

Sage is used as a flavoring and spice in foods around the world. Besides its strong and bitter taste, sage has been traditionally used as an effective solution to many health problems for centuries. It is a rich plant with many bioactive compounds. In this study, instant tea production was performed from the dried samples of the sage plant (*Salvia rosifolia* Sm.) using two drying methods: freeze-drying and spray-drying. The sensorial, physicochemical, antioxidant, and antimicrobial properties of the products obtained by both methods were analyzed. Color values, solubility in water, moisture analysis (21.3% spray-dried; 4.0% freeze-dried), free radical scavenging activity (DPPH), Trolox Equivalent Antioxidant Capacity (TEAC), Ferric reducing/antioxidant power assay (FRAP), aroma analysis, protein analysis (5.5% spray-dried; 5.7% freeze-dried), ash, mineral, antimicrobial, and sensory analyses were performed. Significant differences were found between the two soluble teas obtained by the spray-drying and the freeze-drying methods. In general, the antioxidant capacities are higher in freeze-dried samples. All the 61 aroma components were detected in freeze-dried samples while only 18 of them were detected in spray-dried samples. In addition to their differences, plenty of bioactive components, easy to use, ready-to-drink herbal tea have been produced with both techniques.

Keywords: Freeze-drying, instant tea, PCA, sage, spray-drying

1. Introduction

The sage plant (*Salvia* spp) is a member of the Lamiaceae family also known by the names 'Leadwort' and 'Common sage'. It is rich in bioactive compounds such as thujone, cineol, borneol, pinene, saponin, tannin, fumaric acid, and flavone. Scientific studies reveal that it has antioxidant, antibacterial, anti-diabetic and anti-tumor properties and it has a positive effect on memory and Alzheimer's disease [1,2]. Sage is one of the most important medicinal and aromatic plants used for a long time in the world. In Türkiye *Salvia* species are usually known as Adaçayı and consumed as tea [3]. Sage has been used for the treatment of some diseases such as epilepsy, colds, hemorrhoids, pains, tuberculosis, and menstuous disorderliness since ancient times [4]. There are many studies that report the antibacterial, anti-viral, anti-inflammatory, and anticarcinogenic effects of *Salvia* species [5–7].

Spray-drying is used for producing high-quality products in terms of particle size distribution, residual

moisture content, bulk density, and particle morphology. Spray drying is generally used in the food industry to ensure microbiological stability by reducing the water content and water activity of the products, avoiding the risk of chemical and/or biological degradation, reducing storage and transport costs, and final yield of products with specific properties such as immediate solubility [8].

Freeze-drying is a drying technique of a frozen food product under a vacuum at a low temperature. This process provides the vaporization of frozen water without becoming liquid under a vacuum. Freeze-drying has advantages such as uniformity, high quality, long shelf life, lightweight, and ease of transport for the products. Nowadays, in addition to the protection of food products (coffee, tea, crispy fruits and vegetables, ready-to-eat foods, and some aromatic herbs), biotechnological products and pharmaceutical products are successfully produced by freeze-drying [9].

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Spray and freeze-drying methods have been extensively studied in many food areas, but no comparative study has been reported on instant sage (*S. rosifolia* Sm.) tea that can provide better insight into the potential of these two methods. Hence, this study aimed to compare the effect of both drying methods in terms of sensory, physical, and chemical properties; antioxidant and antimicrobial properties, and explore the potential of both methods. It is also important for producing first-time instant sage tea with a freeze drier and this study can be a preliminary resource for the researchers who work in this area. The produced soluble herbal tea will provide an ability to consume natural herbal tea rich in bioactive components and easy to use.

2. Materials and methods

2.1. Materials

Samples of the sage plant (*S. rosifolia* Sm.) used in the study were collected in Gümüşhane region during the flowering season (40° 16' 19.23" N 39° 28' 53.96" E, above sea level: 1933 m, Kelkit, Gümüşhane). *S. rosifolia* Sm. species were identified by M. Gültepe (Gültepe 722 (KTUB)). The samples were dried in the shadow and natural environment until they reached an 8% moisture level. Samples were stored in locked polyethylene bags during storage.

2.2. Extraction process of the sage leaves

The same extraction process was used for both techniques. For this, 1250 g of plant samples were weighed and steeped for 10 minutes in 10 L of boiled water. During the steeping stage, sage was mixed with a spoon at intervals of several minutes. At the end of 10 minutes, the herbal tea was filtered with filter paper (grade 1: 11 µm; medium flow filter paper) and cooled to room temperature. The obtained herbal tea was stored at +4 °C in capped glass bottles.

2.3. Manufacturing of instant sage tea with freeze-drying

All of the aqueous extracts were divided into splay glass containers and lyophilized for 72 hours after being frozen at -65 °C for 0.1 mbar in the freeze dryer (Scientz-12N Laboratory Lyophilizer, Ningbo Scientz Biotechnology, China). At the end of this period, freeze-dried samples were transferred to dark-capped glass bottles and stored at +4 °C. Production was done in three replicates. 297.0 ± 12.2 g product was obtained.

2.4. Manufacturing of instant sage tea with spray drying

All of the aqueous extract obtained was dried in a laboratory-type spray dryer (The SD-06 spray, Labplant

UK, England). It was conducted for 4 hours at a liquid flow rate of 5 mL/s and the internal temperature was set at 200 °C. Powdered samples were taken from the machine's flask and stored at +4 °C in dark-capped bottles. Production was carried out in 3 replications. 246.0 ± 9.8 g product was obtained.

2.5. Analysis of the spray and freeze-dried sage tea samples

2.5.1. Moisture analysis

Moisture contents of the freeze-dried and spray-dried samples were weighed at 1 g and kept at 70 °C until the fixed weighing of the sample was reached. The measurement was made in triplicate. Moisture content was expressed as gram loss of water/100 g sample [10].

2.5.2. Crude protein and ash analyses

For the protein analysis, a 1.0 g sample was weighed into a Kjeldahl tube and 1 tablet catalyst and 20 mL H₂SO₄ were added and burned for 4 hours. Then 4% H₃BO₃ was added to samples on the distillation unit. Distillate titrated with 0.1 N HCl [11].

For the ash analysis, samples were weighed 2.5 g into the porcelain crucible, and the following pre-burning samples were kept at 550 °C in the ash oven. % Ash was calculated by weighing [12].

2.5.3. Yield % analysis

The yield of products obtained by two different methods using the classical brewing method was calculated according to the following formula.

$$\text{Yield (\%)} = \frac{P}{R} \times 100 \quad (1)$$

P: the amount of the obtained powder (g), R: the amount of the used plant (g).

2.5.4. Color analysis

Color analysis was performed using a Minolta Chromameter (CR-200) (Konica Minolta Sensing, Inc. Japan). For the hunter scale; L (dark/whiteness), a (greens/redness), b (blue/ yellow) are color parameters [13]. About 5 g of instant sage was placed in the sample vessel in front of the light source. The color values of the freeze-dried and spray-dried products were determined by studying on average six replicates. For the calibration of the device, L* 97.96, a* 0.08, and b* 1.78 white tiles were used.

2.5.5. Analysis of solubility in water

Determination of the water solubility of the products; 1.0 g of powder product was weighed and 100 mL of deionized water at room temperature was added. Thereafter, the mixture was stirred in a magnetic stirrer

at 600 rpm for 5 minutes. 20 mL of the solution was transferred to a petri dish and dried at 70 °C for 24 hours, after the drying period petri dish was weighed and the difference was expressed as 100 mL resolution [10].

2.6. Antioxidant activity analysis

Sample instant tea at a concentration of 200 µg/mL in distilled water was used as samples in the analysis. UV-1800 (Shimadzu, Japan) spectrophotometer was used to measure absorbances in all of the antioxidant analyzes.

2.6.1. Free radical scavenging activity (DPPH)

The free radical scavenging activity was determined by the DPPH method [14]. Diluted samples (100 µL each) were mixed with 3000 µL of freshly prepared 80 µg/mL DPPH methanol solution and allowed to stand for 30 min in the dark at room temperature for any reaction to take place. The ultraviolet (UV) absorbance of these solutions was recorded on a spectrometer at 517 nm using a blank containing the same concentration of extracts without DPPH.

2.6.2. Trolox equivalent antioxidant capacity (TEAC)

The ABTS solution was prepared by storing 7 mM aqueous ABTS solution and 2.45 mM potassium persulfate solution (1/1, v/v) for 6 hours in a dark place at room temperature. At the beginning of the analysis, the ABTS stock solution was diluted with methanol and the absorbance at 734 nm was set to 0.700 ± 0.020 . The mixture of methanol (900 µL), stock solutions of isolated molecules (100 µL), and ABTS solution (3mL) were transferred to the spectrophotometer cuvettes. The absorbance of 734 nm was measured after incubation at room temperature for 120 min [15]. Trolox was used as a standard substance.

2.6.3. Ferric reducing/antioxidant power (FRAP) assay

For the FRAP activity assay of water-soluble tea, 300 mM sodium acetate buffer solution (pH 3.6), 20 mM aqueous FeCl₃ solution, and 10 mM aqueous TPTZ solution were mixed in a ratio of 10/1/1. The FRAP solution (3 mL) was mixed with the herbal tea sample (100 µL) and methanol (900 µL) into the spectrophotometer cuvettes, and the measuring of absorbance was done at 593 nm after storing for 30 minutes at room temperature [16].

2.7. Phenolic substance analysis

HPLC-UV-DAD analyzes were performed on the Agilent 1200 series (DAD 1200) instrument for total phenolic content. The device was checked with the Agilent Chemstation program. C18 column (250 mm × 4.6 mm i.d., 5 µm particle) was used for all analyzes. The

mobile phase gradient was A; ultra-pure water, B; ACN (acetonitrile HPLC purity), C; 3% acetic acid + ultra-pure water, D; 3/25% acetic acid + ACN + ultra-pure water. The flow rate was 0.800 ml/min. Gradient flow; C90%-D10% for 0 min, C80%-D20% for 10 min, C50%-D50% for 22 min, C20%-D80% for 32 min, B80%-D20% for 45 min, C90%-D10% for 55 min (5 min).

The injection volume of the samples was used as 20 µL. The flow rate was set to 0.800 mL/min. 232, 246, 260, 272, 280, 290, 308 and 328 nm were recorded with the DAD detector [17]. 5 g of sample was extracted with acetone (10 mL) in an ultrasonic bath for 10 min. It was centrifuged at 1500 g for 10 min at +4 °C. The procedure was repeated using 70% acetone (10 mL). The acetone was evaporated under nitrogen gas at 37 °C and dissolved in 15 mL of purified water and filtered through a 0.45 µm filter (Millipore Corp.) was injected 20 µL into the device [18].

2.8. Mineral analysis of water-soluble tea

For the mineral analysis, the sample was weighed about 0.5 g and acidified with 5 mL of concentrated nitric acid. Burned in a microwave. At the end of the burning, the sample was transferred to a 25 ml balloon and the volume was completed with ultrapure water. Minerals were determined by using a coupled plasma mass spectrometer. The calibration curves were drawn with 5 different concentrations for all the analytes. The calibration standards were analyzed at regular intervals to control instrument drift. Also, ultrapure deionized water blanks were analyzed after each standard to control cross-contamination [19].

2.9. Aroma analysis of the water-soluble tea

Two grams of weighed sage samples for both products were left in 100 mL of hot water for 10 minutes. 40 mL of these solutions were taken into separation funnels, then 10 mL of saturated sodium chloride solution was added. The mixture was extracted four times with 40 mL of binary solvent (diethyl ether and pentane, volume ratio 2: 1). The extracts were then combined and washed two times with 50 mL of saturated sodium chloride solution and 50 mL of deionized water, respectively. The extracts were stirred with 10 g of anhydrous sodium sulfate and filtered. The filtrate was concentrated to the final volume of 0.5 mL at 39 °C with a rotary vacuum evaporator. A 1.0 µL sample was injected into the GC-MS / FID device [20]. Instrument: Agilent 5975 GC-MS Detector. Column: 30 m × 0.25 mm ID, 0.2 µm HP-5MS. Oven: 50 °C, 4 °C/min. to 260 °C, 15 min. Carrier Gas: Helium, constant flow 1.2 mL/min. Injection: 250 °C Detector: MS, 230 °C FID Detector: 250 °C, Gas flows H₂ 40 ml/min and dry air 400 ml/min.

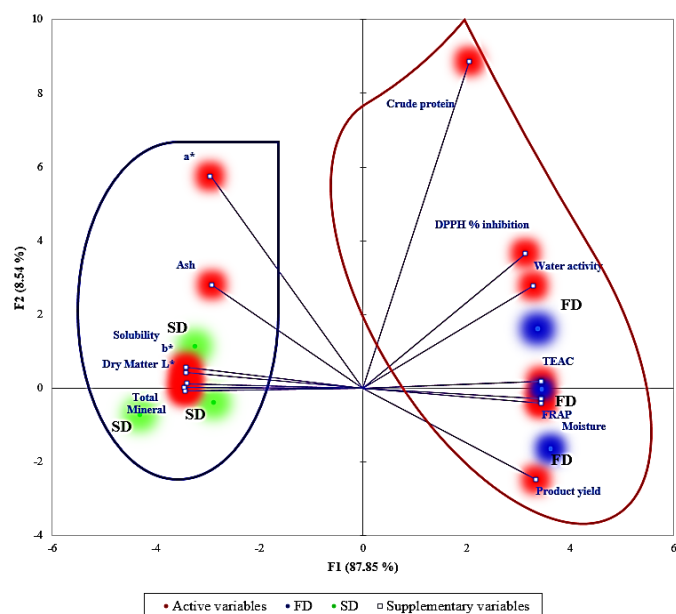


Figure 1. Multivariate analysis of physico-chemical, antioxidant activity and color of two types of instant tea. PC1 explaining 87.85%, versus PC2 explaining 8.54%, FD: Instant sage (*S. rosifolia* Sm) freeze-dried tea, SD: Instant sage (*S. rosifolia* Sm) spray-dried tea

2.10. Sensory analysis

Freeze-dried and spray-dried samples of brewed tea were prepared for sensory analysis according to ISO norms [21]. 0.4 g of each sample was taken and placed in porcelain pots with the addition of 10 ml of boiled water. The analysts were formed from 12 educated people (students of the food engineering department, the panel consisted of 6 male and 6 female, aged 18 to 24, nonsmoker panelists). Descriptive analysis of infused teas; Just the aroma, the color, the taste and smell, the appearance of the instant tea, and the overall acceptance were evaluated and the sensory scores ranged from 1 (1, 2, 3, and 4 = disliked extremely, disliked very much, disliked moderately, and disliked slightly, respectively, 5 = neither liked nor disliked) to 9 (6, 7, 8, and 9 = liked slightly, liked moderately, liked very much, and liked extremely, respectively).

2.11. Antimicrobial analysis

After determining the extractions conditions of the instant activity were assayed by the agar diffusion method.

Antimicrobial activity was tested on 31 different microorganisms. The agar diffusion test was performed according to the selected method [22]. Holes were made using sterile hole openers (6 mm in diameter) and, the sample was loaded in each hole (50 μ L). Streptomycin sulfate and nystatin were used as a positive control. The prepared Petri dishes were kept at room temperature for 2 hours and placed face up in the incubator, incubated at +37 $^{\circ}$ C for 24 hours, and the inhibition zone diameters were measured with a scale.

2.12. Statistical analysis

PCA and AHC were performed to evaluate the possible relationship between the studied parameters using the software package (XLSTAT Addinsoft SARL 2019). All analyses were made triplicates and results were given as mean \pm standard deviation (SD). To compare the significant differences in the mean values at $p < 0.05$ was used ANOVA.

3. Results and Discussion

3.1. Analyses of physicochemical, antioxidant activity and color

Results of the physicochemical analysis, and color are given in Table 1. PCA analysis of instant tea samples was performed on values of fourteen quality parameters aiming to identify similarities and differences among them. PCA, shown in Fig. 1 described 96.39% of the initial data variability of the total variance. On the observation plot, there was determined a clear separation between spray-dried and freeze-dried instant tea samples. This situation can be seen in Fig. 1, clustered in two different colored circles (red and blue colored). In order to describe these characteristics of applied physicochemical properties, antioxidant activity, and color parameters for freeze and spray-dried sage (*S. rosifolia* Sm) instant tea samples and obtain an overview of the main variations among them; product yield (PY), moisture (M), dry matter (DM), water activity (WA), solubility (S), crude protein (CP), ash (A), total mineral (TM), color ((C) (L^* , a^* , b^*)), FRAP, TAC and DPPH% were subjected to PCA analysis together.

Table 1. Changes in the physicochemical properties and of the spray and freeze-dried instant sage (*S. rosifolia* Sm.) tea

Product Type	Product yield (g/100 g)	Moisture (g/100 g)	Dry Matter (g/100 g)	Water activity	Solubility (g/100 g)	Crude Protein (g/100 g)	Ash (g/100 g)	Color		
								L^*	a^*	b^*
SD	20.0 ^b \pm 0.8	4.0 ^b \pm 0.1	96.0 ^a \pm 1.0	0.26 ^b \pm 0.01	97.4 ^a \pm 1.9	5.5 ^a \pm 0.5	12.7 ^a \pm 0.7	66.6 ^a \pm 0.8	3.2 ^a \pm 0.2	29.6 ^a \pm 0.18
FD	24.0 ^a \pm 1.0	21.3 ^a \pm 0.9	78.7 ^b \pm 0.8	0.45 ^a \pm 0.01	80.4 ^b \pm 0.7	5.7 ^a \pm 0.3	11.5 ^b \pm 0.8	25.1 ^b \pm 0.2	2.4 ^a \pm 0.1	-0.9 ^b \pm 0.1

Note: Results are presented as means; \pm standard deviations. Instant sage was expressed as g/100 g dry weight protein and ash, except color. Different letters (a-b) in the same column are significantly different ($p < 0.05$). FD: Instant sage (*S. rosifolia* Sm) freeze-dried tea, SD: Instant sage (*S. rosifolia* Sm) spray-dried tea.

Tea samples were characterized by significant correlations between the 14 variables analyzed while there were negative strong correlations between A, DM, S, TM, L*, a*, b* (blue colored circle) and FRAP, TEAC, DPPH, PY, WA, M (red-colored circle); on the other hand, strong positive correlations were found amongst themselves these clusters. According to PCA analysis, both negative and positive correlations were detected between CP and other analyzes. The correlation matrix is given in Table 2.

The moisture content of the products obtained by freeze dryer was found the moisture 21.3%, the dry matter content 78.7%, product yield 24.0%, and the water activity 0.45. The spray-dried products' moisture content was found the moisture 4.0%, dry matter content 96.0%, product yield 20.0%, and water activity 0.26. Moisture, dry matter content and water activity varied significantly ($p < 0.05$) with spray and freeze-dried instant sage tea (Table 1). It is mentioned that immediately tea powder having < 5 g/100 g moisture content material indicates a greater balance in packaging and storage [23]. Water activity content was found as compatible with the literature (0.23–0.29) for the SD sample [10]. Food processing techniques such as drying, freezing, curing, and baking affect water activity. On PCA analysis, except for moisture and CP ($R = 0.558$), there was a negative strong correlation between moisture and A, S, DM, and color (L*, a*, b*). On the other hand, a strong positive correlation was observed between moisture and WA, PY, and antioxidant capacities. These correlation values were given in Table 2.

When the water solubility ratios were examined, the solubility of the freeze-dried samples was found as

80.4% and it was found as 97.4% in the spray-dried samples. The solubility ratio of the spray-dried samples was found to be significantly ($p < 0.05$) higher than the freeze-dried samples. In the literature; The solubility values of the sage (*Salvia fruticosa* Miller) obtained by spray-dried were found to be in the range of 97.4–99.2 g/100 g [10].

Ash and crude protein content is given in Table 1. The crude protein content of both obtained products was analyzed. Based on dry weight, there was not any significant ($p > 0.05$) difference between the two samples in the crude protein that were found to be 5.5% and 5.7% in the spray-dried and freeze-dried samples, respectively. In PCA analysis crude protein showed weak positive and negative correlations with other analyzes except for DPPH ($R = 0.821$ strong). In spray-dried and freeze-dried dried samples, ash content was found to be 12.7% and 11.5% respectively ($p > 0.05$).

In Table 1, it can be easily determined that there is a discrepancy in color for L*, a*, and b* difference values between spray-dried and freeze-dried samples. These values show that the spray-dried sample is lighter in color, slightly reddish, and much yellow in color than the freeze-dried sample. The brightness of spray-dried products is higher than freeze-dried products. There is no significant ($p > 0.05$) difference between 'a*' value of the two methods. Spray-dried product has higher b* and L* value ($p < 0.05$). The drying technique has an effect on the color of the final product. The total color difference between the two samples is $\Delta E^* = +51.6 \pm 1.6$. It seems that the difference in total color difference value is quite large and it is understood that the spray-dried sample is brighter than freeze-dried sample. These color changes in instant sage tea samples may result from not only

Table 2. Correlations (R values) between physico-chemical, antioxidant activity and color

Variables	Product yield	Moisture	Dry Matter	Water activity	Solubility	Crude protein	Ash	TEAC	FRAP	DPPH% inhibition	L*	a*	b*	Total Mineral
Product yield	1	0.951	-0.942	0.844	-0.937	0.408	-0.940	0.958	0.948	0.841	-0.914	-0.938	-0.946	-0.939
Moisture		1	-0.998	0.941	-0.998	0.558	-0.808	0.988	0.999	0.876	-0.993	-0.857	-1.000	-0.997
Dry Matter			1	-0.924	1.000	-0.528	0.782	-0.976	-0.999	-0.854	0.997	0.859	0.997	0.996
Water activity				1	-0.927	0.763	-0.730	0.962	0.932	0.913	-0.927	-0.689	-0.948	-0.937
Solubility					1	-0.537	0.774	-0.976	-0.999	-0.854	0.998	0.852	0.997	0.997
Crude protein						1	-0.352	0.611	0.546	0.821	-0.547	-0.097	-0.579	-0.579
Ash							1	-0.860	-0.798	-0.787	0.734	0.874	0.805	0.786
TEAC								1	0.983	0.911	-0.965	-0.846	-0.990	-0.981
FRAP									1	0.869	-0.995	-0.858	-0.999	-0.998
DPPH% inhibition										1	-0.843	-0.604	-0.885	-0.886
L*											1	0.828	0.992	0.993
a*												1	0.846	0.833
b*													1	0.997
Total Mineral														1

Note: Values in bold are different from 0 with a significance level alpha = 0.05. Significant correlations are displayed in bold. Correlation coefficients vary -1 and 1. The closer is to 1 or -1, stronger is the link between two variables. **Negative** values indicate negative correlation and **positive** values indicate positive correlation. Values close **zero** reflect the absence of correlation

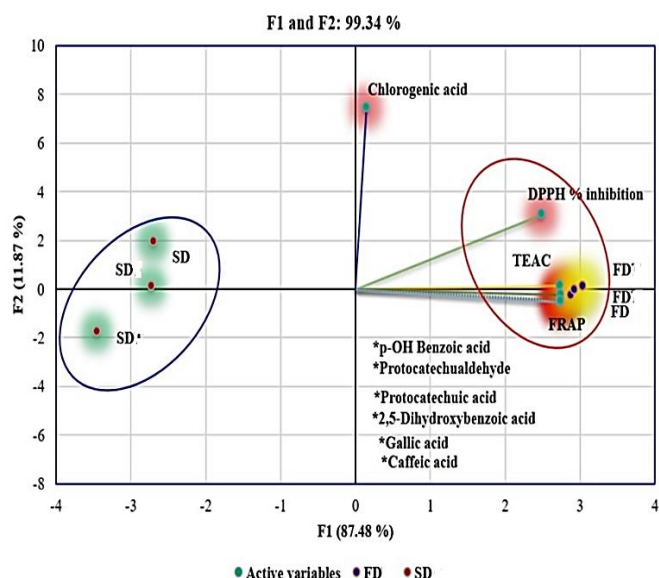


Figure 2. PCA of antioxidant activity and phenolic, flavonoid compounds of two types of instant sage tea. PC1 explaining 87.48%, versus PC2 explaining 11.87%. *Compounds in the bottom right quadrant. FD: Freeze-dried tea, SD: Spray-dried tea

thermal treatments and the non-enzymatic browning reaction but also the removal of volatile components in the spray-dried samples. 18 volatile compounds were detected in the spray-drying sample, while 61 volatile compounds were detected in the freeze-drying sample. In a study, significant differences were observed also for the color indices when comparing samples subjected to low-temperature and high-temperature drying. It was determined that there was a correlation matrix between color indices and volatile compounds [24]. Similar L^* , a^* , and b^* results were also reported as 61.8-79.7, 0.1 to 4.0, and 15.8-20.5, respectively in previous studies [25].

3.2. TEAC, FRAP, DPPH, phenolic, and flavonoid substance analysis

Analysis of PCA, shown in Fig. 2 described 99.34% of the initial data variability of the total variance. The comparison between antioxidant, phenolic, and flavonoid profile of freeze-dried and spray-dried samples are given in (Fig. 2). In this evaluation, two different clusters occurred (red and blue colored circles) which were completely separate from each other.

Table 3. Mineral contents for freeze-dried and spray-dried samples

Sample	Be	Na	Mg	Al	P	S	K	Ca	V
SD	<LOQ	711.7 ^a ±48.2	10337.5 ^a ±299.2	603.5 ^a ±79.8	5464.3 ^a ±154.9	5971.1 ^a ±128.7	72644.5 ^a ±223.43	24597.4 ^a ±126.1	2.2 ^a ±0.5
FD	<LOQ	540.4 ^b ±34.1	9054.7 ^b ±187.9	583.3 ^b ±78.65	4063.2 ^b ±145.87	3964.7 ^b ±156.6	63838.2 ^b ±157.43	20596.5 ^b ±870.9	1.1 ^b ±0.2
	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	As
SD	4.9 ^a ±0.5	161.4 ^a ±21.1	843.6 ^a ±93.3	2.7 ^a ±0.3	17.7 ^a ±2.4	9.1 ^a ±1.0	39.2 ^a ±3.9	8.9 ^a ±1.6	<LOQ
FD	3.6 ^b ±1.0	122.1 ^b ±4.3	708.2 ^b ±65.8	1.4 ^b ±0.1	12.8 ^b ±0.3	7.92 ^b ±0.25	15.95 ^b ±0.7	6.6 ^b ±0.2	<LOQ
	Se	Rb	Sr	Ag	Cd	Cs	Ba	Pb	Hg
SD	0.2 ^a ±0.1	24.4 ^a ±0.8	102.04 ^a ±4.56	0.56 ^a ±0.09	<LOQ	0.3 ^a ±0.1	116.1 ^a ±8.8	<LOQ	<LOQ
FD	0.2 ^a ±0.01	18.2 ^b ±0.2	79.24 ^b ±4.98	0.34 ^b ±0.10	<LOQ	0.2 ^b ±0.01	69.7 ^b ±3.2	<LOQ	<LOQ

Note: Results are presented as means minerals mg/kg ± standard deviations, Different letters (a-b) in the same column are significantly (P < 0.05) different FD: Instant sage (*S. rosifolia* Sm) freeze-dried tea, SD: Instant sage (*S. rosifolia* Sm) spray-dried tea.

Table 4. Phenolic and flavonoid contents for freeze-dried and spray-dried samples

No	Name	R.T.	FD	SD
			Concentration	
1	2,5-Dihydroxybenzoic acid	3.524	17.8 ^a ± 0.2	3.4 ^b ± 0.1
2	Benzoic acid	17.263	n.d.	n.d.
3	Caffeic acid	38.283	550.7 ^a ± 8.2	389.3 ^b ± 7.4
4	Catechin	29.646	n.d.	n.d.
5	Chlorogenic acid	26.122	491.5 ^a ± 9.3	388.9 ^b ± 7.9
6	Ferulic acid	28.334	n.d.	n.d.
7	Flavone	38.952	n.d.	n.d.
8	Galangin	30.123	n.d.	n.d.
9	Gallic acid	37.003	186.3 ^a ± 4.2	156.4 ^b ± 3.7
10	Myricetin	9.003	n.d.	n.d.
11	p-Coumaric acid	30.224	n.d.	n.d.
12	p-OH Benzoic acid	36.339	256.6 ^a ± 4.4	139.29 ^b ± 8.99
13	Protocatechualdehyde	23.371	35.1 ^a ± 2.1	19.2 ^b ± 3.3
14	Protocatechuic acid	20.399	174.3 ^a ± 5.7	88.1 ^b ± 4.2
15	Quercetin	15.993	n.d.	n.d.
16	Rutin hydrate	35.402	n.d.	n.d.
17	Sesamol	41.935	n.d.	n.d.
18	Sinapic acid	29.900	n.d.	n.d.
19	Syringaldehyde	39.822	n.d.	n.d.
20	Syringic acid	35.401	n.d.	n.d.
21	Vanillic acid	30.534	n.d.	n.d.
22	Vanillin	27.795	n.d.	n.d.

Note: Results are presented as means; ± standard deviations; all phenolic and flavonoid compounds are expressed as µg/g dry weight. Different letters (a-b) in the same rows lines are significantly different (p < 0.05). n.d.: Not detected. FD: Instant sage (*S. rosifolia* Sm) freeze-dried tea, SD: Instant sage (*S. rosifolia* Sm) spray-dried tea

There was a strong positive correlation between the antioxidant analysis results (Table 2).

The antioxidant properties of natural products such as plant extracts are originating from the bioactive compound's nature and sometimes-synergistic effects between them. However, it is difficult and time-consuming to determine the contribution of each component to the total antioxidant activity. The general procedure is to measure the total antioxidant capacity of the whole sample. Lamiaceae family has high antioxidant properties [26,27]. They contain a variety of phenolic substances which have both reduction ability (FRAP) and radical sweeping (DPPH) effects. A variety of methodologies is commonly used to assess

antioxidant potential. The average total antioxidant capacities of instant tea obtained by spray-drying and freeze-drying methods are given in Table 5 in dry matter. The antioxidant capacity of the instant tea sample dried with the freeze dryer was found to be higher. The reason for this situation can be interpreted as the expulsion of certain compounds from the product due to the high-temperature treatment in the spray-drying process and this caused the determination of more compounds in the samples dried with a freeze dryer for the analysis of aromatic compounds.

A study demonstrated that the total phenolic amount in *Salvia* species in Türkiye ranged from 50.3 to 167.1 mg GAE/g in the dry matter [28]. DPPH% inhibition study was conducted by Senol and colleagues on 55 *salvia* species in 2010. They found that the DPPH% inhibition rate was 45.56, 77.25, and 90.61, respectively, at concentrations of 25 to 50 and 100 mg/ml prepared from the methanol extract of *S. rosifolia* [29]. In this study, ascorbic acid and Trolox were used as DPPH antioxidant standards. Inhibitions percentages of these standards were determined as 98.50% and 97.99% respectively. When compared to Trolox and Ascorbic acid, inhibition rates of both samples were found quite high in Table 5.

Phenolic and flavonoid compounds were analyzed by extraction of the samples and the HPLC-DAD method (Table 4). In the PCA analysis, except for the chlorogenic acid analysis result, there was a strong positive correlation between the other acid and aldehyde analysis results (Fig. 2, Table 2). In the literature, these phenolics (in Table 4) are found in *salvia* species [30–32]. Sage

antioxidants are an alternative to the one of the common antioxidants of rosemary and can be used for the protection and preservation of certain food and nutraceutical products and can help the extension of their shelf life [44].

3.3. Mineral analysis

In the samples, twenty-seven mineral analyzes were performed by ICP-MS. The results are given in Table 3.

The values of Na, Mg, Al, P, S, K, Ca, Mn, Fe, Sr, and Ba minerals are quite high in both samples. However, the contents of minerals were significantly higher in spray-dried sage tea ($p < 0.05$). This is due to the low probability of moisture content of the SD sample. When the results are given on the basis of dry matter, the results are close to each other. Heavy metal contents of Hg, Pb, Cd, and As were not detected (N.D.) (LOQ, Pb: 1 $\mu\text{g}/\text{kg}$, Hg: 5 $\mu\text{g}/\text{kg}$, Cd: 5 $\mu\text{g}/\text{kg}$, As: 5 $\mu\text{g}/\text{kg}$). The results are in agreement with the Turkish Food Codex and literature [33]. The results show that the sage contains essential minerals in human metabolism that have a vital preserve, prevention, and improvement of diseases in growth and development literature [34].

3.4. Analysis of the aroma content and sensory characteristics

PCA is the most well-known method to explore relationships between both variables and observations, which provide implicated information and can be analyzed graphically by considering all variables simultaneously. In the PCA analysis of instant tea groups, the diagram of PCA1 and PCA2 represented 58.66% of the cumulative variance. The two groups were separated partially, as shown by the score plot in Fig. 4 (red and blue colored circles). Scores were arranged in four areas. The separation between the samples pointed out the differences in certain investigated sensory parameters. Also, the score chart showed that from both groups surveyed, FD got scores for aroma, overall acceptance, taste, and smell while SD were got scores for

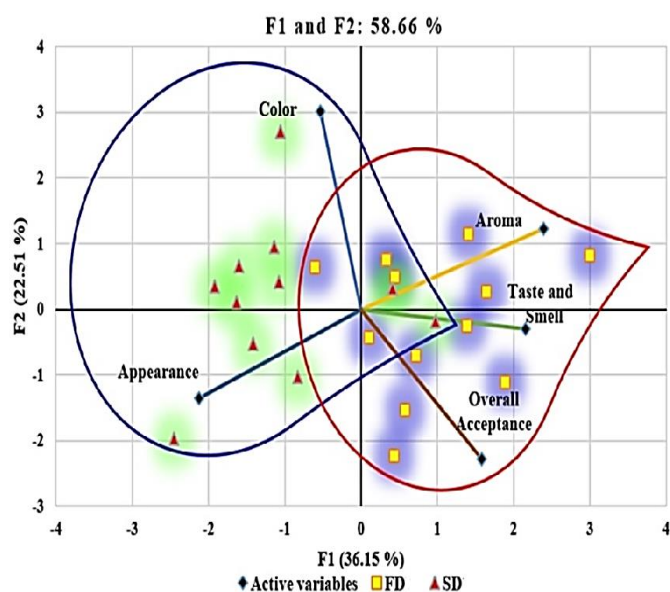


Figure 3. Two-dimensional principal component analysis: Representation of the sensory descriptors and the instant Sage tea samples evaluated by 12 panel members. PC1 explaining 36.15%, versus PC2 explaining 22.51%. FD: Instant sage (*S. rosifolia* Sm) freeze-dried tea, SD: Instant sage (*S. rosifolia* Sm) spray-dried tea

Table 5. Changes in antioxidant capacities of the spray and freeze-dried instant sage (*S. rosifolia* Sm.) tea

Product Type	TEAC	FRAP	DPPH % inhibition
SD	169.1 ^b ± 6.4	916.0 ^a ± 11.0	73.9 ^b ± 4.3
FD	231.9 ^a ± 9.1	1235.8 ^a ± 14.1	78.6 ^a ± 2.9

Note: Values for Trolox equivalent antioxidant capacity were expressed as mg TEAC/g dry weight, antioxidant activities of instant sage tea were expressed as mmol FeSO₄/g dry weight for FRAP, % inhibition for DPPH. Different letters (a-b) in the same column are significantly different ($p < 0.05$). In this study, ascorbic acid and trolox were used as antioxidant standards. Inhibitions percentages of these standards were determined as 98.50% and 97.99% respectively. TEAC: Trolox equivalent antioxidant capacity. DPPH: Sweeping Activity. FRAP Iron-reducing capacity. FD: Instant sage (*S. rosifolia* Sm) freeze-dried tea, SD: Instant sage (*S. rosifolia* Sm) spray-dried tea.

color and appearance by the panelists (red and blue colored circles). The five variables of sensory analysis are characterized by weak correlations in Fig. 3. Aroma and flavor indicators are based on volatile compounds. Aroma perception depends on the number of volatile substances present in food [27]. Therefore, the presence of aroma compounds affects the results of sensory analysis.

In this study, high scores of FD samples by panelists can be attributed to aroma compounds. A total of 61 compounds corresponded to 97.1% of the total area in the study of freeze-dried sample aroma analysis and 18 compounds were identified corresponding to 98.0% of the total area in the spray-dried sample aroma analysis. The compounds, their concentration ratios, and RI Index are given in Table 6 for both the experimental and the literature, according to the definition order of RI, MS. The reduced number of compounds in the spray-dried samples can be attributed to the removal of the compounds from the exhaust of the device due to the high temperature (200 °C) airflow during the applied process. The analysis of PCA obtained data of PCA1 and PCA2 represented 100.00% of the total aroma compound fractions variance. The freeze and spray dried instant

teas were separated by PCA into two main groups in Fig. 4 (red and blue teardrop circles). The first group SD (blue teardrop circle) characterized a total of 18 compounds, and the second group FD (red teardrop circle) total of 61 compounds. According to the literature, Epimanol, Camphor, Aromadendrene, Borneol, Camphor, α -Thujone, caryophyllene oxide, and Carvone oxide were determined as volatile compounds of Sage samples [35,36,43]. On analysis of PCA, amounts of other compounds detected were < 1% lower and showed a negative correlation with compounds with high amounts of compounds.

3.5. Antimicrobial activities

The results of the antibacterial activity test freeze-dried and spray-dried samples according to the disk diffusion method are given in Table 7. Antibacterial activity of the tea extracts was compared with Streptomycin sulfate and Nystatin as positive control and used with a concentration of 10 $\mu\text{g}/\text{mL}$. Instant tea of freeze-dried (1 mg/mL) showed antibacterial activities against *Bacillus cereus*, *Enterococcus faecalis*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enteritidis*, *Salmonella typhimurium*, and *Staphylococcus aureus*.

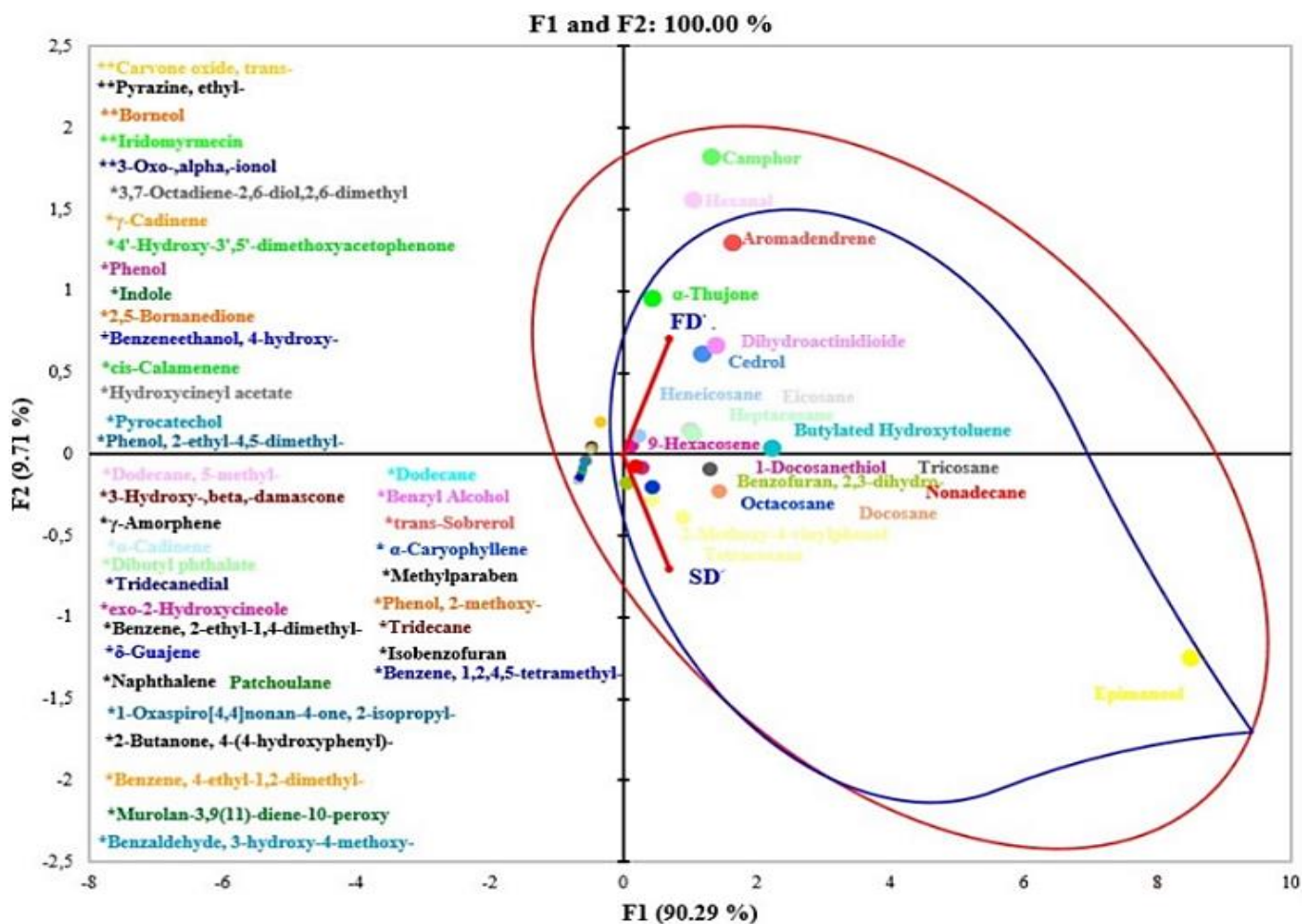


Figure 4. PCA score plot (PCA1/PCA2) of aroma compounds of spray and freeze drying instant sage tea PC1 explaining 90.29%, versus PC2 explaining 9.11% , *Compounds in the bottom left quadrant, **compounds in top left quadrant. FD: Instant sage (*S. rosifolia* Sm) freeze-dried tea, SD: Instant sage (*S. rosifolia* Sm) spray-dried tea.

Table 6. Volatile compounds of instant *salvia* teas, their concentration ratios, and RI Index

No	Compounds	ED. RI	L. RI	GC-MS/FID % area		Identification
				FD	SD	
1	Hexanal	808	808	6.45 ^a ± 0.21	.. ^b	RI, MS
2	Phenol	992	992	0.10 ^a ± 0.01	.. ^b	RI, MS
3	Isobenzofuran	1026	1036	0.07 ^a ± 0.01	.. ^b	RI, MS
4	Benzyl Alcohol	1039	1039	0.34 ^a ± 0.01	.. ^b	RI, MS
5	Benzene, 4-ethyl-1,2-dimethyl-	1061	1062	0.07 ^a ± 0.01	.. ^b	RI, MS
6	Benzene, 2-ethyl-1,4-dimethyl-	1088	1089	0.05 ^a ± 0.01	.. ^b	RI, MS
7	Phenol, 2-methoxy-	1093	1093	0.05 ^a ± 0.01	.. ^b	RI, MS
8	α-Thujone	1118	1118	4.19 ^a ± 0.11	.. ^b	RI, MS
9	Benzene, 1,2,4,5-tetramethyl-	1123	1123	0.05 ^a ± 0.01	.. ^b	RI, MS
10	Camphor	1145	1145	7.50 ^a ± 0.12	.. ^b	RI, MS
11	Borneol	1170	1173	0.78 ^a ± 0.03	.. ^b	RI, MS
12	Naphthalene	1186	1186	0.03 ^a ± 0.01	.. ^b	RI, MS
13	3,7-Octadiene-2,6-diol,2,6-dimethyl	1192	1191	0.18 ^a ± 0.01	.. ^b	RI, MS
14	Dodecane	1200	1200	0.03 ^a ± 0.01	.. ^b	RI, MS
15	exo-2-Hydroxycineole	1215	1218	0.07 ^a ± 0.01	.. ^b	RI, MS
16	Benzofuran, 2,3-dihydro-	1226	1226	1.37 ^a ± 0.04	2.36 ^b ± 0.65	RI, MS
17	Dodecane, 5-methyl-	1249	1249	0.07 ^a ± 0.01	.. ^b	RI, MS
18	Hydroxycineyl acetate	1249	1247	0.20 ^a ± 0.01	.. ^b	RI, MS
19	2,5-Bornanedione	1268	1264	0.05 ^a ± 0.01	.. ^b	RI, MS
20	Carvone oxide, trans-	1273	1277	1.35 ^a ± 0.06	.. ^b	RI, MS
21	Indole	1298	1298	0.07 ^a ± 0.01	.. ^b	RI, MS
22	Tridecane	1300	1300	0.05 ^a ± 0.01	.. ^b	RI, MS
23	Phenol, 2-ethyl-4,5-dimethyl-	1307	1305	0.26 ^a ± 0.01	.. ^b	RI, MS
24	2-Methoxy-4-vinylphenol	1318	1318	1.87 ^a ± 0.09	3.84 ^b ± 0.91	RI, MS
25	1-Oxaspiro[4.4]nonan-4-one, 2-isopropyl-	1343	1350	0.07 ^a ± 0.01	.. ^b	RI, MS
26	Pyrazine, ethyl-	1350	1352	0.72 ^a ± 0.04	.. ^b	RI, MS
27	trans-Sobrerol	1385	1384	0.10 ^a ± 0.02	.. ^b	RI, MS
28	Benzaldehyde, 3-hydroxy-4-methoxy-	1404	1401	0.47 ^a ± 0.03	.. ^b	RI, MS
29	Iridomyrmecin	1426	1422	0.77 ^a ± 0.04	.. ^b	RI, MS
30	Benzeneethanol, 4-hydroxy-	1434	1431	0.33 ^a ± 0.01	.. ^b	RI, MS
31	Methylparaben	1454	1459	0.46 ^a ± 0.01	.. ^b	RI, MS
32	α-Caryophyllene	1461	1461	0.21 ^a ± 0.01	.. ^b	RI, MS
33	γ-Cadinene	1482	1482	0.11 ^a ± 0.01	.. ^b	RI, MS
34	Pyrocatechol	1492	1493	0.33 ^a ± 0.01	.. ^b	RI, MS
35	δ-Guajene	1497	1498	0.10 ^a ± 0.01	.. ^b	RI, MS
36	Butylated Hydroxytoluene	1518	1518	5.87 ^a ± 0.04	8.64 ^b ± 0.82	RI, MS
37	γ-Amorphene	1521	1521	0.11 ^a ± 0.01	.. ^b	RI, MS
38	cis-Calamenene	1530	1530	0.28 ^a ± 0.01	.. ^b	RI, MS
39	Dihydroactinidioides	1539	1539	5.87 ^a ± 0.07	3.97 ^b ± 0.78	RI, MS
40	α-Cadinene	1544	1544	0.03 ^a ± 0.01	.. ^b	RI, MS
41	2-Butanone, 4-(4-hydroxyphenyl)-	1561	1555	0.05 ^a ± 0.01	.. ^b	RI, MS
42	Cedrol	1592	1589	4.99 ^a ± 0.21	3.50 ^b ± 0.77	RI, MS
43	Aromadendrene	1601	1606	7.06 ^a ± 0.12	2.74 ^b ± 0.76	RI, MS
44	Patchoulane	1619	1618	0.13 ^a ± 0.01	.. ^b	RI, MS
45	3-Hydroxy-.beta.-damascone	1621	1627	0.11 ^a ± 0.01	.. ^b	RI, MS
46	3-Oxo-.alpha.-ionol	1654	1656	0.82 ^a ± 0.01	.. ^b	RI, MS
47	Tridecanedial	1692	1690	0.08 ^a ± 0.01	.. ^b	RI, MS
48	Murolan-3,9(11)-diene-10-peroxy	1729	1729	0.16 ^a ± 0.01	.. ^b	RI, MS
49	4'-Hydroxy-3',5'-dimethoxyacetophenone	1741	1740	0.05 ^a ± 0.01	.. ^b	RI, MS
50	Nonadecane	1899	1900	1.79 ^a ± 0.02	2.45 ^b ± 0.69	RI, MS
51	Dibutyl phthalate	1967	1967	0.16 ^a ± 0.01	.. ^b	RI, MS
52	Eicosane	2001	2000	3.75 ^a ± 0.11	4.39 ^b ± 0.87	RI, MS
53	Epimanol	2068	2068	15.16 ^a ± 1.23	32.87 ^b ± 2.02	RI, MS
54	Heneicosane	2101	2100	2.27 ^a ± 0.07	2.07 ^b ± 0.56	RI, MS
55	Docosane	2201	2208	3.85 ^a ± 0.04	6.96 ^b ± 0.75	RI, MS
56	Tricosane	2307	2307	3.83 ^a ± 0.04	6.03 ^b ± 0.45	RI, MS
57	Tetracosane	2400	2400	2.53 ^a ± 0.02	5.70 ^b ± 0.77	RI, MS
58	1-Docosanethiol	2511	2512	1.96 ^a ± 0.01	2.79 ^b ± 0.43	RI, MS
59	9-Hexacosene	2611	2614	1.91 ^a ± 0.01	1.81 ^b ± 0.54	RI, MS
60	Heptacosane	2701	2705	3.78 ^a ± 0.03	4.61 ^b ± 0.76	RI, MS
61	Octacosane	2801	2804	2.00 ^a ± 0.01	3.63 ^b ± 0.54	RI, MS

Note: Results are presented as means; ± standard deviations; all volatile compounds are expressed as % area. Different letters (a-b) in the same rows lines are significantly ($P < 0.05$) different. FDT: Freeze-dried tea, SDT: Spray-dried tea, ED: Experimental determined, L: Literature, a: experimental RI, b: Literature RI

Table 7. Antimicrobial analysis results of 31 microorganisms

Microorganisms	FD	SD	Streptomycin sulfate	Nystatin
	1 mg/mL	1 mg/mL	10 µg/mL	30 µg/mL
Bacteria sp.	Diameter of inhibition zones (mm)			
<i>Aeromonas hydrophila</i> ATCC 35654	-	-	17	ND
<i>Bacillus cereus</i> ATCC 9634	6 ± 1	4 ± 1	16 ± 3	ND
<i>Bacillus subtilis</i> ATCC 6633	-	-	19 ± 3	ND
<i>Citrobacter freundii</i> ATCC 3624	-	-	13 ± 3	ND
<i>Enterococcus faecalis</i> ATCC 29212	12 ± 2	-	4 ± 1	ND
<i>Enterococcus sakazakii</i> ATCC 29544	-	-	21 ± 4	ND
<i>Escherichia coli</i> ATCC 25922	5 ± 1	-	7 ± 1	ND
<i>Escherichia coli</i> O157:H7 35150	-	-	15 ± 3	ND
<i>Klebsiella pneumoniae</i> ATCC 13883	-	-	16 ± 3	ND
<i>Lactobacillus bulgaricus</i> KCTC 3188	-	-	11 ± 3	ND
<i>Lactobacillus plantarum</i> NCDO 343	-	-	10 ± 3	ND
<i>Listeria monocytogenes</i> ATCC 7644	6 ± 1	-	19 ± 3	ND
<i>Micrococcus luteus</i> KCTC 10240	-	-	10 ± 3	ND
<i>Proteus vulgaris</i> FMC 1	-	-	14 ± 3	ND
<i>Pseudomonas aeruginosa</i> ATCC 27853	-	-	17 ± 3	ND
<i>Salmonella enteritidis</i> ATCC 13076	4 ± 1	-	16 ± 3	ND
<i>Salmonella typhimurium</i> ATCC 23566	6 ± 1	4 ± 1	13 ± 3	ND
<i>Shigella flexneri</i> ATCC 12022	-	-	8 ± 2	ND
<i>Staphylococcus aureus</i> ATCC 25923	7 ± 1	5 ± 1	12 ± 3	ND
<i>Staphylococcus epidermidis</i> KCTC 1917	-	-	18 ± 3	ND
<i>Streptococcus pneumoniae</i> ATCC 49619	-	-	9 ± 2	ND
<i>Vibrio parahaemolyticus</i> ATCC 17802	-	-	11 ± 3	ND
<i>Yersinia enterocolitica</i> ATCC 1501	-	-	7 ± 1	ND
Yeast and Molds				
<i>Aspergillus flavus</i> ATCC 46283	-	-	-	11 ± 3
<i>Aspergillus niger</i> ATCC 9142	-	-	-	14 ± 3
<i>Candida albicans</i> ATCC 10231	-	-	-	12 ± 3
<i>Fusarium oxysporum</i> ATCC 44187	-	-	-	12 ± 3
<i>Penicillium expansum</i> ATCC 7861	-	-	-	10 ± 3
<i>Rhizopus oryzae</i> ATCC 4858	-	-	-	20 ± 3
<i>Sacc. cerevisiae</i> S288C	-	-	-	18 ± 3
<i>Zygosaccharomyces bailii</i> ATCC 66825	-	-	-	13 ± 3

FD: Instant sage (*S. rosifolia* Sm) freeze-dried tea, SD: Instant sage (*S. rosifolia* Sm) spray-dried tea

Instant tea of spray-dried (1 mg/mL) showed antibacterial activities against *Bacillus cereus*, *Enterococcus faecalis*, *Salmonella typhimurium*, and *Staphylococcus aureus* (Table 7). Quantitative chemical differences due to differences in production during the samples showed the effect on antimicrobial activity of samples; antimicrobial activity of freeze-dried samples was higher. This is because the freeze-dried sample has excess volatile oil compounds. The tea samples did not show any effect on the yeast and the mold. Analyzed flavonoids, phenols, terpenes, alkaloids, etc. components are responsible for biological activity [37]. There have been many studies in the literature on the antimicrobial properties of salvia species [38]. In literature, the oils of *S. rosifolia*, *S. fruticosa*, *S. tomentosa*,

S. ringens and *S. officinalis* were reported to have strong antimicrobial activity [35,39–42].

4. Conclusions

The present work was carried out to produce freeze-dried and spray-dried instant tea from *S. rosifolia* Sm. The obtained results showed that instant teas dried by freezing and spraying have some properties different from physicochemical, antioxidant, and antimicrobial aspects, at the same time these results revealed negative effects of heat treatment on tea compounds. Drying techniques used affected the composition, aroma, sensory properties, antioxidant activity, and antimicrobial activity and physical properties of

instant sage. This is a step in the technological development of herbal-based tea products freeze-drying and spray-drying. According to our overall results, instant sage tea represents an important dietary source of minerals and antioxidants. It may be concluded that the spray and freeze-dried instant sage can be used as a functional drink.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Declaration of Competing Interest

None

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