Comparative study of ginger (*Zingiber officinale* Roscoe) as raw and herbal tea: microbiological, mycotoxin, and phytochemical quality

Emad A. H. Guirguis
*General Organization of Teaching Hospitals and Institutes (GOTHI), dr_emad_atef@yahoo.com*

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Comparative study of ginger (Zingiber officinale Roscoe) as raw and herbal tea: microbiological, mycotoxin, and phytochemical quality

Emad A.H. Guirguis
Department of Food Hygiene, National Nutrition Institute (NNI), General Organization of Teaching Hospitals and Institutes (GOTHI), Cairo, Egypt

Abstract

Background
Ginger, commonly available as fresh rhizomes and dry powder, is prone to be contaminated either in spice or herbal tea form.

Materials and methods
Microbiological profile, total aflatoxin content, and phytochemical component were assessed using standard methods. Moreover, disc diffusion method was applied to investigate the antimicrobial ginger extract activity against foodborne pathogens.

Results
The most abundant identified compound was zingiberene in fresh (38.59%) and in dry powder (43.93%). The antimicrobial activity of ginger extract was more effective against gram-positive bacteria when compared with the results obtained by gram-negative bacteria. The microbiological quality revealed high contamination of dry samples with total aerobic mesophilic bacteria, mold and yeast count as a group, coliforms, and Bacillus cereus, which exceeded the Egyptian standards, unlike the fresh rhizomes and herbal tea samples (P < 0.05), which were in acceptable levels. Furthermore, Salmonella spp., Staphylococcus aureus, Escherichia coli, and Clostridium perfringens were not detected in any of the samples. Total aflatoxin was detected within the acceptable levels (6.2 μg/kg) in dry samples only.

Conclusion
Contamination was more pronounced in dry samples which need monitoring and control to fit the critical limits.

Keywords: Ginger, herbal tea, microbiological quality

INTRODUCTION
Ginger (Zingiber officinale Roscoe), which belongs to the family Zingiberaceae, is a tropical rhizomatus plant originated in South-East Asia, being used as spice and herbal tea with medical value [1]. It has a beneficial effect in either fresh or dried form attributed its volatile compounds [2]. The most abundant active compounds is shogaols in dry ginger, whereas is gingerols in fresh ginger, because the heat treatment during drying degrades gingerols to shogaols [3]. Moreover, it contains biological active compounds (i.e. zingiberene, caffeic acid, curcumin, hogoals, bisabolene, salicylate, and capsaicin) and other types of lipids [4].

Ginger, as for all spices, is susceptible to be contaminated with microorganisms and mycotoxins during growth, processing, and storage steps; however, it has antimicrobial activity [5-7].

Ginger harbors a wide variety of contaminants and consequent food-borne infections during the growth, processing, and storage progresses [7, 8]. When the spice is added to foodstuffs without being subjected to thermal treatment, there is increased risk of growth of pathogens [5]. Ginger had the lowest microbial contamination (1.5 × 10^4 cfu/g) among the studied spices by Moore et al. [9]. The microbiological tolerance levels of raw and...
ginger herbal tea were coined by the Egyptian standards (ES: 2986) [10] as total aerobic mesophilic bacteria (TAMB, 10⁴ and 10⁵ cfu/g), mould and yeast count (10⁴ and 10⁵ cfu/g), Escherichia coli (10⁴ and 10⁵ cfu/g), and Enterobacteriaceae (10⁴ and 10⁵ cfu/g), whereas Clostridium spp., Salmonella spp., and Shigella spp. should not be detected. Powdered ginger is found to be an adequate growth substrate for moulds, for example, Aspergillus spp., Penicillium spp., and Fusarium spp. [11]. Heavily contaminated with Aspergillus flavus suggests being prone to aflatoxin content [12]. The prevalence of aflatoxins in fresh ginger during winter and summer was 86 and 98% of positive aflatoxin B1, with mean values of 0.165 and 1.21 µg/kg, respectively [13]. Moreover, a few publications reported that total aflatoxins were detected in genger rhizomes with various levels up to 25 µg/kg [14, 15]. It shall not contain total aflatoxin exceeding the maximum level (10 µg/kg) set out in European commission regulation (EC: 1881) [16] and the Egyptian standards (ES: 7136) [17]. Yang et al.[7] inferred a certain linkage between producing mycotoxins and active compounds reduction, which is beneficial for fungal growth.

Antimicrobial activity is generally applied by using the plant extract and essential oils to control bacterial disease. In this context, Hossain et al.[18] and Irfan et al.[4] discussed the various inhibition effect of food-borne bacterial multiplication against the ginger extract. Fresh and dried ginger extracts inhibit the growth of E. coli and Staphylococcus aureus, similar to some standard antibiotics [19]. Its extracts exhibit antibacterial activity against gram-negative and gram-positive bacteria owing to the presence of gingerols [20]. Ginger methanol extract contained steroids and flavonoids, which are antimicrobial agents [21].

The aim of this study was to compare the possible microbiological contamination, mycotoxin content, and phytochemical components between dried and fresh ginger rhizomes in spice and infusion tea form, in addition to investigate the antimicrobial activity against food-borne microorganisms.

### MATERIALS AND METHODS

#### Samples

Ethics committee approval was taken. A total of 100 samples representing 500 g each of powdered and fresh ginger rhizomes (Z. officinale Roscoe) were collected in 2019 from retail spice markets of Egypt. Samples were kept in sterile insulated containers at ~4°C and analyzed upon arrival. Samples were examined as spice and tea forms for the microbiological, mycotoxic, and phytochemical content in addition to antimicrobial activity.

#### Preparation of ginger tea

Ginger tea was prepared by boiling 0.5 g/250 ml of powdered or peeled sliced fresh rhizomes with water for 10 min [22, 23].

#### Microbiological analysis

Spice and tea form samples (25 g) were homogenized in 0.1% peptone water (225 ml) using stomacher 3500 series (Seward, England), and then serial decimal dilutions up to 10⁻¹⁰ were (ISO 7218) [24]. Quantitative analysis was performed with sterilized standard media purchased from Difco and Oxoid, as described in Table 1.

#### Mycotoxic analysis

Ground samples (2 g) were carried out through competitive enzyme immunoassay kit (RIDASCREEN Art. No. R4701, R-Biopharm AG, Darmstadt, Germany), following its instruction for the quantitative determination of total aflatoxins forming yellow color [25]. The intensity of the color is measured photometrically at 450 nm using MRX microwell reader (Dynatech Laboratories, Guernsey, Channel Islands, Great Britain) with software version 1.2 to indicate the total aflatoxin content in µg/kg or ppb.

#### Phytochemical analysis

The samples were prepared and subjected to the gas chromatography-mass spectrometry based on their retention indices and relative area percentage to the total areas according to Sharma et al. [26].

### Table 1: Methods for enumeration and detection of microbiological analysis

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Media and incubation conditions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic mesophilic bacteria</td>
<td>Plate Count Agar (35°C/48±2 h)</td>
<td>AOAC 966.23/2000[27]</td>
</tr>
<tr>
<td>Mould and yeast count</td>
<td>Sabouraud D-glucose Agar (25±1°C/5 days)</td>
<td>ISO 21527-2:2008[28]</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Baird-Parker’s medium (37°C/24 and 48 h) and Brain Heart Infusion Broth (37°C/1-24 h)</td>
<td>ISO 6888-1: 1999/Amd. 1: 2003[29]</td>
</tr>
<tr>
<td>Salmonella spp. a</td>
<td>Lactose Broth, LIA and TSI (37°C/24 h); Selenite Cystine Broth and Tetrathionate Brilliant Green Broth (45°C/24 h) and Bismuth Sulphate Agar and Brilliant Green Agar (37°C/24 and 48 h)</td>
<td>ISO 6579-1: 2017[31]</td>
</tr>
<tr>
<td>Shigella spp. a</td>
<td>Shigella broth contained 0.5 µg/ml novobiocin (anaerobic a 41.5±1°C/16-20 h); MacConkey agar, XLD agar and Hektoen enteric agar (37±1°C/20-24 h) and TSI (37±1°C/24±3 h)</td>
<td>ISO 21567: 2004[32]</td>
</tr>
<tr>
<td>Clostridium perfringens a</td>
<td>OPSP+selective supplement A and B (35°C/18-24 h)</td>
<td>ISO 7937: 2004[33]</td>
</tr>
<tr>
<td>Coliform group</td>
<td>VRBL (ISO) Agar (30-37°C/27±2 h) and Brilliant Green Bile Broth 30-37°C/24+durhams tube</td>
<td>ISO 4832: 2006[34]</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>EMB (37°C/24 h), Tryptone water (37°C/24 h)+indol reagent, MRVP (37°C/5 days)+Methyl red solution and (37°C/48 h)+α-naphthol solution, Simon Citrate Agar (37°C/48 h)</td>
<td>ISO 16649-2: 2001[35]</td>
</tr>
</tbody>
</table>

aSerological identification using antiserum agglutination test (Difco). bIncubated with H₂/CO₂ gas generating pack in a conventional gas-jar.
Fresh or dry powder *Z. officinale* (1 kg) was hydrodistilled for 4 h in a Clevenger glass apparatus. The resultant oil was dried over anhydrous sodium sulphate and stored at 4°C in the dark until analyzed using gas chromatography-mass spectrometry. It was carried out using ThermoScientific (Model ITQ 900), employing the following conditions: column HP88 (30 m × 0.25 mm i.d., film thickness 0.22 µm), injection temperature (240°C), detector temperature (280°C), injection volume (0.3 µl), mass scan range m/z (40–850 amu), ionization energy voltage (70 eV), split flow (101 ml/min), and split ratio (1: 80). Nitrogen with flow rate 1.21 ml/min was the carrier gas, oven column temperature 60°C/10 min, increased at rate of 4°C/min up to 230°C/10 min then 1°C/min up to 260°C/min.

**Antimicrobial activity**

**Bacterial cultures**

Food-borne pathogens included two gram-negative bacterial cultures (*Salmonella typhimurium* ATCC 14028 and *E. coli* ATCC 10536) and two gram-positive bacterial cultures (*Bacillus cereus* ATCC 10876 and *S. aureus* ATCC 6538), which were used to determine the bacterial sensitivity against the dry powder and fresh ginger rhizome extracts.

According to Hossain *et al.* [18], the bacterial cultures were adjusted to 0.5 McFarland standards (1.5 × 10⁸ CFU/ml) with sterile saline, and then spread on Mueller-Hinton Agar (BIO-RAD), Marnes-la-Coquette, France.

**Plant extract**

Referred to Mostafa *et al.* [36], 50 g of dry powder or minced fresh rhizomes were soaked in ethanol 200 ml/48 h, filtered through double muslin layers, centrifuged at 9000 rpm/10 min (Centurion Scientific, UK), filtered through Whatman filter paper No. 41, evaporated under reduced pressure at 40°C (IKA, Germany), re-dissolved (50 mg/ml), and finally sterilized through 0.22 µm filter (Millipore, Massachusetts, USA). The yield extract was then stored at 5°C in refrigerator for further disk diffusion method.

**Disk diffusion method**

Sterile filter disks were impregnated with 10 µl of the extract, placed in the inoculated Petri-dishes, then inoculated at 37°C/24 h, which was performed in three replicates. Gentamycin 10 µg disk was used as a positive control. The zone of inhibition was measuring as the clear zone diameter and recorded in millimeters [36].

**Statistical analysis**

The observed values were expressed in mean. The significant difference (*P* < 0.05) between the ginger spice and tea form samples was implemented by *t*-test for paired comparison using statistical software (IBM-SPSS, 20; SPSS Inc., Chicago IL, USA).

**Results and discussion**

The phytochemical analyses of fresh and dry powder ginger rhizomes representing volatile components and retention time are illustrated in Figs. 1 and 2. The major volatile components in fresh ginger were zingiberene (38.59%), citral (13.77%), cedrene (10.13%), and cineol (7.04%), whereas in dry powder ginger were zingiberene (43.93%), cedrene (15.23%), cuparene (11.31%), and farnesene (9.73%). High percentages were found in dry powder ginger compared with fresh ginger regarding to zingiberene and cedrene, which agreed with those reported by Mahboubi [37]. Phytochemicals in fresh samples are lower than in dry samples owing to the enzymatic actions, which degrade the bioactive compounds [38].

Most of these components had antimicrobial activity. Zingiberene has defending action in some plants against oxidation and antimicrobial properties [39]. Cedrene isomers are a selective inhibitor against harmful bacteria [40]. Citral is hydrophobic and unstable under storage conditions, which easily loses its bactericidal effects [41].

Ginger ethanol extract showed a broad observation of antibacterial activities against food-borne pathogenic bacteria (Fig. 3). The dry powder ginger extract exhibited the maximum inhibition zone toward the gram-positive bacterial strains more than the fresh ginger extract, in which *S. aureus* was 13.3 and 12 mm and *B. cereus* was 10.6 and 8 mm, respectively. On the contrary, no bioactive action was observed toward the gram-negative bacteria. Positive control, gentamycin 10 µg, showed inhibition zone as 19.3, 20, 14, and 15.6 mm against *B. cereus*, *S. aureus*, *S. typhimurium*, and *E. coli*, respectively.
Gram-negative bacterial strains showed a weak antibacterial activity against both fresh and dry powder ginger essential oil owing to the fact that the outer membrane possesses hydrophilic polysaccharides which hamper the hydrophobic component diffusion of the ginger essential oils [18]. Moreover, it showed higher resistance owing to the complexity of the cell wall, and its external membrane provides highly hydrophilic surfaces, whereas gram-positive bacteria have negative charge on the wall surface, which reduces their antimicrobial resistance [20].

Concerning the microbiological quality, Fig. 4 shows a comparison between dry powder and fresh raw ginger and their herbal tea to evaluate and assess TAMB, mould and yeast count as a group, coliform group, *E. coli*, *B. cereus*, *S. aureus*, *Salmonella spp.*, *Shigella spp.*, and *Clostridium perfringens*. These findings showed that dry powder ginger was the most contaminated samples, whereas fresh rhyzomes and herbal tea were within the acceptable levels set by the Egyptian standards (ES: 2986) [10]. The above mentioned results enforce the consumer to reject the former sample group or not to be intended for direct human consumption while enable the later accepted groups to be used.

The average microbial contamination of TAMB, mould and yeast count, coliform group, and *B. cereus* ranged between $6.8 \times 10^3$ and $5.3 \times 10^6$ cfu/g, $3.1 \times 10^2$ and $9.9 \times 10^3$ cfu/g, $1.7$ and $1.9 \times 10^3$ cfu/g, and $4.5$ and $3.2 \times 10^1$ cfu/g in fresh and dry powder samples, respectively. These results showed columns with different characteristics indicate a significant difference ($P > 0.05$) between dry powder samples and the other samples including fresh rhyzomes and the herbal ginger tea. As the safety criteria required by the standards, *S. aureus*, *E. coli*, *Salmonella spp.*, *Shigella spp.*, and *C. perfringens* were not detected in any of the examined samples.

No microbial count was found in the herbal tea samples. Heating the samples reduced the microbial load, whereas boiling for 15 min killed all the microorganisms [8].

Raw plant materials generally associated broad range of microbial contaminants, which affects its quality. These are consequences of the agriculture and processing conditions [42]. Microbial population varies owing to the year of production, the region, and the conditions before drying. The observed count reflects the original bio-load and the die-off which enhanced the presence of active compounds and oxidation [43]. Impurities in medical herbs as well as their preparations and products, causes biological contamination which involve yeasts, moulds, bacteria and their spores [44]. Dry powder samples harbor high microbial count owing to the surface area of the dry particles, and wholesome non-damage rhizomes contained low count.

Spices are a vehicle that may be contaminated with spore-forming bacteria (*Bacillus* spp.), Enterobacteriaceae, and fungi [45, 46]. Coliform group bacteria are usually found in spices sporadically in small population, associated with fecal contamination [43]. Mould and yeast count agreed with that reported by Nahemiah *et al.* [47], which was $2.21 \times 10^3$ cfu/g.

Only the dry powder ginger samples contained total aflatoxin (6.2 µg/kg), whereas no detectable levels were found in fresh samples or ginger tea. However, adding hot water to form ginger tea could have diluted the total aflatoxin content. Approximately 30–40% of total aflatoxins in the contaminated ginger may migrate to surrounding liquid ginger tea [48].

The γ-terpinene and citral in ginger essential oil showed potent antifungal properties against *Aspergillus* flavus and reduced the expression of some genes related to aflatoxin biosynthesis [2]. Ginger oleoresin, a complex mixture extracted from ginger (*Z. officinale* Roscoe), is rich in gingerols and shogaols. Some previous studies showed that ginger oleoresin had good capability of inhibiting the growth of certain types of fungi [49].
Figure 4: Microbiological contamination of fresh and dried ginger as spice and herbal infusion tea (in logarithmic scale).

CONCLUSION
The results indicated that dry powder samples contained the highest phytochemical volatile components, bioactive effect, microbiological population, and total aflatoxin content. Fresh ginger rhizomes as well as ginger tea samples comply with the standards, whereas dry powder samples were unacceptable, which needs further processing steps before use.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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