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Effect of infrared drying on the various biochemical and antioxidant properties of fermented garlic

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Abstract
As health consciousness and demand for functional foods increase, it becomes imperative to investigate the various options for the production of processed functional foods. The bioactive compounds in fermented garlic as a source of functional food possess unique texture and flavor, which make it more nutritious than raw garlic. The different incubation periods preserved during the ageing process of fermented garlic changes its hardness, color and bioactive properties produced various health benefits. The incubation period of 50 days for the development of fermented garlic had better properties than that aged for 30 and 40 days' treatment compared to raw garlic. The content of Allin was reduced by 77% in fermented garlic, and 88% in infrared dried garlic. The surface etching that occurs during infrared treatment increases the total phenols, total flavonoids content and decreases the colour, hardness and Allin content of fermented garlic after treatment. The results obtained for DPPH radical scavenging activity (DPPH) and ferric reducing antioxidant power (FRAP) of Fermented Garlic after infrared treatment indicate that there is an increase in bioactive compounds with an increase in infrared treatment time. Thus, infrared treatment has proven to be a great technique for processing the Fermented Garlic for commercial purposes.

Keywords: Fermented garlic, infrared, allin, texture, antioxidant

1. Introduction

In the Alliaceae family, garlic *(Allium sativum* L.) is a bulbous herb with a spicy, aromatic flavour that becomes noticeably sweeter with cooking. Garlic's distinctive scent is derived from sulfur compounds. Garlic's whole bulb emits minimal scent, but allicin is released when it is cut, producing an intense, pungent, spicy, and mellow scent (Rahman and Lowe, 2006) [18]. It has antibacterial, antiparasitic, antiviral, anticancer, anti-inflammatory, immunomodulatory, and cardioprotective properties. As a result of its carminative, anti-rheumatic, and digestive properties, garlic is used in Ayurvedic formulations for relieving giddiness, muscle pain, ulcers in the intestines and lungs (Gebreyohannes *et al.*, 2013) [7]. However, its pungent taste and characteristic odour limit garlic consumption, especially unprocessed raw garlic (Karnjanapratum *et al.*, 2020) [10].

Fermented Garlic has improved organoleptic properties and expanded its health benefits without an unpleasant odour or pungent taste and used as a traditional garlic product in Southeast Asia. Due to the recent awareness of nutritional and therapeutic foods, the consumption of Fermented Garlic has a significant increase in recent years. Fermented Garlic is generally produced by aging the whole garlic bulb for several days under controlled temperatures (60–90 ^{0} C) without adding additives (Toledano *et al.*, 2016; Kimura *et al.*, 2017) [23, 12]. Due to thermal incubation, the colour of the garlic gradually changes from whitish-yellow to dark brown due to a variety of chemical reactions, including non-enzymatic browning, phenol oxidation, and polysaccharide depolymerization (Bae *et al.*, 2014) [1].

Maillard reaction occurs during production, where amino acids and reducing sugars in garlic combine (Li *et al.*, 2020) [13]; During Maillard reaction, melanoidin and other brown-coloured polymers are produced, which gives Fermented Garlic its colour, and their stability determines the duration of their presence in Fermented Garlic. Fermented garlic's flavour and nutritional properties change significantly during processing. The decomposition of allin content in Fermented Garlic leads to the formation of many sulfur-containing compounds, which have various health benefits when consumed (Liu *et al.*, 2019) [14]. Allicin, a component Fermented Garlic formed the enzymatic decomposition of allin by the enzyme allinase, the odour Fermented Garlic (Rosin, Tuorila, & Uutela, 1992) [19]. Raw garlic's strong, pungent odour is eliminated because the allicin content in Fermented Garlic reduces to insoluble antioxidants like S-allyl cysteine and S-allyl-mercapto cysteine (Corzo-Martínez *et al.*, 2007) [5].

When Fermented Garlic is thermally, the conditions during the process determine the bioactivity. In different locations, enzymes that are intact with reducing sugars are isolated. A drying procedure is needed to release the enzymes from the localized sites and bring them in contact with substrates.

Drying food products using infrared energy (IR) is considered as a promising technique. Infrared radiation does not penetrate the food to a greater depth; instead, heat is generated on contact with the materials (Semwal and Meera, 2020) [21]. Heating with infrared radiation provides an efficient method of heat transfer, which reduces processing times and energy costs. Heat transfer is unaffected by surface irregularities during IR heating, resulting in uniform heating, high heat transfer capacity, direct heating and fast process control, make it an attractive alternative thermal processing technique over other methods (Huang *et al.*, 2021) [8]. The aim of the present study is to determine the effect of infrared radiation on the drying process and quality aspects of the Fermented Garlic.

2. Materials and Methods

2.1 Preparation of Fermented Garlic

Fresh raw garlic was procured from a local market in Thanjavur and stored at room temperature. Fermented Garlic was prepared by incubating whole garlic at a temperature of 70^0 C and relative humidity of 80% for 30, 40 and 50 days in a Humidity chamber, Technico Lab Products (Thirumudivakkam, India).

2.2 Infrared Treatment of Fermented Garlic

Infrared treatment of the black garlic was done by using the equipment developed by Enerzi Microwave Systems for continuous drying of food grains (Belgaum, India), equipped with infrared heater section having power of 4.4 KW. Fermented Garlic was treated with infrared with 1484 KW with time intervals of 5, 10 and 15 minutes.

2.3 Physiochemical Properties

2.3.1 Colour

The samples were completely homogenized in distilled water. The sample was measured using Hunter lab Color Flex EZ Spectrophotometer (Virginia, United States) in reflectance mode. The amount of blackening and colouration of Fermented Garlic was determined by measuring the colour difference (ΔE) in the sample (Li *et al.*, 2020) [13].

2.3.2 Texture

The texture of the black garlic before and after infrared treatment was determined using Texture Analyzer (Stable Micro Systems TA.HD plus C Texture Analyser (Godalming, United Kingdom) with the sample size of garlic 10mm each of width, length and height. Probe P/35 was used with 0.5 mm/s test speed, 0.5 mm/s return speed, 5 g trigger weight, and 5 mm probe distance.

2.4 Functional Properties

2.4.1 Extract Preparation

Fermented Garlic extract is prepared by the method described by (Karnjanapratum *et al.*, 2020) [10] with some modifications. 0.2 gm Fermented Garlic is mixed with 70% methanol (v/v) and sonicated for 15 minutes in 5 ml of the solvent, after which the matter was heated for 10 minutes at 70 ^{0} C. In addition, the same extraction procedure was repeated twice using Whatman no 4 filter paper as the next step. The three supernatants were combined with 70% methanol (v/v) for the measurement of total phenolics and flavonoids, along with determining antioxidant activity.

2.4.2 Total Phenolic Content

The phenol content present in the Fermented Garlic was determined using the method ISO 14502-1 (ISOC 2005), using Gallic acid as a standard. Extracts from samples were transferred to separate tubes in triplicates of 1.0 ml each and diluted with F-C reagent in water to a concentration of 1/10. Add 4 ml of 7.5% sodium carbonate solution to the test tubes and incubate at room temperature for f 60 minutes, and then the absorbance was taken at 765 nm using Shimadzu UV-1800 UV/Visible Scanning Spectrophotometer (Kyoto, Japan). The values are expressed in mg GAE/Kg (gallic acid equivalents per kg) dry matter of Fermented Garlic sample.

2.4.3 Total Flavonoids Content

The total flavonoid content in the black garlic was estimated by the procedure given by (Ordoñez *et al.*, 2006) [16]. Add 0.5 ml of 2% $AlCl_3$ ethanol solution to the 0.5 ml of sample extract. Incubate for 1 hr ar room temperature and read the absorbance spectrophotometrically at 420 nm. The TFC of samples were expressed as mg Rutin equivalents (RE/g) of the sample.

2.4.4 DPPH Radical Scavenging Assay

The DPPH radical scavenging activity of the Fermented Garlic samples was analyzed by using (Chang *et al.*, 2021) [3] with slight modifications. To determine the effect of the Fermented Garlic extract on the DPPH radical, the absorbance of the extract was measured spectrophotometrically.

1 ml of Fermented Garlic extracts were taken in a test tube, and add 4 ml of 0.1 mMol/L DPPH in methanol. Incubate at room temperature in dark place and read the absorbance at 517 nm spectrophotometrically. The results were expressed as millimoles of Ascorbic acid equivalent (AE) of antioxidant activity per gm of sample (mMol AE/L).

2.4.5 FRAP

Ferric Reducing Antioxidant Power Assay measures the reduction of ferric TPTZ (Fe (III)-TPTZ) to the ferrous complex at low pH spectrophotometrically (Benzie and Strain, 1999) [2]. FRAP reagent was prepared in a ratio of 10:1:1 using acetate buffer of 300 mMol/L, ferric chloride of 20 mMol/L and TPTZ of 10 mMol/L. To 0.1 ml of sample, 3 ml of FRAP solution were added, and absorbance was read at 593 nm, and after 4 mins of incubation at 37 ^{0} C to calculate the reduction. The data was expressed in terms of Ascorbic acid Equivalents (mMol AE/L).

2.5 Determination of Allin content

Using HPLC method described by Li *et al.*, 2020 [13] is used to estimate the alliin content of the Fermented black garlic and fresh garlic samples with small modification. In detail, Shimadzu HPLC Nexera X2 (Kyoto, Japan) was used for quantitative analysis of allin in samples. A slurry of 10 g sample was made grinding in 30 ml of milli Q water using motor and pestle for 10 minutes, followed by centrifugation for 15 minutes at 4,000 rpm, at a temperature of 4 °C. By using an aqueous membrane of 0.45 µm, the supernatants were collected and filtered to remove any impurities before injecting the samples into the HPLC system. An HPLC column with a Shimpack (4.6 mm x 250 mm/5.0 mm) was used to separation. Methanol: water in the ration of 30:70 (v/v) is used as mobile phase with a flow rate of 0.7 ml/min. 10 µl of the sample is injected into the injection port of HPLC keeping the oven temperature as constant at 25 °C. Detection is done by using a DAD detector in the wavelength of 214 nm. The quantification of the alliin was made by using corresponding standard curve.

3. Results and Discussion

3.1 Texture

The hardness of a sample is defined as the peak force required for the first compression and the maximum compression force obtained when compressing a sample is considered the hardness of the sample (Karnjanapratum *et al.*, 2020) [10]. According to Toivonen and Brummell, (2008) [22] structural changes, like those occurring in the cell walls and changes in sugar and starch molecules, contribute to the textural changes in Fermented Garlic compared with raw garlic. The results suggest that incubation days had a significant effect on Fermented Garlic hardness and increasing incubation days decreased the hardness of Fermented Garlic. Comparing Fermented Garlic with raw garlic, the hardness values are reduced to a greater extent, and the infrared treatment time also directly affects the hardness values. Infrared treatment reduces the internal contraction force of Fermented Garlic, and thus, the time of treatment decreased the hardness values of the Fermented Garlic. As a result of the decrease in hardness values obtained during treatment, more chemical compounds are released, and the antioxidant and phenolic properties of treated samples are enhanced (Bae *et al.*, 2014; Yuan *et al.*, 2016) [1, 24] Accordingly, the 15 minutes of infrared treatments did increase the bioactive contents of the Fermented Garlic, which correlates with the results obtained for the total phenols and flavonoids. The values of hardness are provided in Table 2.

Table 1: Symbols and descriptions of garlic and Fermented Garlic in this study

Symbol	Description
Raw	**Untreated raw garlic**
T1	garlic maintained at 70oc,80%RH for 30 days
T2	garlic maintained at 70oc,80%RH for 40 days
T3	garlic maintained at 70oc,80%RH for 50 days
I1	Fermented Garlic treated with IR for 5 minutes
I2	Fermented Garlic treated with IR for 10 minutes
I3	Fermented Garlic treated with IR for 15 minutes

Table 2: Hardness and color-Lab values of raw, untreated and treated Fermented Garlic

	Hardness	L*	a*	b*
Raw	$6791.17±0.78^b$	$70.38±0.16^a$	$-1.03±0.01^e$	$19.19±0.06^a$
T_1	$143.03±0.66^g$	$16.55±0.34^d$	$0.25±0.05^d$	$-0.42±0.03^g$
T_2	$9110.99±0.6^a$	$19.33±0.09^b$	$1.28±0.06^a$	$1.02±0.01^e$
T_3	$670.87±0.56^d$	$19.11±0.17^b$	$1.26±0.05^a$	$1.16±0.02^d$
IR_1	$792.32±1.02^c$	$18.38±0.12^c$	$0.94±0.02^c$	$0.58±0.01^f$
IR_2	$391.57±0.39^f$	$16.69±0.26^d$	$1.14±0.03^b$	$1.74±0.01^b$
IR_3	$593.57±1.15^e$	$15.53±0.28^e$	$0.84±0.01^c$	$1.34±0.12^c$

Statistical difference between each values is expressed using different alphabets in same column

3.2 Colour

Colour is a physicochemical property that significantly influences consumers' perception regarding food products (Ryu and Kang, 2017) [20]. The total colour difference (ΔE) values of all the samples were compared (Li *et al.*, 2020) [13] to determine the extent of blackening in Fermented Garlic. The Maillard reaction that occurred during the aging process leads to the formation of dark-coloured compounds, which significantly led to the dark colour of Fermented Garlic. The production of Fermented Garlic changed the L*, a*, and b* values significantly, and the white-yellow color of the garlic changed to the dark brown colour (Kang, 2016) [9]. The colour values of Fermented Garlic suggest that the number of incubation days is directly proportional to the ΔE value; the colour change is maximum for 50 days incubation compared to 30 and 40 days. Therefore, 50 days incubation represents high-quality Fermented Garlic with desired brown colour. Chi *et al.* (2014) reported that Fermented Garlic compared to raw garlic had increased colour redness (a* value) and reduced lightness (L* value) and yellowness (b* value). When compared with raw garlic, the lightness of Fermented Garlic decreased significantly as it reduced from 70.55 to 16.92, 18.82, and 19.31 after incubation for 30 days, 40 days and 550 days, respectively. Infrared treatment time is inversely proportional to the total colour difference values since it decreases as treatment time increases. The L*, a*, b* values are given in Table 2.

3.3 Total Phenolic Content

The total phenolic content in the garlic samples was presented in Table 3. Compared to raw garlic, the total phenolic content of black garlic was increased with the period of incubation. Present results show the highest phenolic content was 147.12 mg GAE/kg in T_3 and the lowest in 39.69 mg GAE/kg in T_1.
The present study results are in accordance the Kim *et al.*, 2013 [11], showed that phenolic content of 83.76 – 139.02 mg GAE/kg in Fermented Garlic. The total phenolic content of IR treated Fermented Garlic (I_3) was increased by 23% compared to the T_3 sample. The increase in phenolic content in Fermented Garlic occurs as phenolic compounds are released from the bound form in raw garlic. IR treatment of the Fermented Garlic has allowed to release more bound phenolic compounds. Hydrolysis of polyphenolic macromolecules liberates more phenolic hydroxyl groups when heated during the processing. The Maillard reaction is known to result in the production of phenolic compounds (Qinzhu *et al.*, 2018) [17].

Table 3: Total Phenol content, total flavonoid content and antioxidant properties of raw, untreated and treated Fermented Garlic (mean)

	TPC GAE/kg	TFC RE/kg	FRAP mMol AE/L	DPPH mMol AE/L
Raw garlic	$6.45±0.26^g$	$7.69±0.17^f$	$0.09±0.01^f$	$9.93±0.06^e$
T_1	$38.93±0.66^f$	$32.53±0.67^e$	$0.67±0.02^e$	$24.29±0.48^d$
T_2	$95.61±0.39^e$	$44.06±0.38^d$	$0.85±0.04^d$	$32.19±0.35^c$
T 3	$146.74±0.34^d$	$57.21±0.22^c$	$1.37±0.06^c$	$42.33±0.58^b$
IR 1	$161.05±1.11^c$	$58.05±1.24^c$	$1.48±0.06^c$	$42.99±0.82^b$
IR 2	$181.6±1.03^b$	$68.71±0.91^b$	$1.9±0.09^b$	$46.22±0.52^a$
IR 3	$197.4±1.91^a$	$97.11±2.27^a$	$3.08±0.07^a$	$47.17±0.39^a$

Statistical difference between each values is expressed using different albhabets in same column

3.4 Total Flavonoids Content

The total flavonoid content of the samples was presented in Table 3. According to the study, increased duration of the incubation has increased the flavonoid content in the Fermented Garlic. The flavonoid content was increased by 5 times after incubation.
During production of Fermented Garlic, the incubation

temperature have released the bound polyphenols and flavonoids. The highest value was seen in T_3 as 57.42 mg RE/kg and lowest in T_1 as 32.84 mg RE/kg. The present study results are in accordance the Kim *et al.*, 2013 [11], showed the flavonoid content in the range of 31.99-103.7 mg QE/kg. IR treated Fermented Garlic (I_3) has flavonoid content of 99.11 mg QE/kg, this may due to thermal treatment the bound flavonoid compounds are released. Choi *et al.* (2014) [4] also suggested that the garlic during thermal treatment has liberated the polyphenols and flavonoids.

3.5 Antioxidant activity

The DPPH scavenging activity and ferric reducing antioxidant power of raw garlic, Fermented Garlic before and after IR treatment are presented in Table 3. Basically, radial scavenging shows the ability of a compound to donate electrons to DPPH, which produces a more stable molecule with a reduced absorbance. A significant difference was seen between fermented garlic samples and raw garlic samples in terms of DPPH radical scavenging. The results presented shows that as increase in the incubation time has improved the antioxidant activity of Fermented Garlic. The strongest antioxidant activity was found in T_3 sample and weaker in T_1 sample. The IR treated Fermented Garlic also showed an increase in the DPPH radical scavenging activity compared to the untreated samples. However, there is no significant difference ($p< 0.05$) in the increase in the DPPH scavenging activity between IR3 and IR2. DPPH radical scavenging is generally associated with a reduction in power. Ferricyanide is reduced to its ferrous form by antioxidants, which gives it reducing power. The reducing power of the heat-treated garlic samples increased significantly over time, reaching its peak after 60 days. Fermented garlic treated with IR had a 5-6 fold increase in reducing power.

The increase in antioxidant activity may be due to the liberation of phenolic compounds and flavonoids during thermal treatment. Additionally, the degradation of alliin results in the formation of sulfur compounds such as diallyl sulfide, trisulfide, and SAC, which contribute to Fermented Garlic's antioxidant activity. (Leelarungrayub *et al.*, 2006). The antioxidant activity of garlic may be enhanced if it is heated at a high temperature. The hydrogen atoms donated by browning products appear to promote antioxidant activity. Furthermore, browning products have been demonstrated to inhibit free radical formation through their antioxidant action (Eichner, 1980; Manzocco *et al.*, 2000) [6, 15]. The comparative study of DPPH and FRAP on Fermented Garlic shows that the incubation time and IR treatment has improved the antioxidant properties.

3.6 Alliin content

Alliin is a significant component of garlic. The figure 1 below shows how alliin content changed after Fermented Garlic was processed. Alliin levels decreased significantly after the formation of Fermented Garlic in all three treatments ($p< 0.05$). Fermented Garlic's alliin content decreased by 70% compared with raw garlic, while IR-treated Fermented Garlic's alliin content decreased by 88%. As the time of the incubation increases the alliin content was decreased (Li *et al.*, 2020) [13]. Highest value was found in T_1 as 1355.76 mg/kg, whereas the T_3 sample contains lowest alliin content of 519.232 mg/kg. The results are in accordance with the previous study conducted by Li *et al.*, 2020 [13] were the HHP and HPCD processed Fermented Garlic contains the alliin content of 279.44 and 356.01 µg/g. The degradation of alliin resulted in its decline in concentration, which was converted to S-allyl cysteine and arginine along with other degraded products (Bae *et al.*, 2014) [1]. Additionally, non-enzymatic browning takes place contributing to the maillard reaction due to the reaction of alliin with fructose during fermentation.

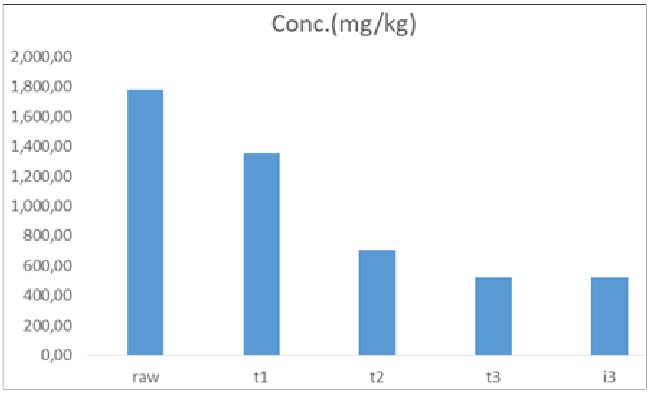
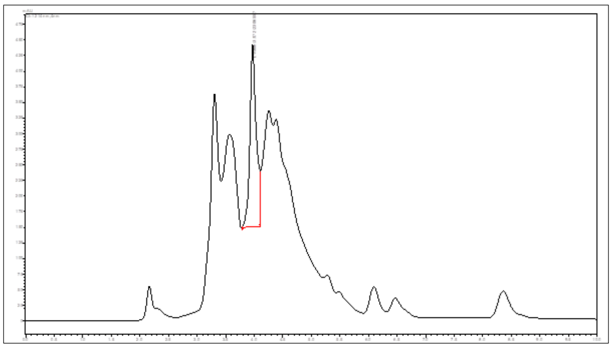
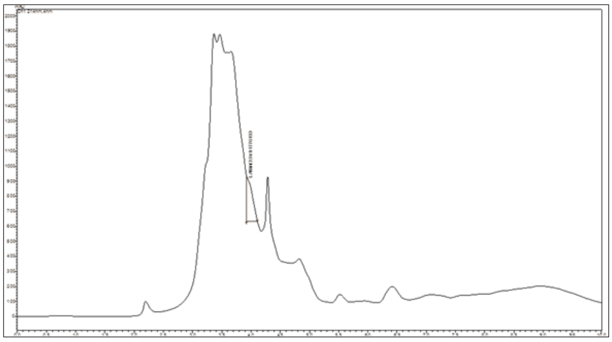


Fig 1: Allin content of Raw, T1, T, T3, I3

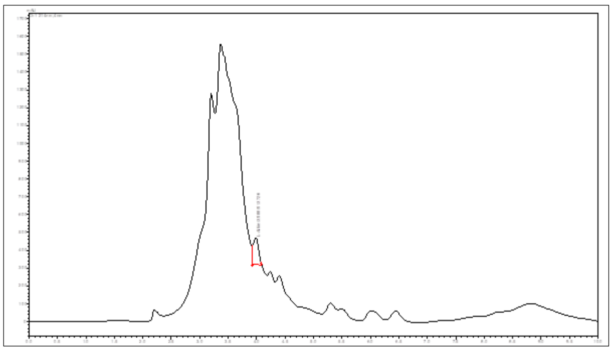
Supplementary data



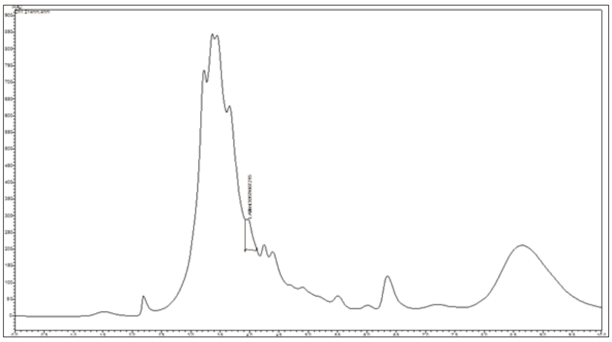
Raw garlic



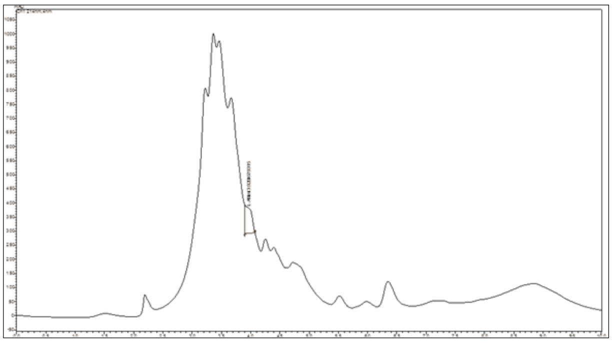
T1



T2



T3



IR_3

4. Conclusion

The study demonstrated that infrared treatment increased the biochemical and antioxidant properties of Fermented Garlic. A longer incubation time improves the quality of the Fermented Garlic by enhancing the maillard reaction. The Fermented Garlic with 50 days treatment has improved texture, colour and bioactive compounds among other treatments. In contrast, IR treatment led to increased free radical scavenging activity by DPPH and reducing power primarily through the production of browning products, SAC, total phenols, and total flavonoids. The infrared treatment I_3 sample showed enhanced antioxidant activity, as the polyphenols and flavonoids liberated are more. Multiple compounds are significantly associated with Fermented Garlic's antioxidant activity, according to these findings. This study may also serve as a basis for future research on improving the functional properties of Fermented Garlic using infrared treatment.

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