

Impact of Infra-red drying temperature on total phenolic and flavonoid contents, on antioxidant and antibacterial activities of ginger (*Zingiber officinale Roscoe*).

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Abstract: In this work, the effect of infrared drying temperatures between 40°C and 70°C on the antioxidant capacity and antibacterial activity of *Zingiber officinale* was studied. The total phenolics content (TPC) and total flavonoids content (TFC) were analyzed. The evaluation of antimicrobial activity of ginger extracts was determined using a disc diffusion method against six bacterial cultures. The antioxidant activity was evaluated in vitro by means of DPPH and β -carotene assay. When comparing the fresh with the corresponding dehydrated ginger samples, it was shown that the antioxidant activity of the ginger extracts decreased as the drying temperature increased. Thermal degradation, especially at 60 and 70°C resulted in a notable reduction in TPC. However, TFC was not affected by drying temperatures. Results have shown a minor loss of antibacterial activity when drying temperature increased.

Keywords: *Zingiber Officinale*, Infrared drying, Ginger, Antioxidant, Antimicrobial.

I. INTRODUCTION

Ginger or its scientific name *Zingiber officinale Roscoe*, is a monocotyledon belonging to the family Zingiberaceae. The rhizome of ginger (*Zingiber officinale Roscoe*) is widely consumed as a common spice throughout the world and used in traditional oriental medicine [1]. Its major pungent constituent, [6]-gingerol has been reported to exhibit antioxidative activity against linoleic acid autoxidation and peroxidation of phospholipid liposomes and to scavenge trichloromethylperoxyl- and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals [2]; [3].

The chemical composition of fresh ginger are different due to different geographical locations [4]; [5]; [6]; [7], varieties [8] extraction methods [9]; [10]; [11] and processing [12]; [13]; [14].

Dehydration is one of the most widely used methods for fruits and vegetables preservation. Its main objective is the removal of water to the level at which microbial spoilage and deterioration reactions are minimized. However, it is well known that during hot-air drying, vegetables undergo physical, structural, chemical and nutritional changes that can affect quality attributes like texture, color, flavor, and nutritional value [15]; in addition, the removal of water from the agricultural or food materials is very energy consumption process. The efficiency of the drying of waste materials is an important economic consideration with respect to both energy and time. Therefore, in this study, infrared (IR) drying technique was used to achieve an increase of the effective thermal processing.

IR drying is based on the action of infrared wavelength radiation from a source, which interacts with the internal structure of the sample and thus increases its temperature and favors the evaporation of its moisture content. Moreover, IR energy is transferred from heating element to the sample product without heating surrounding air. Thus, in this radiating process the temperature of the inner layers of the sample is higher than that of the surrounding air. As a result, the drying of the sample takes place from inner to outer layers via both radiation and convection thermal phenomena. This leads to a high rate of heat transfer with respect to conventional drying.

The basic requirements for a vegetable dryer are that it must achieve the required amount of drying in a reasonable time, obtain a product of acceptable quality, and minimize operative costs [16]. Besides, the increasing demand for high-quality shelf-stable dried products requires the optimization of the drying process conditions, especially temperature, with the purpose of accomplishing not only the efficiency of the process but also the final quality of the dried product [17].

Therefore, the aim of this work is to study the effect of IR drying temperature on total phenolic and flavonoid content, on antioxidant and antibacterial activities that resulted of the drying process of *Zingiber officinale*.

II. Materials And Methods

1. Plant Material

Raw ginger (*Zingiber Officinale*) was purchased from local market. The rhizomes of ginger samples were prepared manually. The edible parts were divided into five parts: one of them was used as fresh (raw) ginger (FG), and four others (G-40; G-50; G-60; and G-70) were dried at 40°C, 50°C, 60°C, and 70°C, respectively. After drying, samples were pulverized into fine powder using a grinder (MF 10 basic mill, GMBH & Co., Staufen, Germany).

2. Drying Process

Infrared drying is manifested by an electromagnetic field conveyed by frequencies which excites the water's molecules. The molecular agitation which results from it causes intermolecular shocks that involve a heating of the product and thus the vaporization of the water's molecules. The procedure consists in placing a sample of 40±0,1g of the product, cut in thin layer (thickness of 3 to 5 mm) inside the device and on an aluminum support placed on the top of the balance. A schematic diagram of the experimental apparatus is shown in "Fig.1".

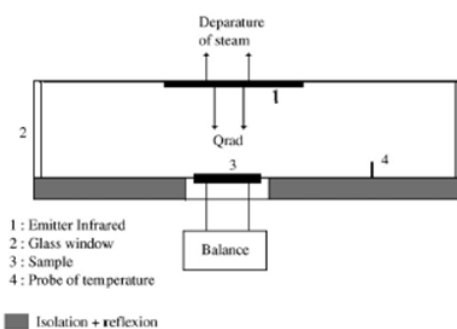


Fig. 1 - Experimental apparatus: IR moisture analyzer

The technical data of the above infrared dryer and moisture analyzer equipment is as following:

Frequency: 50-60 Hz

Power consumption (max): 660 VA

Temperature range: 40-160 °C

Reproducibility of the temperature: 1 %

Graduation: 1°C

Time switch (range): 0-300 min

Reproducibility (sample=1g): 0.2%

Resolution of balance: 1 mg

The amount of evaporated water during drying was determined at about 2 min interval in each drying temperature. The samples were dried until they reached a constant weight. Drying tests were replicated three times at each temperature and averages weight loss are reported. Moisture content (g water/g dry matter) was determined using the following equation:

$$M = \frac{(W_0 - W) - W_1}{W_1} \quad (1)$$

Where M is moisture content (g water/g dry matter), W_0 is initial weight of sample, W is the amount of evaporated water, W_1 is the sample dry matter mass.

3. Extracts Preparation

After IR drying, 5 g of dried samples (G-40, G-50, G-60, and G-70) were extracted in magnetic stirring with 50 mL of methanol (100%) for 30 min. Extracts were kept for 24 h at 4°C, filtered through a Whatman filter paper, and evaporated to dryness under vacuum. Then, the extract was tested for total polyphenolic content, total flavonoid content, antioxidant and antibacterial activity. In each assay, the activities of the extracts dried by infrared were compared with the value of the extract from raw ginger (FG).

4. Total Polyphenol Content (TPC)

Total phenolic content of the (methanol) extracts was determined using Folin-Ciocalteu method [18] slightly modified by [19]. An aliquot of diluted sample extract was added to 0.5 mL of distilled water and 0.125 mL of the Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 10 min, before addition of 1.25 mL of 7% w/v sodium carbonate. The solution was then adjusted with distilled water to a final volume of 3

mL and mixed thoroughly, and held for 90min at room temperature. After incubation in dark, the absorbance of the resulting blue color was measured at 760 nm. Gallic acid was used as a standard, and total phenolic content of plant extracts was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW) through the calibration curve with gallic acid. Data reported of three replications.

5. Total Flavonoid Contents (TFC)

Total flavonoid content was measured using a colorimetric method developed by Chang and al. [20]. A 500 μ L aliquot of diluted sample or standard solution of (+)-quercetin were mixed with 1.5 mL methanol, 0.1 mL of AlCl₃ solution (10%), 0.1 mL of potassium acetate (1M), and 2.8 mL of distilled water. After 30 min incubation at room temperature, the absorbance of the mixture was determined at 415 nm. Total flavonoid content was expressed as mg of quercetin equivalents per gram of dry weight (mg QE equivalent/g DW). All samples were analyzed in triplicates.

6. Determination of Antioxidant Assays

6.1. Scavenging ability on DPPH radical

The radical scavenging activity of the extracts against DPPH (2, 2'-diphenylpicrylhydrazyl) radical was measured according to [21], slightly modified as follow: One milliliter of different dilutions of extracts were added to 0.25 mL of daily prepared methanol DPPH solution (0,1 mM). The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark. The absorbance of the resulting solution was then measured at 517 nm and corresponded to the ability of extracts to reduce the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine. Control sample was prepared without adding extract. The antiradical activity was expressed as IC₅₀ (μ g/mL), the extract dose required to cause a 50% inhibition. A lower IC₅₀ value corresponds to a higher antioxidant activity of plant extract. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH} \cdot \text{scavenging effect (\%)} = [(A_0 - A_1)/A_0] \times 100 \quad (2)$$

Where A₀ is the absorbance of the control, and A₁ is the absorbance of the sample. All samples were analyzed in triplicate.

6.2. β -carotene bleaching test

The method described by [22] was used with a slight modification. A β -carotene and linoleic acid solution was prepared by dissolving 0.5 mg of β -carotene in 1 mL of chloroform then adding 25 μ L of linoleic acid and 200 mg Tween 40. Chloroform was evaporated under vacuum and 100 mL of aerated distilled water were added.

300 μ L of each extract were added to 2.5 mL of this mixture and incubated in a 50 °C water bath for 2 h. Reading of all samples were performed immediately (t=0) and after 2 h of incubation. Distilled water was used as a control, instead of the sample extract, and prepared in the same manner as the samples with antioxidants from ginger. The absorbance was measured at 470 nm

$$AA\% = \frac{\beta\text{-carotene content after 2h assay}}{\beta\text{-carotene content}} \times 100 \quad (3)$$

Tests were carried out in triplicate. Extract concentration providing 50% inhibition (IC₅₀) was obtained by plotting inhibition percentage versus extract concentrations.

7. Antibacterial Activity

The antimicrobial activity of *Zingiber Officinale* extracts was determined against six bacterial cultures using a paper disc diffusion method. Three bacteria Gram-positive: *Listeria monocytogenes* (ATCC19111); *Bacillus cereus* (ATCC6633), *Staphylococcus aureus* (ATCC12600), and three bacteria Gram-negative: *Escherichia. Coli* (ATCC25922), *Salmonella enteritidis* (ATCC13075) and *Pseudomonas aeruginosa* (ATCC17588), were used.

The bacterial strains were cultured in a nutrient broth (Merck) for 24 h and diluted with sterilised peptone water. Then, 100 μ l of each culture (10⁶ CFU) was spread onto the surface of Mueller–Hinton agar (Oxoid) to create a bacterial lawn. Sterile blank filter paper discs of 6 mm in diameter (Oxoid) were wetted with 20 μ l crude extract of *Zingiber Officinale* (100 mg/ml) and left to dry before being placed on the microbial lawn. The plates were incubated at 37°C for 24h. The antibacterial activity was compared with 20 μ l of 10% acetic acid. Antimicrobial activity was determined based on the diameter of the clear zone surrounding the paper discs. This was the diameter of the zone visibly showing the absence of growth, including the 6-mm disk. Three replicate discs were prepared for each extract in this study.

8. Statistical Analysis

All quantitative analyses were expressed as mean value ± SD for three replicates. Differences among treatments for each parameter studied in ginger were determined by using the one-way ANOVA test at 95% confidence interval. The statistical differences between the treatment groups were estimated using the Tukey test for multiple comparisons. Statistical analysis were conducted using SPSS, version 14.0

III. Results And Discussion

1. Evolution of moisture content of ginger slices during drying

Initial moisture content of (FG) was 88.34±2.40g per 100g fresh ginger and final moisture content of four drying samples was as follows 0.52927; 0.31992; 0.08888 and 0.043857 (g water/g dry matter), for G-40, G-50, G-60 and G-70, respectively. The drying time for reaching the equilibrium moisture contents at different drying temperatures is shown in Table 1.

Table 1 - Drying time and equilibrium moisture content of samples at different drying temperatures

| Drying Temperature (°C) | Drying time (min) | Equilibrium moisture content (g water/g dry matter) |
|-------------------------|-------------------|---|
| 40 | 200 | 0.52927 ± 0.092 |
| 50 | 190 | 0.31992 ± 0.066 |
| 60 | 166 | 0.08888 ± 0.025 |
| 70 | 114 | 0.043857 ± 0.013 |

Values are means ± standard deviation

The moisture content values obtained for the drying temperatures 40-50-60-70°C were converted into the moisture ratio (MR). The dimensionless moisture ratio might be obtained using the following [23]: $MR = (M_t - M_e) / (M_0 - M_e)$. Nevertheless, the moisture ratio was simplified to M_t / M_0 , where M_t and M_0 are the moisture content at any given time and the initial moisture content, respectively.

Dehydration curves of ginger at 40, 50, 60 and 70°C are presented in “Fig. 2”. Drying time decreased considerably when temperature was increased from 40 to 70°C. At higher temperature the rate of water evaporation from the food increases thus causing a reduction in drying time. The decrease in the drying time with an increase in drying temperature for ginger slices have been observed by [24], [25], and [26] with different methods of drying.

The effect of temperature on the dehydration rate of ginger at 40, 50, 60 and 70°C temperatures is indicated in “Fig. 3”. It can be seen that higher drying rates were obtained at higher drying temperatures. The moisture decreased faster at higher drying temperatures in all cases, as expected. Therefore, a higher drying temperature produced a higher dehydration rate and consequently the drying time decreased due to the increase of heat transfer inside the ginger slices, and the acceleration of water migration inside them.

A constant drying rate period was not observed in all cases. The same results have been found for other vegetables in previous reports, in which only the falling drying rate period has been observed. ([27]; [28]; [29]; [30]; [31]).

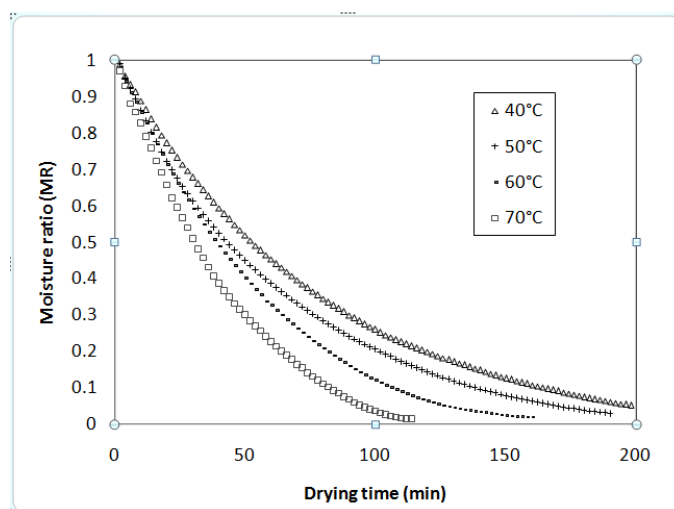


Fig. 2 - Drying curves of ginger at different temperatures

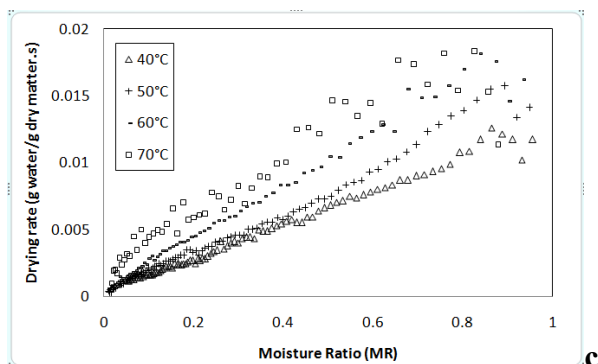


Fig. 3 - Drying rates versus moisture content of ginger at different temperatures

2. Total Phenolic And Flavonoid Contents

Table 2 shows total phenolic content (TPC) (mg GAE/g dry weight) and total flavonoid content (TFC) ($\mu\text{g/g}$ dry weight) for FG and dehydrated samples respectively. The TPC, calculated using the standard curve of gallic acid ($R^2=0.998$; $y=0.0047x+0.0635$) in all dehydrated samples were inferior than that of FG (4.60 mg GAE/g DW), it ranged from 0.77 to 2.59 (mg GAE/g DW).

Our values were higher than those reported by [32], who compared TPC of 12 different ginger varieties, and found that the TPC ranging from (0.7-1.58 mg tannic acid eq./ g dry weight), but lower than those reported by ([33]), who found that the total phenols of the alcohol extracts of ginger were 870.1 mg/g dry extract.

However, all extracts of ginger (dehydrated and fresh) showed almost same total flavonoid content. TFC ranged from (0.24 to 0.15 mg QE/g DW) (Table 2), the initial content is comparable to the results reported by [32] who obtained a TFC ranged from 0.13 to 0.38mg quercetin eq./g DW. Based on our finding, TFC was not affected by drying temperature; nevertheless, the meteorological conditions, season and post-harvest conditions have been reported as additional source of variance in the TFC [34].

Table 2 - Total phenol and flavonoid contents for the studied extracts of ginger

| Ginger extract | Total phenolics (mg GAE/g DW) | Total flavonoids (mg QE/g DW) |
|----------------|-------------------------------|-------------------------------|
| FG | 4.60 ± 0.66 ^a | 0.24 ± 0.09 ^a |
| G-40 | 2.59 ± 0.17 ^b | 0.18 ± 0.03 ^b |
| G-50 | 1.94 ± 0.32 ^b | 0.15 ± 0.04 ^c |
| G-60 | 0.80 ± 0.07 ^c | 0.17 ± 0.01 ^b |
| G-70 | 0.77 ± 0.067 ^c | 0.20 ± 0.04 ^a |

Values are means ± standard deviation

Processing methods are known to have variable effects on TPC of plant samples. The influence of temperature on TPC for fresh and dehydrated ginger is shown in table 2 ($p<0.05$). It can be observed that an increase in IR drying temperature had an important effect on the total phenolic content, leading to a notable reduction in these components, especially at higher temperatures (60 and 70°C). Losses of TPC due to thermal degradation have been also reported by other authors ([35]; [36]). In addition, decreases in TPC during dehydration may be ascribed to the binding of polyphenols with other compounds or the alterations in the chemical structure of polyphenols which cannot be extracted and determined by available methods ([37]; [38]).

3. Antioxidant Activity

The total antioxidant activities of vegetables cannot be evaluated by any single method, due to the complex nature of phytochemicals [39]. Two or more methods should always be used in order to evaluate the total antioxidative effects of vegetables. Accordingly, the antioxidant activities of the methanol extracts of ginger was determined by two methods: DPPH (1,1-diphenyl-2-picrylhydrazyl) assay and β -carotene bleaching test.

DPPH is a free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [40]; The reduction capability of DPPH radical was determined by the decrease in absorbance induced by plant antioxidants.

Results in "Fig.4" demonstrated that all samples exhibited dose-dependent DPPH radical scavenging activities expressed as IC_{50} values. The free radical-scavenging capacity was different between the fresh ginger and the dried ginger extracts. Most of the extracts from ginger dried by infrared showed slightly lower activities than that of FG. The methanolic extract of FG showed higher activity ($IC_{50}= 0.13 \mu\text{g/mL}$) followed by dehydrated sample at 70°C ($IC_{50}= 0.28 \mu\text{g/mL}$), which showed higher activity than those of other dehydrated

samples followed by G-40 ($IC_{50}= 0.32 \mu\text{g/mL}$), G-50 ($IC_{50}=0.35\mu\text{g/mL}$) and G-60 ($IC_{50}= 0.49 \mu\text{g/mL}$). Some authors reported a correlation between the TPC and DPPH assay [41]. However, in this study the correlation coefficient between DPPH scavenging activity ($R^2= 0.602$) was found to weak, indicating that probably other phenolics or non-phenolics compounds might be also contributors to the antioxidant activity.

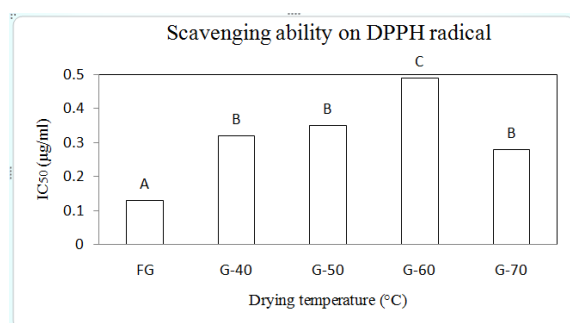


Fig.4 - Scavenging activity, expressed as IC_{50} values ($\mu\text{g/mL}$), on DPPH radical of fresh and dried extracts of ginger.

In general, the antioxidant activity of vegetable extracts is associated with specific compounds or classes of compounds, which are affected by the operating condition. Kelly et al [42] have found that the major bioactive compounds present in the ginger extracts were gingerols, α -zingiberene and shogaols, the amounts of these compounds were significantly affected by temperature, pressure and solvent. On the other hand, the antioxidant activity of the ginger extracts determined using the coupled oxidation of linolenic acid and β -carotene, decreased to 60% in the absence of gingerols and shogaols [42].

Dugasani et al., [43] comparing the antioxidant activities of gingerols and their natural analogues, they found that [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol exhibited substantial scavenging activities with IC_{50} values of 26.3, 19.47, 10.47 and 8.05 μM against DPPH radical. Thus, [6]-shogaol has exhibited the most potent antioxidant propertie. Furthmore, according to Jolad et al [44], dry ginger contains more of [6]-shogaol than fresh ginger. This may justifies the increase of antioxidant at higher drying temperature (70°C).

Based on the β -carotene bleaching test, undergoes rapid discoloration in the absence of an antioxidant. The presence of an antioxidant such as phenolics can hinder the extent of β -carotene destruction by “neutralizing” the linoleate free radical and any other free radicals formed within the system [45]. “Fig. “5 depicts the inhibition of β -carotene bleaching by the fresh and dried extracts of ginger. All the extracts showed good scavenging activity for reduction of the radicals generated by the oxidation of linoleic acid and the activity with IC_{50} ranging from 2.52 to 4.63 mg/mL. FG extract showed the highest ability to prevent the bleaching of β -carotene.

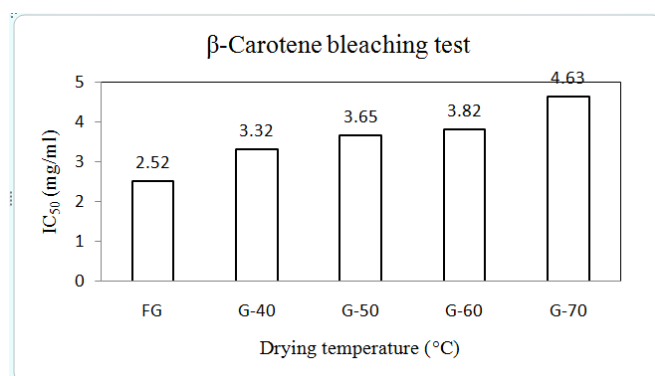


Fig. 5 - Antioxidant activity expressed as IC_{50} (mg/mL) of fresh and dehydrated samples from ginger in β -carotene bleaching test

4. Antibacterial Activity

The extracts were screened at concentration of 2mg /disc in a disc diffusion test for their antibacterial activities against six species of bacteria. The diameters of the inhibition zones obtained are presented in Table3. The results from the bioassays revealed that the different extracts of *Z. Officinale* possessed significant antibacterial activity against *S. aureus*, *B.cereus* et *S. enteritidis*, and moderate inhibition against *L. monocytogene* et *E. coli*, which could have resulted from the presence of geraniol, geranial, linalol, camphene, β -caryophyllene [46] caryophyllene oxide, α -pinene, α -terpineol, and 1,8-cineol [47]: essential oils known to

possess antibacterial activity. The yield of volatile oil from rhizomes varies from 1% to 3% depending on the place of origin and whether the rhizomes are fresh or dry [48].

However the fresh extract of *Z. Officinale* present the best antibacterial activity (strong inhibition: +++). When comparing the dehydrated samples, the higher inhibition was observed at low temperature (40°C and 50°C), rather the extracts dried at a higher temperature (60°C and 70°C) which presents a weak inhibition against *B.cereus* and they are without activity against the rest of bacteria. Losses of antibacterial activity could be due to the denaturation of various compounds mentioned above, responsible for it. Based on our findings, an increase in drying temperature leads to a reduction in the antibacterial activity. Thus we can say that antibacterial activity of *Z. officinale* is heat sensitive.

Ding et al [49], have studied different drying method of ginger: air drying (AD), microwave drying (MD), vacuum drying (VD), and freezing drying (FD). Volatiles were extracted by gas chromatography-mass spectrometry. Results indicated that (AD) decreased the relative content of Camphene from 6.675 in fresh ginger, to 0.559 and 0.275 in (AD50°C) and (AD60°C) respectively. This is in accordance with our finding indicating that compounds known to possess antibacterial activity are heat sensitive.

It is well known that methanol and other alcohols possess bactericidal activity due to their lipophilicity which enhances penetration into target organisms [50]. Because of this fact, we use only pure water to dissolve all the extracts, thus eliminating the influence of alcohol and DMSO usually used to dissolve extracts before being tested for their bactericidal activities. Therefore, in this experiment, the bactericidal effect could be only attributed to the antimicrobial compound(s) present in the extracts.

Generally, the high concentrations of phenol compounds in these natural products accounts for their antioxidant property [51]. Addition to the enormous scientific studies reported by several researchers on the antimicrobial activities of these herbs, there is also enough documented data suggesting the high positive correlation between antimicrobial activity, total phenolic content and antioxidant property ([52]; [53]).

Table 3. Inhibition zone indicating the antibacterial activity of *Z. Officinale*

| | Extracts | | | | | Acetic acid |
|-------------------------------|----------|------|------|------|-----|-------------|
| | G-70 | G-60 | G-50 | G-40 | FG | |
| Gram positive bacteria | | | | | | |
| <i>S. aureus</i> | - | + | ++ | ++ | +++ | + |
| <i>B.cereus</i> | + | - | - | ++ | ++ | + |
| <i>L. monocytogenes</i> | - | - | - | + | + | + |
| Gram negative bacteria | | | | | | |
| <i>S. enteritidis</i> | - | - | - | ++ | +++ | - |
| <i>E. coli</i> | - | - | - | + | ++ | +++ |
| <i>P. aeruginosa</i> | - | - | - | + | + | - |

Key : (-): no inhibition ; (+): weak inhibition (<8 mm) ; (++) : modest inhibition (0mm<=x<=8mm); (+++): strong inhibition (diamètre> 10mm) ; all readings were inclusive of 6mm disc diameter.

IV. Conclusion

The effect of Infrared drying temperature on total phenolic content, total flavonoid content and antioxidant and antibacterial activities between 40 and 70 °C was investigated. Results have shown an alteration in the TPC of ginger, particularly at 60 and 70°C drying temperatures. However, the radical scavenging activity showed higher antioxidant activity at high temperatures 70°C compared to low temperatures (40–50°C). This is in accordance with the weak correlation coefficient above found between DPPH scavenging activity and TPC (R²= 0.602), indicating that probably non-phenolics compounds might be also contributors to the antioxidant activity. However, we showed losses of antibacterial activity when drying temperature increased, indicating that compound(s) known to possess antibacterial activity are heat sensitive. In consequence, the infrared drying at higher temperature was unable to eliminate the antioxidant activity of the extracts, but able to decrease antibacterial activity

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