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SCIENTIFIC PAPER

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EFFECT OF THE DIFFERENT INFRARED LEVELS ON SOME PROPERTIES OF SAGE LEAVES

Article Highlights

- Five different infrared power were used to dry Sage leaves
- TPC and AA were protected better in dried at 88 W than the other IP powers
- Rosmarinic acid, caffeic acid, gallic acid, and luteolin were the major phenolics of Sage leaves
- All samples had antibacterial activity
- This study suggested 88 W for drying Sage leaves

Abstract

This study aims to investigate the effect of different infrared powers (IP) (38 W, 50 W, 62 W, 74 W, and 88 W) on drying kinetics, total phenolic content (TPC) and individual phenolics, antioxidant activity (AA) and antibacterial activity, and color quality of sage leaves. IP level significantly affected ($p<0.05$) drying kinetics, bioactive contents, and color quality of sage leaves. Higher TPC and AA were obtained from the sample dried at 88 W. Rosmarinic acid, caffeic acid, gallic acid, and luteolin were found as major phenolic compounds, and their higher levels were obtained from the samples dried at an IP level of 88 W. All samples showed antibacterial activity on test pathogens. A higher correlation was observed between TPC, rosmarinic acid level, and antibacterial activity ($P>0.80$). This study suggested that sage leaves should be dried at 88 W regarding lower drying times and color changes, lower phenolic degradation, and higher antibacterial activity.

Keywords: infrared drying, rosmarinic acid, phenolic profile, antibacterial activity, color.

Sage (*Salvia officinalis*) is a well-known aromatic herb from the mint family. It is an evergreen and fastest-growing plant. Its leaves have a strong aromatic smell. In the Latin name of sage, "*Salvia*" means to cure, and "*Officinalis*" means medicinal [1]. It is usually used for various purposes, such as antiheroic, carminative, expectorant, disinfectant, analgesic, and diuretic [2]. Sage also has been commonly used as filter tea [3].

In addition to medicinal properties, sage leaves have strong antioxidant effects and are used for food preservation due to their antioxidant properties [4,5]. The antioxidant properties of sage have been investigated in many studies, and these studies, especially diterpenoids, triterpenoids, phenolic acids (exclusively caffeic acid derivatives), and flavonoids which form the majority of the phenolics in the *Salvia* species, have been emphasized [5–7]. The antioxidant property of *Salvia officinalis* originates from the abietane-type diterpenoids (carnosic acid and carnosol) and caffeic acid derivatives, particularly rosmarinic acid [8]. In addition to antioxidant activity, sage has antiproliferative [9], antimicrobial [10], and antitumor activities [11]. Besides, sage reduces or prevents lipid oxidation in some foods [12].

Like many other agricultural products, sage leaves have a high moisture content. In addition, it is a

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seasonal and highly perishable plant herb. Therefore, postharvest technological processes, such as drying, should be applied to sage to provide all-year-round consumption and preservation [13]. Drying, as a preservation method, is a critical aspect of food processing. The purpose of drying is to reduce the product's water activity, thus preventing the growth of microorganisms, reducing chemical reactions, and extending the shelf life of food at room temperature [14]. Also, less space is required for storage, and dried product is lighter for easy transportation.

Because of its simple and easy application, hot air drying is the most used method for drying foodstuffs. However, hot air drying takes a long time due to low heat and mass transfer rates, and bioactive food components are damaged during the long drying process. Alternative drying methods with a higher drying rate and less damage to bioactive compounds should be applied to overcome these disadvantages [15]. High energy efficiency and short drying times are among the advantages of the infrared method. Water molecules absorb energy quickly. Therefore, quick water evaporation occurs, providing high food drying rates. Furthermore, using low-energy, better-quality dried foods can be obtained [16].

The effect of drying methods was investigated in many studies. The herbs' characteristics and the volatile components' concentrations depend on various factors, such as the drying method and the herb [2,13]. Some studies on hot-air drying have been reported in the literature [14,17,18] and microwave drying of the behavior of sage leaves [18]. These studies focused on drying kinetic different drying processes and their effect on some bioactive compound behavior of sage leaves. Hamrouni-Sellami *et al.* [14] conducted a comprehensive study focused on some drying methods for individual phenolics of sage leaves. Doymaz and Karasu [17] dried sage leaves using a cabinet dryer and found the highest TPC and AA at the lowest temperature (45 °C). Sadowska *et al.* [18] dried sage leaves under natural conditions, convective drying, and freeze-drying. They reported that drying conditions strongly affected drying duration and bioactive properties [19]. Jonas *et al.* [20] investigated the effect of oven-drying conditions on the key aroma content of sage leaves. However, there have been no studies on the effect of different infrared drying conditions on total bioactive content and individual phenolic components of sage leaves.

In the present study, the effects of different infrared levels on TPC, AA, phenolic profile, antibacterial activity, and color quality of sage leaves were investigated extensively. However, there is no comprehensive study of the effects of infrared drying on the quality properties of sage leaves.

MATERIALS AND METHODS

Materials

Fresh sage (*Salvia officinalis* L.) leaves were collected in September 2021 from a house garden in Arsuz, Southern Turkey. The harvested leaves were stored in a refrigerator at 4 °C ± 1 °C, brought to the laboratory without breaking the cold chain, and stored in a refrigerator at 4 °C ± 1 °C until the drying experiments were started. The moisture content of fresh sage leaves was calculated as 70.58%, w. b. (2.399 kg water/kg dry matter, d. b.) an oven at 105 °C for 24 h. Triplicate samples were used to calculate initial moisture content, and the average values were reported.

Experimental procedure

Drying experiments were carried out in a moisture analyzer with one 250 W halogen lamp (Snijders Moisture Balance, Snijders b.v., Tilburg, Holland). Sage leaves (33 g ±0.5 g) were separated homogeneously over the drying chamber for the drying process. The drying process was conducted at the infrared power level varying from 38 W to 88 W. The IP level was adjusted in the control unit of equipment. Sample weight loss was recorded at 15 minute intervals during drying by a digital balance (model BB3000, Mettler-Toledo AG, Grefensee, Switzerland), which has a 0 g–3000 g measurement range with a reading accuracy of 0.1 g. Drying was ended when the moisture content of the sample reached 0.03 ± 0.01 kg water/kg dry matter (d.b.). The dried samples were cooled and packaged in low-density polyethylene bags and then heat-sealed (SMVK 126, Sonkaya Corporation, Istanbul, Turkey). The drying experiments were conducted in triplicate. Drying data were analyzed using a two-way analysis of variance at $p<0.05$.

Extraction procedure

Fresh and dried sage samples were ground, and an aqueous methanol solution (20 vol.%) was added to grounded sage samples with the ratio 1:50. This mixture was shaken for 2 h in a shaking incubator (Memmert WB-22) at room temperature [20]. After the extraction process, the solid/liquid mix was centrifuged (Hettich, Universal 320R, Tuttlingen, Germany) at 4,000 rpm for 10 min. Finally, samples were filtered by Whatman No. 1 and 0.45-μm microfilter. The extracts were kept at 4 °C for further analysis.

Total phenolic content (TPC)

The TPC of the sage samples was determined by the Folin-Ciocelteau method [21]. First, 2.5 mL of tenfold diluted Folin–Ciocelteau's phenol reagent was

added to tubes containing 0.5 mL of extract. Then, 2 mL of 7.5% Na_2CO_3 was added to the tubes. After 30 min incubation at room temperature in the dark, the absorbance at 760 nm was measured using a UV/VIS spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The calculations were made using Eq. (1) obtained from the created calibration curve for TPC:

$$A = 11.291C + 0.0442 R^2 = 0.997 \quad (1)$$

where A and C indicate the absorbance and concentration values, respectively. TPC was expressed as mg gallic acid equivalent (GAE) per g dry samples.

Antioxidant activity (AA)

AA was determined using the DPPH method previously described by Si *et al.* [22]. 4.9 mL DPPH solution dissolved in 0.1 mM of methanol was added to the tubes containing 0.1 mL of extract. This mixture was incubated at room temperature for 40 min in a dark place. The absorbance of the mixture was read at 517 nm. The calculations were made using Eq. (2) obtained from the created calibration curve for DPPH:

$$A = 0.0018C + 0.017 R^2 = 0.9961 \quad (2)$$

where A and C indicate the absorbance and concentration values, respectively. The results were expressed as μmol Trolox/g samples.

Individual phenolic compounds

HPLC coupled to a diode array (HPLC-DAD, Shimadzu Corp., Kyoto, Japan) was used to analyze individual phenolic components in fresh and dried sage samples according to the method reported by Kayacan *et al.* [23]. The extracts for use in TPC analysis were filtered through a 0.45-μm membrane filter, and the samples were analyzed in an HPLC system (LC-20AD pump, SPDM20A DAD detector, SIL-20A HT autosampler, CTO-10ASVP column oven, DGU-20A5R degasser, and CMB-20A communications bus module; (Shimadzu Corp., Kyoto, Japan).

Separations were performed at 40 °C on Intersil® ODS C-18 reversed-phase column (250 mm × 4.6 mm length, 5 μm particle size). The mobile phases were solvent A (distilled water with 0.1% (v/v) acetic acid) and solvent B (acetonitrile with 0.1% (v/v) acetic acid). Gradient elution was 10% B (2 min), 10% to 30% B (2 min to 27 min), 30% to 90% B (27 min to 50 min), and 90% to 100% B (51 min to 60 min) and at 63 min returns to initial conditions. The flow rate was set to 1 mL/min. Chromatograms were recorded at 254 nm and 356 nm. Identification and quantitative analysis were carried out based on retention times and standard curves. The result of individual phenolics was expressed as mg/kg for fresh and dry sage samples.

Antimicrobial activity of sage extracts

The antimicrobial activity of sage extracts was determined using the disc diffusion method [24] against *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Salmonella* Typhimurium ATCC 14028, and *Listeria monocytogenes* ATCC 13932 pathogens. The pathogen cultures were cultivated overnight and spread onto Petri dishes containing nutrient agar. After waiting for bacterial penetration, sterile paper discs impregnated with sage extracts at 50 mg/mL concentration were placed on the agar. 20% methanol (v/v) was used as a negative control. Petri dishes were incubated at 37 °C for 24 h. Inhibition zones were measured and expressed as millimeters (mm).

Color

Fresh and dried sage leaves color values surface were measured by a chromameter (CR-13, Konica Minolta, Tokyo, Japan). The measurement was performed at four different edge spots on the surface of each sample [23]. The color values of the samples were expressed as L^*(whiteness/darkness), a^*(redness/greenness), and b^*(yellowness/blueness). The total color change (ΔE) was calculated using Eq. 3:

$$\Delta E = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2} \quad (3)$$

Statistical analysis

Statistical evaluation was performed using the software program Statistica (StatSoft, Inc., Tulsa, OK). All analyzes were performed three times. First, the standard deviation and mean values were presented. Second, ANOVA was carried out to determine differences between samples. Third, Duncan, multiple comparison tests at a 95 % significance level was used to evaluate the effect of different infrared power on the TPC, AA, phenolic profile, antimicrobial activity, and color value of sage leaves. Finally, Pearson's coefficient of correlation was performed to determine the relation between TPC and antioxidant and antimicrobial activities.

RESULTS AND DISCUSSION

Analysis of infrared drying curves

Figure 1 shows the effect of IR power on the drying characteristics of the sage leaves during infrared drying. As seen in the figure, the drying curves are similar to the characteristic drying behavior of agricultural products [25]. As expected, the product's moisture content decreased exponentially with the drying time, and the moisture removal rate was higher at higher IR power.

As can be seen, an increase in the time required

for the samples to reach the targeted moisture content was 120 min, 80 min, 60 min, 50 min, and 40 min at the infrared power levels of 38 W, 50 W, 62 W, 74 W, and 88 W, respectively. In addition, the average drying rate of samples increased 3.0 times when the infrared power level increased from 38 W to 88 W. The faster drying rate and lesser drying time at higher IR power could be explained by the higher heat absorption resulting in higher product temperature and higher mass transfer driving force [26,27].

Change in TPC and AA

Table 1 shows the change in TPC and AA depending on the applied infrared power. The TPC values were significantly affected by applied infrared power. The TPC value of the fresh samples was found to be 24.5 mg GAE per g of dry samples. The TPC value of the dried samples ranged from 10.74 mg to 18.99 mg GAE per g dry samples. A significant loss of phenolic compounds was observed during the drying process ($P<0.05$), and this loss was higher at low infrared power (56.16 %). The high reduction in TPC in sage leaves during the drying process was reported in previously published studies [19,28]. Sadowska *et al.* [28] reported that fresh sage leaves had the highest polyphenols (1,773.20 mg/100 fresh weight–chlorogenic acid equivalent). Also, the highest amount of polyphenolic compounds was significantly in freeze-dried sage samples compared to other dried sage (naturally dry, dried at 35 °C and 45 °C) and thyme samples. In contrast, the lowest amount of these compounds was recorded in sage leaves dried at 35 °C. This high reduction might be due to heat-sensitive phenolic compounds such as carnosic acid and rosmarinic acid [28]. The change in phenolic compounds and b^* value showed a positive correlation (>90%), indicating that low infrared power cause higher degradation in TPC and pigments. The long drying process could explain the higher degradation during low power infrared drying. The higher retention of TPC at higher infrared power might be due to the disruption effect of the high temperature on the cell wall and releasing the phenolic compound from the insoluble part of the plant [29,30].

AA value was 19.76 μmol Trolox/g for fresh samples and 12.32 μmol Trolox/g–18.06 μmol Trolox/g. As shown, AA significantly decreased during the drying process. The change in AA showed a similar trend with TPC during drying. A higher AA value was observed from the sample dried at high infrared power. Bioactive substances' degradation could explain the AA reduction during the drying process.

An increase in TPC and AA with increasing infrared power was also reported in the study. Adak *et al.* [31] reported that increasing infrared power from 100 W to 300 W increased AA from 2.88 g/g DPPH to 5.81 g/g DPPH and TPC from 35171 mg/kg GAE to 44993 mg/kg GAE. This study suggests that sage leaves must be dried at 88 W to maintain TPC and AA.

Effect of IP on phenolic profile of sage leaves

Table 2 shows the individual phenolic distribution of the fresh and dried samples. Rosmarinic acid and luteolin were found as major phenolic compounds, and their levels were 1.92 mg/kg–13.60 mg/kg and 2.61 mg/kg–5.01 mg/kg, respectively. Gallic acid and

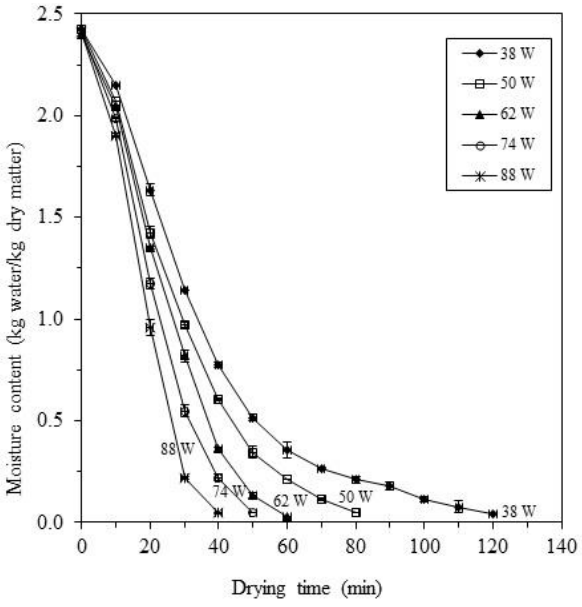

Figure 5. Variations of moisture content with drying time of sage leaves at different infrared powers.

Table 1. Effect of infrared power on the TPC and AA value of the sage leaves.

Infrared power (W)	TPC* (mg GAE /g dry samples)	AA* (μmol Trolox/g)
Fresh	24.50±0.52^{a}	19.76±0.01^{a}
38	10.74±0.19^{f}	12.32±0.20^{c}
50	17.34±0.10^{c}	12.92±0.76^{c}
62	14.35±0.05^{e}	13.18±0.84^{c}
74	15.20±0.57^{d}	16.34±2.85^{bc}

The different lowercase letters in the same column show statistical differences ($p<0.05$). *TPC: Total phenolic content, AA: antioxidant activity.

caffeic acid were other abundant phenolic components with lower levels than rosmarinic acid and luteolin. The IP level significantly affected both the numbers of the phenolic compounds and their concentrations ($p<0.05$). IP levels of 38 W and 50 W showed a high number of phenolic compounds. The degradation of heat sensitive phenolic compounds benzoic acid and vanillin could explain these results. However, benzoic acid was not detected for the samples dried by high IP (62 W, 74 W, and 88 W), and vanillin was not found for the IP level of 88 W.

Rosmarinic acid and luteolin levels were higher for the IP levels of 50 W and 88 W. The higher level of rosmarinic acid and luteolin values for high IP values can be explained by two reasons. First, infrared radiation dramatically increased the phenolic compound accumulation. The other reason is the short drying time due to the high drying rate [32,33]. Phenolic compounds were less damaged because samples dried at 88 W had the lowest drying time. Exposure to prolonged heating leads to irreversible changes in temperature-sensitive phenolic substances [33]. Caffeic acid showed similar trends with rosmarinic acid and luteolin. Gallic acid levels increased with increased IP levels. A high correlation was observed with rosmarinic acid, luteolin, and caffeic acid. This study concluded that high infrared power should be conducted to preserve the major phenolic compounds.

Table 2. Phenolic profiles of the sage leaves.

Phenolic compounds (mg/kg)	Fresh	Infrared Power (W)				
		38	50	62	74	88
Gallic acid	2.22^{b}	1.32^{e}	1.51^{d}	1.90^{c}	1.67^{d}	2.90^{a}
Protocatechic acid	1.10^{a}	0.45^{c}	0.31^{c}	0.55^{b}	0.33^{c}	0.30^{c}
Caffeic acid	3.94^{a}	1.75^{d}	3.10^{b}	2.27^{c}	3.03^{b}	3.74^{a}
Vanilin	0.74^{a}	0.33^{c}	0.51^{b}	0.33^{c}	0.53^{b}	nd
p-Coumaric acid	0.31^{a}	0.13^{c}	0.40^{a}	0.33^{a}	0.25^{b}	0.37^{a}
Ferulic acid	1.00^{a}	0.27^{c}	0.81^{b}	1.37^{a}	0.90^{b}	1.10^{a}
Benzoic acid	3.00^{a}	1.53^{c}	2.37^{b}	nd	nd	nd
o-Coumaric acid	1.20^{a}	0.57^{c}	0.75^{b}	0.77^{b}	0.80^{b}	0.50^{c}
Rosmarinic acid	13.60^{a}	1.90^{e}	5.87^{c}	2.10^{d}	5.44^{c}	10.10^{b}
Luteolin	5.01^{a}	3.31^{c}	4.00^{b}	3.01^{c}	2.62^{d}	3.95^{b}

The different lowercase letters in the same row show statistical differences ($p<0.05$). *nd: not detected.

Antibacterial effect of dried sage

The antimicrobial activity of sage extracts is given in Table 3. In general, all extracts inhibited the test pathogens. The inhibition zone diameters of extracts ranged from 12.33 mm to 23.50 mm. Phenolic compounds were less damaged because the IP of 88 W ensured the lowest drying time. Also, sage extracts were more effective on Gram-positive bacteria than Gram-negative bacteria. Similar results were reported previously [34,35]. The IP level significantly affected ($p<0.05$) the antibacterial activity of dried sage leaves. The highest antibacterial effect on the tested pathogens was found for the fresh sample, and the lowest was at the IP of 62 W. The antibacterial activity of the sage leaves is due to phenolic substances, such as rosmarinic acid, caffeic acid, gallic acid, and ferulic acid, which are major phenolic components of sage [36].

The Pearson correlation was applied to explain the relationship between TPC and antimicrobial activity. A high correlation was observed between TPC (>0.80), rosmarinic acid level (>0.85), and antibacterial activity. As reported, rosmarinic acid is responsible for the strong antimicrobial activity of sage [37]. Also, Klancnik *et al.* [38] found that plant extracts with carnosic and rosmarinic acid as major components were more effective on Gram-positive than Gram-negative bacteria.

Color evaluation

Color is one of the most determinative quality parameters affecting consumers' choices. The L^*, a^*, and b^* values of the fresh sage leaves were recorded to be 52.08, -8.21, and 12.55. The negative a^* and positive b^* value of the sage leaves is related to green and yellow pigments, respectively. Table 4 shows the

Table 3. Antimicrobial activity of sage extracts against common foodborne pathogens (inhibition zone, mm).

Infrared Power (W)	*S. aureus* ATCC 29213	*E. coli* ATCC 25922	*S.* Typhimurium ATCC 14028	*L. monocytogenes* ATCC 13932
Fresh	24.6±1.8^{a}	22.7±2.4^{a}	24.3±1.2^{a}	29.7±1.4^{a}
38	15.7±2.1^{bc}	13.8±1.3^{d}	16.2±1^{c}	17.8±1.0^{d}
50	18.8±1.3^{b}	17±1.3^{b}	18±1b^{c}	21.7±1.5^{bc}
62	12.3±2.5^{c}	12.3±1.5^{d}	14.5±0.5^{d}	15±1.0^{e}
74	17.7±1.5^{b}	15±2.0^{b}	17.5±0.9^{bc}	19.3±0.6^{cd}
88	19.5±1.5^{b}	18±1.0^{b}	19.2±1.3^{b}	23.5±1.3^{b}

*The different lowercase letters in the same column show statistical differences ($p<0.05$).

Table 4. Results of color values for sage leaves.

Infrared power (W)	*L**	*a**	*b**	*ΔE*
38	42.88±0.30^{b}	0.95±0.03^{a}	8.41±0.27^{a}	13.63±0.03^{c}
50	43.81±0.51^{b}	1.13±0.01^{a}	8.19±0.02^{a}	13.22±0.26^{d}
62	44.92±0.46^{a}	1.73±0.06^{a}	8.02±0.04^{b}	13.06±0.21^{d}
74	41.34±0.14^{c}	2.32±0.14^{b}	7.87±0.02^{c}	15.75±0.17^{b}
88	40.93±0.16^{d}	2.51±0.03^{c}	5.86±0.01^{d}	16.33±0.12^{a}

*The different lowercase letters in the same column show statistical differences ($p<0.05$).

*L**, *a**, *b**, and *ΔE* values of sage leaves dried at different IPs. As is seen in the Table, infrared power significantly affected color values ($p<0.05$). The *L** decreased with increased infrared power; the lowest *L** value was obtained from the sample dried at 88 W. The lower *L** and higher *a** values could be explained by an increase in the formation of brown pigment with increasing power levels due to a non-enzymatic browning reaction [39]. *a** and *b** values of the dried sage leaves significantly differed ($p<0.05$). The highest change in the *a** and *b** values was obtained from the sample dried at the highest infrared power. The reduction of the *b** value might be due to the degradation of the color pigments such as carotenoids and chlorophyll [39,40]. Therefore, the high infrared power could result in the degradation of the pigments. For this reason, *ΔE* was calculated to describe the total color change in *S. officinalis* leaves. *ΔE* value shows total color differences and gives information about the perceptible color change. *ΔE* value of all samples was higher than 3, indicating that perceptible change was observed after drying [41]. The highest result was obtained from the sample dried at 88 W, indicating that the formation of brown pigment could be considered the main factor determining color change during infrared drying. Similar results have already been reported [31,42].

CONCLUSION

In this study, the infrared drying technique was applied to drying sage leaves as an alternative drying method. The effects of various IP values on the sage leaves' drying time and bioactivity properties were studied. As the IP value increased, the drying time decreased significantly. The effect of the IP value on total phenolic compounds, phenolic profile, antioxidant, antimicrobial properties, and color quality was found to be significant ($p<0.05$). Therefore, the IP of 88 W was recommended as the most suitable IP value in this study due to the resulting low drying time and color change, high phenolic content, and antioxidant and antimicrobial properties. Further analysis, such as SEM images and volatile profile, should be conducted to understand the effect of IP on the quality of sage leaves in more detail.

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NAUČNI RAD

UTICAJ RAZLIČITOG INTENZITETA INFRACRVENOG ZRAČENJA NIVOA NA NEKA SVOJSTVA LISTA ŽALFIJE

Ova studija ima za cilj da istraži uticaj različite snage infracrvenog zračenja (38-88 W) na kinetiku sušenja, ukupni sadržaj fenola i pojedinačnih fenola, antioksidativnu aktivnost i antibakterijsku aktivnost, i kvalitet boje listova žalfije. Nivo snage infracrvenog zračenja je značajno uticao ($p<0,05$) na kinetiku sušenja, bioaktivni sadržaj i kvalitet boje listova žalfije. Viši ukupni sadržaj fenola i antioksidativne aktivnost su dobijeni iz uzorka osušenog na 88 W. Glavna fenolna jedinjenja su rozmarinska, kafeinska i galna kiselina i luteolin, a njihovi viši nivoi su dobijeni iz uzoraka osušenih pri snazi infracrvenog zračenja od 88 W. Svi uzorci su pokazali antibakterijsku aktivnost na patogene mikroroganizme. Uočena je veća korelacija između ukupnog sadržaja fenola, koncentracije rozmarinske kiseline i antibakterijske aktivnosti ($p>0,80$). Ova studija je sugerisala da listove žalfije treba sušiti rpi snazi od 88 W radi kraćeg vremena sušenja i manje promene boje, manje degradacije fenola i veće antibakterijske aktivnosti..

Ključne reči: infracrveno sušenje, rozmarinska kiselina, fenolni profil, antibakterijska aktivnost, boja.