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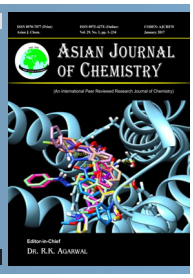

Essential Oil of Fennel Seeds as Natural Preservative in Butter and its Shelf Life Assessment

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The efficacy of essential oil of fennel seeds was examined in terms of its antioxidant and antimicrobial activity. The antioxidant activity of essential oil was determined by DPPH method along with the comparative analysis with ascorbic acid as a standard. Chemical composition of fennel seed oil was determined by GC/MS analysis. The essential oil of fennel seed was incorporated in butter in varying concentrations as natural preservative to enhance antibacterial and antioxidant activities. Total bacterial count, zone of inhibition, water activity of butter samples along with titrimetric analysis *viz*. peroxide value, acid value and free fatty acids were determined at regular intervals in order to have all round assessment on the storage stability of butter.

Keywords: Fennel seeds, Butter, Antioxidant activity, Antimicrobial agent, Essential oils.

INTRODUCTION

Foeniculum vulgare Mill, commonly known as fennel, belongs to family Apiaceae which is a small genus of annual, biennial or perennial herbs cultivated for its aromatic fruits, which are used as culinary spices, originated in Mediterranean (used for medical and culinary purposes); preliminary grows in coastal climates and on riverbanks [1]. It is widely used as carminative, digestive, lactagogue and diuretic and in treating respiratory and gastrointestinal disorders [2]. Its seeds are used as flavourings. Fennel (*Foeniculum vulgare L.*) has a long history of herbal uses and widely cultivated, both in the native habitat, India and Egypt and elsewhere, for its edible strongly flavoured leaves and seeds [3].

It contains phenols, phenolic glycosides and volatile aroma compounds *viz*., *trans*-anethole, estragole and fenchone as the major phytoconstituents, with a characteristic anise odor [4]. The nutritional profile of fennel consists of 9.5 % protein, 10 % fat, 13.4 % minerals, 18.5 % fibre and 42.3 % carbohydrates. The minerals and vitamins present in *F. vulgare* are calcium, potassium, sodium, iron, phosphorus, thiamine, riboflavin, niacin and vitamin C [5]. Fennel increases lactation, acts as a stimulant to circulatory and digestive system and also it is an antiaging agent [2].

The essential oil of fennel seeds has been proven as a potential food conservative due to its high antioxidant, antimicrobial properties [6] and hepatoprotective activity [7]. Essential oils (volatile oils) are concentrated, hydrophobic liquids containing volatile aromatic compounds. They are rich in biologically active compounds, their antimicrobial potential is due to compounds synthesized during secondary metabolism of plants [8]. Essential oils are more effective against food borne pathogens and spoilage bacteria and could be used as natural antibacterial agents in food preservation [9]. The best method to extract essential oils is steam distillation from commercial point of view [10].

Essential oils of spices and herbs at first place are safe and stable as natural foodstuffs have been added to food since antiquity. Recently there has been considerable emphasis on studies involving essential oils and extracts of spices and herbs on inhibiting the growth of microbes. Spices essential oils appeal to all who question safety of synthetic food additives and at the same time demand high-quality. The antimicrobial effects of spices are mostly due to the essential oils present in their composition-potent source of new bioactive secondary metabolites [11]. Consumer awareness of natural food products and a growing concern of microbial resistance towards conventional preservatives have led to exploring naturally-occurring antimicrobials for food preservation [12]. The main advantage of natural sources is that they do not enhance the antibiotic resistance, commonly encountered with the long-term use of synthetic antibiotics. Essential oil of fennel (EOF) is a relatively stronger antimicrobial agent against broad range of pathogens as compared to clove oil, except in case of certain *Aspergillus* strains and *E. coli* [13]. The essential oil and seed extracts of *F. vulgare* could be a source of pharmaceutical materials required for the preparations of new therapeutic and antimicrobial agent. The growth of food spoilage and food-

borne pathogens on/in food can decrease nutritional quality of the food by consuming fat, protein and carbohydrate that are present in the food, subsequently causes food discolouration, heating, mustiness, biochemical changes, weight loss and toxicity [13]. The production of such essential oil and bioactive components from indigenous resources and their utilization as potential natural food preservatives could be of economic value advocating its consumption in food and pharmaceutical preparations of local industries.

This work is our approach of using fennel seeds essential oil as a natural food preservative and its implementation into butter at different concentrations with a close check on its antioxidant and antimicrobial potentials with a purpose of enhancing the shelf life of butter along with the value addition in terms of its flavour profile.

EXPERIMENTAL

The essential oil of fennel seeds (EOF) and butter was procured from Mumbai and Chennai, India, respectively. They were further stored in non-commercial refrigerator for further use.

Antioxidant activity: The antioxidant activity of essential oil of fennel was measured by using DPPH (2, 2-diphenylpicryl hydrazyl) radical scavenging method with slight modifications [14]. Three concentrations of essential oil of fennel (50, 250 and 1000 mg/10 mL) were prepared in methanol and 1 mL of each was then mixed with 3 mL of DPPH solution having concentration of 0.1 mM. The mixture was then incubated in dark for 30 min at room temperature and the absorbance (scavenging capacity) at 517 nm was recorded as (A_{sample}) spectrophotometrically. Analyses were carried out in triplicates. A blank experiment was also carried out applying the same procedure to a solution without the test material and the absorbance was recorded as (A_{blank}). The free radical scavenging activity of each solution was then calculated as percent inhibition according to the following equation [15]:

$$\text{Inhibition (\%)} = \frac{A_{blank} - A_{sample}}{A_{sample}} \times 100$$

Shelf life studies: The butter was procured at the end month of August, 2015 and zeroth reading was taken on the next day of procurement followed by incorporation of essential oil of fennel. Daily trials on butter incorporated with essential oil of fennel were conducted for 5 days, on observing minimum negligible changes, its average was considered as post addition week 1, followed by zeroth trial. Seven weekly trials were conducted on the part of shelf life assessment.

Peroxide value: It is commonly used to determine the rancidity of sample containing fat or oil subjected to oxidative deterioration. The method employed to determine peroxide value is as per AOAC with slight modification. 1 g of all the four butter samples (control, 1, 2 and 3 % essential oil) was added to 25 mL of 2:1 glacial acetic acid: chloroform, to which 1 mL of potassium iodide was added and sample was left in dark for a minute or two in order to incubate it. Thereafter 30 mL of distilled water was added to sample and titrated against 0.1 N sodium thiosulfate using starch as an indicator. The analysis was done in triplicates.

$$\text{Peroxide value} \left(\frac{\text{mEQ}}{\text{kg}}\right) = \frac{(\text{Sample} - \text{Blank}) \times 0.1\text{N} \times 100}{\text{Weight of the sample}}$$

Acid and free fatty acid value: The acid value is the number of milligrams of potassium hydroxide required to neutralize a gram of lipid [16]. The method employed to determine acid value is as per AOAC with slight modification. 0.8 g of all the four butter samples (control, 1, 2 and 3 % essential oil) was added to 10 mL of 1:1 ethanol:diethyl ether and subsequently titrated against 0.1 N potassium hydroxide with phenolphthalein as the titration indicator.

$$\text{Acid value (mg)} = \frac{56.1 \times \text{Normality of KOH} \times \text{Titer volume}}{\text{Weight of sample}}$$

$$\text{Free fatty acid (FFA)} = 0.503 \times \text{Acid value (mg)}$$

Microbiological analysis: Total plate count (TPC) is measure of biological activity in the sample. This is a count of all (heterotrophic) bacteria, fungi (molds) and yeast that will grow in aerobic or microaerophilic conditions. All the four butter samples of 1 mL were serially diluted in 0.9 % of saline and 0.1 mL of 10^{10th} dilution was inoculated in Nutrient agar medium, followed by 24 h of incubation. Analysis was done in triplicates.

Zone of inhibition: This method is used for measuring the effectiveness of an antimicrobial agent against bacteria grown in culture, also known as Kirby-Bauer disk-diffusion method [17]. 0.1 mL of pathogenic broth was swabbed uniformly across a culture plate. Then a filter-paper disk, impregnated with essential oil of fennel (2, 3 and 4 µL/mL) was placed on the surface of agar. The compound diffuses from the filter paper into the agar. The concentration of the compound will be higher next to the disk and will decrease gradually as the distance from the disk increases. If the compound is effective against bacteria at a certain concentration, no colonies will grow wherever the concentration in the agar is greater than or equal to that effective concentration. This region is called the "zone of inhibition." thus, the size of the zone of inhibition is a measure of the compound's effectiveness; the larger the clear area around the filter disk, the more effective the compound.

Sensory evaluation: Hedonic scale was used for sensory evaluation for all the four butter samples. Its evaluation was done using statistical package of social sciences (SPSS) (Version 20.0). A total of 37 panelists participated in the sensory evaluation. The analysis of the data was done using ANOVA.

GC-MS: Components detected are fenchyl acetate ($C_{12}H_{20}O_2$), phenol, 2,4-bis(1,1-dimethylethyl) ($C_{14}H_{22}O$), anethole ($C_{10}H_{12}O$), estragole ($C_{10}H_6O$), L-α-terpineol ($C_{10}H_{18}O$), L-fenchone ($C_{10}H_{16}O$), γ-terpinene ($C_{10}H_{16}$), D-limonene ($C_{10}H_{16}$), *o*-cymene ($C_{10}H_{14}$), β-myrcene ($C_{10}H_{16}$), β-pinene ($C_{10}H_{16}$), β-phellandrene ($C_{10}H_{16}$), camphene ($C_{10}H_{16}$) and α-pinene($C_{10}H_{16}$) [18,19].

Water activity of butter: Unbound water in food can support growth of bacteria, yeasts and molds (fungi). The term water activity (aw) refers to this unbound water, moisture content is quantity of water whereas aw is quality of water present in food.

RESULTS AND DISCUSSION

A total of 4 butter samples were taken *i.e.* control, 1 % essential oil of fennel, 2 % essential oil of fennel and 3 % essential oil of fennel incorporated in butter. It was observed from Table-1 that the inhibition trait of essential oil of fennel bears the direct exponential relationship with its concentration. However, its inhibition potential as compared to ascorbic acid is significantly low. The tendency of retarding deterioration in butter, in terms of peroxide values is shown to increase with increasing oil concentration. However, trend analysis shows a sharp rise in 3 % essential oil of fennel in Fig. 1. This can be due to improper mixing of essential oil of fennel in the butter.

TABLE-1
OBSERVATION TABLE FOR ANTIOXIDANT ACTIVITY

Compound	Inhibition (%)		
	5 mg/mL	25 mg/mL	100 mg/mL
Ascorbic acid	94.41	97.02	98.87
Essential oil of fennel seeds	57.18	60.98	73.96

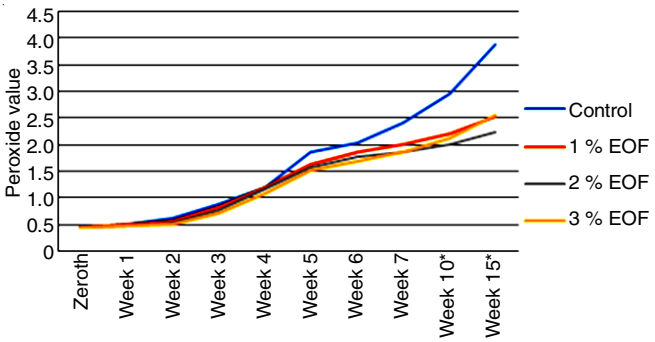

Fig. 1. Weekly observation and trend analysis of peroxide value

The presence of essential oil of fennel is successfully reducing the formation of acids of deterioration. Same holds true for free fatty acids also as acid value (Fig. 2) and free fatty acid (FFA) (Fig. 3) are inter-related.

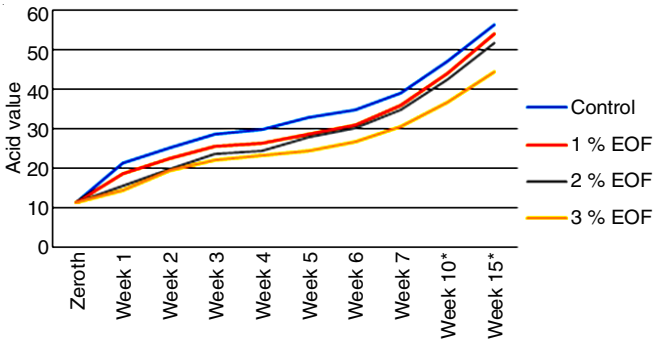

Fig. 2. Weekly trial and trend analysis of acid value

Statistical analysis was done using SPSS software using ANOVA method. Using the hedonic scale it was observed that more acceptance was given to butter sample 4 *i.e.* 3 % essential oil of fennel (Fig. 4).

Water activity of butter represents the ratio of water vapour pressure of the food to water vapour pressure of pure water under the same conditions and is expressed as a fraction. Multiplying this ratio by 100, we obtained an equilibrium relative humidity (ERH) that the foodstuff would produce if enclosed with air in a sealed container at constant temperature The values of which ranges from 0 (bone dry) to 1.0 (pure water).Water activity of all the butter samples was measured with water activity meter. The results are given in Table-2.

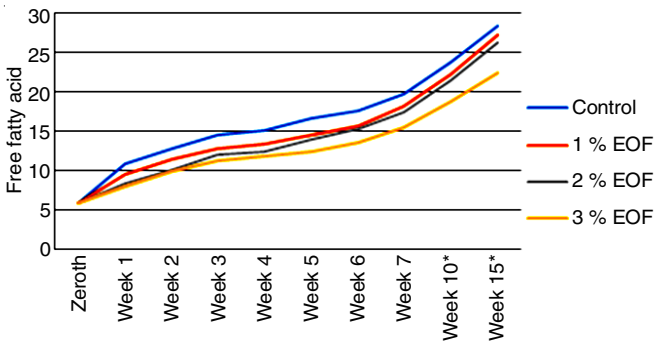

Fig. 3. Weekly trial and trend analysis of free fatty acid

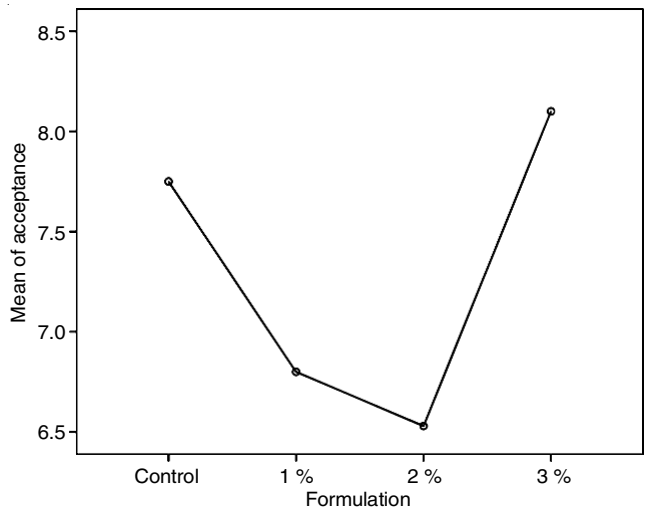

Fig. 4. ANOVA result

TABLE-2
OBSERVATION TABLE FOR WATER ACTIVITY OF ALL THE BUTTER SAMPLE

Samples	Water activity (a_w)	Temperature (°C)
Control	0.956	27.9
1 % Essential oil of fennel seeds	0.969	28.6
2 % Essential oil of fennel seeds	0.970	28.3
3 % Essential oil of fennel seeds	0.968	28.1

Conclusion

In the present study, essential oils of fennel exhibited significant antioxidant property with respect to ascorbic acid standard, which justifies its potential role in delaying oxidative and hydrolytic rancidity especially in high fat content foods.

The spoilage-indicator parameters *viz.* total plate count (TPC), peroxide value, acid value and free fatty acid value were shown to be drastically reduced with the increasing concentration of essential oil of fennel seeds, thus it was proven to retard/delay aforementioned changes. Pertaining to the fact that essential oil of fennel seeds has sweet flavour profile; the higher concentration of essential oil of fennel (> 3 %) quantity will hamper the organoleptic profile of butter, which can be demonstrated with sensory evaluation results.

REFERENCES

1. M. Oktay, I. Gülcin and Ö.I. Küfrevioglu, *Lebenson. Wiss. Technol.*, **36**, 263 (2003); https://doi.org/10.1016/S0023-6438(02)00226-8.
2. C. Saviuc, I. Marina, A.M. Grumezescu, C. Bleotu, C. Chifiriuc, D. Mihaiescu and V. Lazar, *Biointerface Res. Appl. Chem.*, **2**, 300 (2012).
3. I. Parejo, F. Viladomat, J. Bastida, A. Rosas-Romero, N. Flerlage, J. Burillo and C. Codina, *J. Agric. Food Chem.*, **50**, 6882 (2002); https://doi.org/10.1021/jf020540a.
4. M.C. Díaz-Maroto, M.S. Pérez-Coello, J. Esteban and J. Sanz, *J. Agric. Food Chem.*, **54**, 6814 (2006); https://doi.org/10.1021/jf0609532.
5. M.A. Rather, B.A. Dar, S.N. Sofi, B.A. Bhat and M.A. Qurishi, *Arabian J. Chem.*, **9**, S1574 (2016); https://doi.org/10.1016/j.arabjc.2012.04.011.
6. S. Cosentino, A. Barra, B. Pisano, M. Cabizza, F.M. Pirisi and F. Palmas, *J. Food Prot.*, **66**, 1288 (2003); https://doi.org/10.4315/0362-028X-66.7.1288.
7. H. Ozbek, S. Ugras, H. Dulger, I. Bayram, I. Tuncer, G. Ozturk and A. Ozturk, *Fitoterapia*, **74**, 317 (2003); https://doi.org/10.1016/S0367-326X(03)00028-5.
8. S. Purkayastha, R. Narain and P. Dahiya, *Asian Pacific J. Trop. Biomed.*, **2**, S1625 (2012); https://doi.org/10.1016/S2221-1691(12)60465-1.
9. A. Sheeladevi and N. Ramanathan, *Int. J. Pharm. Biol. Arch.*, **3**, 1106 (2012).
10. Essential oil/http://www.wikipedia.org.
11. D.S. Arora and J. Kaur, *Int. J. Antimicrob. Agents*, **12**, 257 (1999); https://doi.org/10.1016/S0924-8579(99)00074-6.
12. R.K. Upadhyay, *Am. J. Plant Sci.*, **6**, 1058 (2015); https://doi.org/10.4236/ajps.2015.67110.
13. J. Shabnam, M. Sobia, K. Ibatsam, A. Rauf and M.S. Haider, *Afr. J. Biotechnol.*, **11**, 16065 (2012); https://doi.org/10.5897/AJB11.3058.
14. S.A. Mahmoud, S. Clark, B. Woodard, S.A. Deolu-Sobogun, *Ethn. Dis.*, **20(S1)**, 78 (2010).
15. R.Y. Tasheva-Petkova, V.L. Christova-Bagdassarian, K.S. Bagdassarian, and M.S. Atanassova, *Mintage J. Pharm. Med. Sci.*, **2**, 26 (2013).
16. E. Choe and D.B. Min, *J. Food Sci.*, **72**, 77 (2007); https://doi.org/10.1111/j.1750-3841.2007.00352.x.
17. A. Dua, G. Garg and R. Mahajan, *Eur. J. Exp. Biol.*, **3**, 203 (2013).
18. A. Zeller and M. Rychlik, *J. Agric. Food Chem.*, **54**, 3686 (2006); https://doi.org/10.1021/jf052944j.
19. F. Anwar, M. Ali, A.I. Hussain and M. Shahid, *Flavour Fragrance J.*, **24**, 170 (2009); https://doi.org/10.1002/ffj.1929.