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Original paper

Fennel and turmeric powders' effectiveness as natural preservatives in beef burgers

SALEH S.M. [1], KASSAB H. A.[1], MARIAM A. RAMADAN [2], EL-BANA M. A.[2]

[1] Food Technology Department, Faculty of Agriculture, Kaferelsheikh University, Egypt.
[2] Food Tech. Res. Institute, Agricultural Research Center, Giza, Egypt.

Abstract This investigation was performed to study the possibility of the utilization of fennel seed powder (FSP) and turmeric seed powder (TSP) at levels of (1.5, 3.0, and 4.5%) as natural preservatives during the preparation of burgers stored at a refrigeration temperature (4 ± 1oC). The obtained results revealed that, FP and TP significantly reduced the total count of bacteria, pH, and thiobarbituric acid (TBA). Furthermore, the results showed that by increasing the concentration of tested powders, the bacterial counts, pH, and TBA values were dropped, with the concentration of 4.5% providing the highest effectiveness. Comparatively, the antioxidant and antibacterial activities of Turmeric ether extract (TEE) were higher than of Fennel ether extract (FEE). In conclusion, fennel and turmeric can play an important role as antioxidants and antibacterial agents in refrigerated burgers.

Keywords fennel, turmeric, refrigerated storage, burger

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*Corresponding author: EL-BANA MA, Food Tech. Res. Institute, Agricultural Research Center, Giza, Egypt.
E-mail: dr.elbana@yahoo.com

Introduction

The progress experienced in meat industrialization and the rise in the social and economic status of the population in the last years concurred to an increase in the exhaustion of meat products well as in its quality requirement **(Ramos and Gomide, 2007).** Synthetic additives are intentionally added to food during production or processing to improve organoleptic quality and/or to prevent deterioration **(**Sedlacek-bassani ***et al.*, 2020).** Despite the fact that food additives provide technological benefits to food, there is still concern about the dangers associated with their use, such as allergic reactions, carcinogenicity, and behavior problems including hyperactivity (Honorato et al., 2013). Today's consumers look for healthier and more practical meat products, preferably with no synthetic chemical additives but still with pleasant and attractive color and taste, and it rests with the food technologists' challenge to develop new products to meet that demand **(Sales et al., 2015).** The burger has attracted great consumer interest as it is composed from fresh and tasty ingredients and maintains the nutritional value and convenience in the preparation **(Baugreet et al., 2017).** However, burgers are subject to deterioration, mostly due to the action of microorganisms and to lipid oxidation, which may happen through processing and/or storage. The use of natural sources bioactive components seems to be preferred to prevent these undesirable effects like deterioration in foods **(Garcia et al., 2012).** Fennel seeds (*Foeniculum vulgare*) have been known as aromatic and therapeutic herbs, widely used in the flavoring of fish, bread, cheese, and salads **(Kaur and Arora, 2010).** These herbs are good source of bioactive components like phenolic acids, flavonoids, coumarin, tannin, and hydroxycinnamic acids **(Rahimi and Ardekani, 2013).** Turmeric is a spice that comes from the root of Curcuma longa, and followed ginger family, Zingiberaceae **(Gupta *et al*., 2015).** It is bright yellow and has been used as a coloring and flavoring agent in foods. The curcuminoids are the principal phenolic compounds that involved in all bioprotective characteristics of Tumeric **(Braga *et al.*, 2018).** Fennel and turmeric extracts are exploited to inhibit *Staphylococcus aureus* through methicillin-resistant **(Mashareq *et al*., 2016).** Thus, the aim of this study was to investigate the antimicrobial properties of FP and TP as active additives to raise the shelf life of burgers. Also, their effect on microbiological and chemical attributes of the product under refrigeration (4±1˚C) storage was evaluated.

Materials and Methods

Materials

A. Fennel (*Foeniculum vulgare*) seeds and turmeric rhizomes were obtained from the Agricultural Seed, Spices, and Medicinal Plants Co. (Harras), Cairo, Egypt. Season 2020 and stored in a deep freeze at-20°C until further use.

B. Microbial strains: Four standard microbial strains were used in the experiments; *Escherichia coli (ATCC 25922), Salmonella Typhimurium (ATCC 14028), Staphylococcus aureus (ATCC 25923) and Pseudomonas aeruginosa (ATCC 27853).* were kindly provided by the Plant Pathology Department, Faculty of Agriculture, Kafr El-sheikh University.

C**.** Merck Co. Ltd. (Darmstadt, Germany) provided the C-Trypticase soy agar (TSA), Trypticase soy Broth (TSB), nutrient agar medium (NAM), and potato dextrose agar (PDA) used in the microbiological examination.

D. The meat of beef and other components to prepare it were procured from local market.

E. Chemicals: All chemicals and reagents were procured from El-Gomhoria Company Tanta, Egypt.

Methods

The gross chemical composition of samples

Fennel seed powder (FSP) and turmeric seed powder (TSP) were analyzed for moisture, crude protein, ether extract, ash, and crude fiber content according to **A.O.A.C. (2005).** Total carbohydrates were calculated by difference.

Preparation of ethanolic extracts

The prepared ground materials (10 g) of each sample were soaked in 100 ml of ethanol (80%) overnight in a shaker at room temperature according to protocol of Mohdaly et al. (2010). The extracts were filtrated through Whatman No.1 filter paper. The process of re-extraction was typically repeated three times for residues. The combined filtrates were evaporated under vacuum in a rotary evaporator below 40ºC. The extracts obtained after evaporation of organic solvents called fennel ethanolic extract (FEE) and turmeric ethanol extract (TEE) were stored -18±2oC until further analysis.

Determination of total phenolic compounds

Total phenolic compounds of the FEE and TEE were calculated according to the method given by **(*Salem et al*., *2018*)** using Folin-ciocalteau reagent and used to estimate the phenolics-acid content using a standard curve prepared using gallic acid.

Determination of total flavonoids

Total flavonoids of FEE and TEE were determined by the method of **Ordonez *et al.* (2006)** by using standard curve prepared by catechol acid.

FEE and TEE DPPH radical scavenging assay

The 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of sample extracts was determined by spectrophotometer according to a modified method described by **Lee *et al.* (2003)** at 517 nm (HITACI, U-1900). The total antioxidant activity (TAA) is expressed as a % reduction of DPPH.

The potential antibacterial capabilities

The potential antibacterial capabilities of FEE and TEE against examined bacteria were screened qualitatively using agar/disc diffusion as described by **Shihabudeen *et al.* (2010).** The appropriate media were poured into sterile

plates (12 cm diameter), left to solidify, at room temperature. The organisms were inoculated on the surface of the prepared media. A sterile disc, 6 mm in diameter, of Whatman No. 1 filter paper, was dipped in the appropriate solutions, blotted, and then stabilized on the surface of the inoculated petri plates. The inhibitory effect of the ethanol was 80% and ampicillin (25 µg/ml) positive control. The plates of bacteria were preserved for incubation at 37° C for 48 hrs. At the end of the incubation period, the generated inhibition zones were measured with a ruler. All tests were completed in triplicate with four discs per plate. The bacteria were cultured on nutrient agar.

Preparation of beef burgers and their formulae

The method of **Sorour *et al.* (2021)** was used to prepare the beef burger samples. Blends containing 1.5, 3.0, and 4.5 grams of FSP and TSP were used as additions to beef burgers. The formulas for beef burgers were prepared using 65g of the beef meat, 15g of fat, 10.50g of ice water, 1.5 g of spice mixture, 3g of dried onion, 3g of dried garlic, and 2g of NaCL. After that, the petri dishes were exploited to form rounded discs of burger with 9 cm diameter and 1 cm thickness. The burger discs were transferred inside polyethylene films prior to freeze at -18 °C.

Cooking of Beef Burger

The prepared beef burger samples were cooked using an electrical grill (Arcelik Mini Firin, Turkey) at 300 °C (the distance between heat source and the sample was 4 cm) for a total of 10 min, 6 min one side and 4 min in the other side **(Turhan *et al*., 2005).**

Antimicrobial activity

Antimicrobial activity in burgers was supplemented with FSP and TSP at levels of 1.5%, 3.0%, and 4.5%. Reference with TBHQ and a control product were prepared. The tested products and control were packed in a polyethylene bag and stored at (4 ±1°C) for 12 days after preparation. The samples were analyzed chemically and examined microbiologically every three days during the storage period **(Najeeb et al., 2014).**

pH values

A digital pH meter (HAANA, HI902 meter, Germany) was exploited to determine pH values by recording two readings from each of beef samples (**Yassin, 2003**).

Thiobarbituric acid (TBA)

(TBA) was performed according to the method recommended by **Vyncke(1970).**

Statistical analysis

Data was analyzed according to **Steel and Torrie (1980)** procedures (Duncan's multiple range test DMRT).

Results and discussion

Chemical composition of FSP and TSP

The approximate chemical composition of FSP and TSP is given in Table (1). The obtained results found that FSP had higher contents of crude protein and ash (23.22, and 10.10%, respectively) than TSP (11.47, and 8.99%, respectively). Meanwhile, TSP had a higher concentration of ether extract, crude fiber, and total carbohydrates (8.45, 11.77, and 71.09%) than FSP (6.24, 6.75, and 60.44%). The obtained results partially agree with those of **Hegazi et al. (2009) and Al-Nazawi and El-Bahr (2012).** The results showed that TSP had a higher total phenolic content, total flavonoid content, and DPPH (820.90 mg gallic acid/100 g extracts, 411.20 mg catechin/100 g extracts, and 89.13 %, respectively) than FSP (760.35 mg gallic acid/100 g extracts, 330.75 mg catechin/100 g extracts, and 84.65 %, respectively). These results are in the same trend of those found by **Liu et al. (2008), Ghasemzadeh et al. (2012), and Salama et al. (2015).**

Table 1. Chemical composition (%) and bioactive compounds of fennel seeds and turmeric rhizome powder (on a dry weight basis).

Compounds	fennel seeds	Turmeric rhizomes powder
Moisture %	11.23±0.19	11.50±0.13
Ether extract %	6.24±0.31	8.45±0.25
Crude protein %	23.22±0.25	11.47±0.17
Ash %	10.10±0.16	8.99±0.14
Crude fiber %	6.75±0.37	11.77±0.35
*Total carbohydrate %	60.44±0.42	71.09±0.52
Total phenolic contents (mg gallic acid /100 g extracts)	760.35 ± 1.4	820.90 ± 1.70
Total flavonoid contents(mg catechin /100 g extracts)	330.75 ± 2.35	411.20 ± 1.66
DPPH(%)	84.65 ± 0.93	89.13 ± 0.87

Each value is an average of three determinations ± SD.
Total carbohydrate*

Antimicrobial activity of TEE and FEE

To inhibit food-borne pathogens and to extend shelf life, synthetic chemicals with antimicrobial properties are often used as preservatives in food processing and storage. Concerns over the potential risks of synthetic food additives to human health and consumer awareness have directed interest in using naturally occurring alternatives. The market of health and herbal nutraceuticals is addressing its attention to rich plant sources offering functional efficacy. Al-Saiqali et al. (2016).

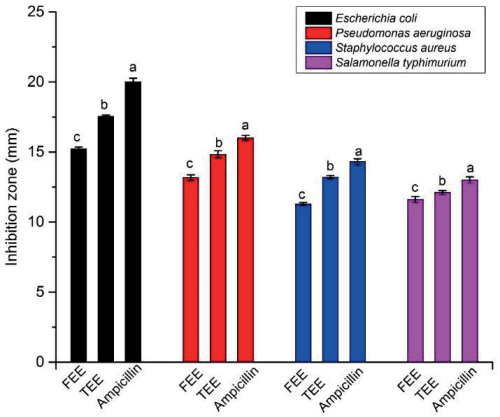

Figure 1: Anti-bacterial activity of fennel seeds and turmeric rhizomes plant extracts against foodborne pathogens, measured as the diameter of inhibition zones (ZOI, mm).

The antimicrobial potential of the TEE and FEE compared with Ampicillin as a reference material or positive control for the antibacterial activity is shown in Fig 1. The results indicated that, TEE and FEE have antibacterial activity against Gram-negative and Gram-positive bacteria. The most effective extract against Gram-negative bacteria was the TEE, which was less effective than ampicillin, while the most effective extract against Gram-positive bacteria was the FEE, which gave a larger inhibition zone than ampicillin. The ethanolic extract gave the highest wide inhibition zones (17.55mm) with *Escherichia coli*, (14.83mm) with *Pseudomonas aeruginosa*, (13.20mm) with *Staphylococcus aureus*, and (12.11mm) with *Salmonella Typhimurium*. These data coincide with those of Gul et al. (2015); Maharjan et al. (2019) and Tayel et al (2021) . They reported that fennel and turmeric displayed significant antibacterial activity, as determined by the agar diffusion method. Overall, the effectiveness of FEE and TEE is higher in Gram-positive than in Gram-negative bacteria. Furthermore, Grace et al. (2017) mentioned that the outer membrane of Gram-negative bacteria could work as selective permeability barrier that restrict larger molecules to penetrate cells and admit of small hydrophilic molecules like phenolic components to pass causing its antibacterial effect.

Besides these, they also possess multidrug-resistant pumps which exclude some of the antibacterial compounds. Furthermore, Floridi et al. (2003) showed that the presence of phenolics in food is particularly important for their oxidative stability and antimicrobial protection. From clinical point of view, there is numerous evidence of sweet fennel to alleviate diseases.

Chemical and microbiological quality assessment of burgers supplemented with FSP and TSP during 12 days of storage at 4 ± 1°C

pH changes

The results in Table (2) showed that there was a significant (at $P \leq 0.05$) increase in pH mean values for different treatments during storage by using different rates of the FSP and TSP, and the highest incremental rates (pH values) were found in the untreated (control). The samples treated with 4.5% FSP and 2.5% TSP had the most significant (at $P \leq 0.05$) effect on pH, with lower values than those of the control samples, followed by samples treated with 3.0% lowering the pH values of treated burgers can enhance microbial inhibition. Finally, the samples were treated with 1.5% FSP and TSP, respectively, till reaching the end of the storage period. There was a significant (at $P \leq 0.05$) increase in pH mean values of all untreated and treated samples with fennel or turmeric at all concentrations for the 12 days of the storage period. Similar findings were found in burgers containing ginger powder during frozen storage, as reported by **(Awad, 2018).** Furthermore**,** the increase in pH could be attributed to the activation effect of microbial load, which may cause protein hydrolysis with the appearance of alkyl groups (**Yassin, 2003**).

Table 2. pH values of burger supplemented by fennel and turmeric powder during storage at 4 ± 1°C for 12 days

Storage period (days)		PH values			
Treatment		0	4	8	12
Control		5.28 ± 0.17^{Da}	6.11 ± 0.10^{Ca}	6.69 ± 0.25^{Ba}	7.09 ± 0.12^{Aa}
TBHQ		5.27 ± 0.21^{Ba}	5.34 ± 0.13^{ABa}	5.77 ± 0.18^{Abc}	5.93 ± 0.11^{Ab}
Fennel	1.5	5.28 ± 0.23^{Ba}	6.05 ± 0.17^{ABa}	6.10 ± 0.19^{Ab}	6.29 ± 0.10^{Ab}
	3.0	5.27 ± 0.16^{Ba}	5.87 ± 0.19^{ABa}	5.93 ± 0.22^{ABa}	6.17 ± 0.17^{Ab}
	4.5	5.26 ± 0.11^{Ba}	5.59 ± 0.24^{ABa}	5.83 ± 0.23^{ABb}	6.03 ± 0.31^{Ab}
Tumeric	1.5	5.29 ± 0.19^{Ba}	6.01 ± 0.31^{ABa}	6.09 ± 0.29^{Ab}	6.26 ± 0.34^{Ab}
	3.0	5.27±0.18Ba	5.83 ± 0.10^{ABa}	5.89 ± 0.20^{ABb}	6.15 ± 0.10^{Ab}
	4.5	5.26±0.23Ba	5.56 ± 0.30^{ABa}	5.81 ± 0.22^{ABbc}	6.00 ± 0.19^{Ab}

Data are means ± SD for 3 replicates. Means with different superscript capital letters(within group at different storage period "row") and small letters (between groups at the same storage period "column") are significantly different at $p<0.05$.

TBA changes

According to the data in Table (3). There were significant differences ($P<0.05$) between the beef burger control and all the low-fat beef burger formulas prepared with different levels of FSP and TSP during storage at 4 ± 1°C for 12 days for TBA values. The amount of thiobarbituric acid in the

prepared beef burger decreased as the level of FSP and TSP increased. TBA values ranged between 0.15 and 0.81. These results may be due to the fact that FSP and TSP have antioxidants that inhibit lipid oxidation throughout storage time. These results are in agreement with those mentioned by **Awad (2018) and Bassano *et al.*(2019).**

Table 3. Thiobarbituric Acid (T.B.A) levels in burgers supplemented with FSP and TSP during 12 days of storage at 4 ± 1°C.

Storage period (days) Treatment		TBA values (mg of malonaldehyde /kg) 0	4	8	12
Control		0.17±0.019^{Da}	0.36±0.033^{Ca}	0.54±0.041^{Ba}	0.81±0.017^{Aa}
TBHQ		0.15±0.034^{Ba}	0.17±0.028^{Bb}	0.21±0.013^{ABb}	0.30±0.023^{Ab}
FSP	1.5	0.16±0.017^{Ca}	0.22±0.017^{BCb}	0.27±0.011^{Bb}	0.36±0.034^{Ab}
	3.0	0.16±0.035^{Ca}	0.21±0.020^{BCb}	0.25±0.024^{Bb}	0.34±0.018^{Ab}
	4.5	0.15±0.027^{Ca}	0.19±0.014^{BCb}	0.23±0.029^{Bb}	0.33±0.054^{Ab}
TSP	1.5	0.16±0.045^{Ca}	0.21±0.018^{BCb}	0.24±0.0337^{Bb}	0.36±0.036^{Ab}
	3.0	0.15±0.064^{Ca}	0.20±0.026^{BCb}	0.23±0.042^{Bb}	0.33±0.047^{Ab}
	4.5	0.15±0.011^{Ca}	0.18±0.026^{BCb}	0.22±0.010^{Bb}	0.31±0.043^{Ab}

Data are means ± SD for 3 replicates. Means with different superscript capital letters(within group at different storage period "row") and small letters (between groups at the same storage period "column") are significantly different at p<0.05.

Total bacterial counts values

According to the data in Table (4). There were significant differences (P<0.05) between beef burger control and all low-fat beef burger formulas prepared with different levels of FSP and TSP during storage at 4 ± 1°C for 12 days for Total count values. The results show that the control samples showed the highest total bacterial counts compared to the other samples. Furthermore. The total bacterial counts of prepared beef burgers supplemented with FSP and TSP in different ratios of 1.5, 3.0, and 4.5 % were 7.10 to 8.31 cfu/g. It is worthy to mention that total bacterial counts were decreased as the level of FSP and TSP increased. These results are in agreement with those found by **Wakoli et al., (2014) and Mancini et al. (2017)** discovered a delay in the TBC of pork burgers with powdered ginger at 1% and 2% during 7 days of storage at 4 °C. During the storage period, the total bacterial count was gradually reduced as the storage time proceeded, until it reached between 7.23 and 9.88 cfu/g. This conclusion was in agreement with Igbinosa et al. (2009). This result is due to the effect of cold storage on microbial load.

Table 4. Total bacterial counts (log CFU */g) of burger supplemented with FSP and TSP during 12 days of storage at 4 ± 1°C.

Storage period (days) Treatment		Total bacterial counts (log CFU */g) 0	4	8	12
Control		7.23±0.22^{Da}	8.17±0.28^{Ca}	9.21±0.15^{Ba}	9.88±0.017^{Aa}
TBHQ		7.09±0.25^{BCa}	7.23±0.15^{Bb}	7.28±0.27^{Bb}	7.91±0.20^{Aa}
FSP	1.5	7.19±0.16^{BCa}	7.44±0.12^{Bb}	7.56±0.18^{Bb}	8.31±0.13^{Aa}
	3.0	7.17±0.18^{BCa}	7.35±0.34^{Bb}	7.42±0.18^{Bb}	8.25±0.19^{Aa}
	4.5	7.12±0.15^{BCa}	7.30±0.19^{Bb}	7.38±0.34^{Bb}	8.17±0.23^{Aa}
TSP	1.5	7.16±0.30^{BCa}	7.39±0.17^{Bb}	7.50±0.11^{Bb}	8.29±0.22^{Aa}
	3.0	7.15±0.37^{BCa}	7.33±0.26^{Bb}	7.39±0.14^{Bb}	8.19±0.28^{Aa}
	4.5	7.10±0.11^{BCa}	7.22±0.23^{Bb}	7.30±0.17^{Bb}	8.11±0.20^{Aa}

Data are means ± SD for 3 replicates. Means with different superscript capital letters(within group at different storage period "row") and small letters (between groups at the same storage period "column") are significantly different at *p<0.05*.

Conclusion

Based on the mentioned results, it could be concluded that fennel and turmeric powders can play an important role as antioxidants and antibacterial agents and can be used to extend the shelf life of beef burgers, especially when kept under refrigeration.

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