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Microbial Assessment of Fenugreek Paste during Storage and Antimicrobial Effect of Greek Clover, *Trigonella foenum-graecum*

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***Abstract*—**In this study, antimicrobial effect of Greek clover was determined with usage of MIC (minimum inhibition concentration) and agar diffusion method. Moreover, pH, water activity and microbial change were determined during storage of fenugreek paste. At first part of our study, microbial load of spices was evaluated. Two different fenugreek pastes were produced with mixing of Greek clover, spices, garlic and water. Fenugreek pastes were stored at 4 °C. At the second part, antimicrobial effect of Greek clover was determined on *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Debaryomyces hansenii*, *Aspergillus parasiticus*, *Candida rugosa*, *Mucor* spp., when the concentrations of Greek clover were 8%, 12% and 16%. According to the results obtained, mould growth was determined at 15th and 30th days of storage in first and second fenugreek samples, respectively. Greek clover showed only antifungal effect on *Aspergillus parasiticus* at previously mentioned concentrations.

***Keywords*—**Antimicrobial, fenugreek, Greek clover, minimum inhibition concentration.

I. Introduction

MICROBIAL activity is one of the major factors causing food spoilage that emerges great challenges to the food industry in preserving food [1]. Microbial safety of foods is one of the most concerning issue for consumers and food industry. Although there are many traditional food preservation methods, microbial contamination and spoilage of foods is still a problem. Even though synthetic antimicrobials are approved in many countries, in the recent years, there has been a rising interest to use of natural preservatives, which emphasizes the exploration of alternative sources of safe, effective and acceptable natural preservatives. Plants contain several constituents and are valuable sources of new and biologically active molecules that have antimicrobial properties [2].

Fenugreek (*Trigonella foenum-graecum* L.) is an annual plant and a member of the leguminous family. It can be grown in Mediterranean countries, the Middle East, and India. Fenugreek is an important spice and its dried seeds have wide applications in food and beverages. Fenugreek has been reported to be having important properties such as antimicrobial and nutritive properties [1], [3], [4]. Fenugreek, like other legumes, is a good source of dietary protein for consumption by human and animals [5]. There are active components in fenugreek, such as alkaloids, flavonoids, steroids [6], [7]. The seeds of fenugreek are commonly used in some countries as a spice in food preparations due to their strong flavour and aroma [8].

Fenugreek paste is a spreadable product and produced from Greek clover, several spices, garlic, salt and water. Fenugreek is the most important ingredient of pastrami processing which is a traditional meat product. It gives characteristic taste and aroma to pastrami, and it also plays a very important role on physicochemical and microbiological profile of pastrami. It is reported that fenugreek paste shows protective effects against microbial contamination from external environment [9].

In this study, antimicrobial effect of Greek clover on pathogen microorganisms was investigated by MIC and agar diffusion method. Furthermore, microbial loads of spices (spice mix, cummin, salt, hot pepper, sweet pepper and Greek clover) that composing fenugreek and 3 different fenugreeks for breakfast that purchased from different local markets in Adana, Turkey were determined. Fenugreeks were produced as spreadable with mixing of spices, garlic and water. Water activity, pH and microbial changes were determined during storage of fenugreeks**.**

II. Material and Method

Escherichia coli, *Staphylococcus aureus*, *Bacillus subtilis*, *Candida rugosa*, *Debaryomyces hansenii*, *Mucor* spp., *Aspergillus parasiticus* used in this study. To activate cultures, the method previously used by Hwang et al., [10] has been adapted to our study [10]. Bacterial cultures were inoculated in Nutrient Broth (NB) and incubated at 37 °C for 48 h, yeast and mould cultures were inoculated in Yeast Malt Extract Broth (YME) and incubated at 25 °C for 3-7 days. Cell concentrations were adjusted to 10^6 CFU/mL for bacterial cultures, 10^4 CFU/mL for yeasts and 10^2 CFU/mL for moulds [10].

After spices, needed for 1 kg fenugreek production, were purchased, total mesophilic aerobic bacteria (TMAB), spore, yeast, mould, coliform and *E. coli* loads of each spice were determined. Mediums and incubation conditions are shown in Table I. After these analyses, spices, garlic and water were mixed in order to produce 2 different fenugreeks in spreadable consistency. Fenugreek compositions are shown in Table II. Fenugreeks were stored at 4 °C.

NA and PDA media were used to determine antimicrobial effect with agar diffusion method. Microorganism cultures are inoculated and spread onto agar plate. Bacteria, yeast and

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mould cell concentrations were adjusted to 10^6 CFU/mL, 10^4 CFU/mL and 10^2 CFU/mL, respectively. After wells were cut into the agar plate with a core drill, 0.1 mL of fenugreek paste and greek clover are added into the wells separately at three different concentrations. Agar plates incubated at 37 °C for 2 days and 25°C for 3-7 days to determine bacteria, yeast and mould growth, respectively. After incubation, the plates were examined for the detection of inhibition zones. Agar diffusion method was made in three replicates [14], [15].

TABLE I
MEDIUMS AND INCUBATION CONDITIONS [11]-[13]

Analysis	Medium	Incubation Conditions
TAMB and Spore	Nutrient Agar (NA)	30°C, 48 h
MIC	NB	37°C, 48 h
Yeast -Mould	Potato Dextrose Agar (PDA)	25°C, 72-120 h
Coliform - *E. coli*	YME Broth Fluorocult Kovacs' indole reagent	37°C, 24 h

TABLE II
FENUGREEK PASTE COMPOSITIONS (%)

Composition	Fenugreek 1	Fenugreek 2
Spice mix	2.9	2.9
Cummin	1.9	2.5
Salt	2.5	-
Hot pepper	5.6	-
Sweet pepper	21.4	-
Greek clover	21	29.1
Red pepper	-	19.9
Garlic	10.3	9.2
Water	34.3	36.4

TABLE III
MICROBIAL LOADS OF SPICES COMPOSING FIRST FENUGREEK

Spice	TAMB *	Yeast *	Mould *	Spore *	Coliform **	*E. coli* **
Spice mix	5.82	5.30	4.60	6.11	>110.00	12.00
Greek clover	5.48	5.14	2.87	5.49	110.00	46.00
Cummin	5.32	5.04	3.69	5.30	2.30	2.30
Sweet pepper	5.79	5.80	4.30	6.14	>110.00	15.00
Hot pepper	3.30	5.86	4.07	5.79	>110.00	15.00

* log CFU/mL, ** MPU/g

TABLE IV
MICROBIAL LOADS OF SPICES COMPOSING SECOND FENUGREEK

Spice	TAMB*	Yeast *	Mould *	Spore *	Coliform **	*E. coli* **
Greek clover	3.57	5.04	5.69	5.04	<0.30	<0.30
Red pepper	6.69	6.14	6.47	5.87	>110.00	110.00
Spice mix	5.84	5.76	6.47	5.78	2.30	0.92
Cummin	5.23	4.69	4.30	5.25	2.30	0.92

* log CFU/mL, ** MPU/g

Greek clover concentrations were adjusted to 8%, 12% and 16% to determine antimicrobial effect with MIC method and NB medium was used. At the end of incubation, effect of greek clover concentrations were evaluated [16].

III. RESULTS AND DISCUSSION

In this study, microbial loads of two different fenugreeks, which were made from various spices, were determined. Antimicrobial effects of fenugreek and Greek clover on *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Debaryomyces hansenii*, *Aspergillus parasiticus*, *Candida rugosa*, *Mucor* spp. were determined with agar diffusion and MIC methods. Water activity, pH and microbial change of fenugreeks were determined during storage at 4 °C.

A. Microbial Load of Raw Material

Microbial loads of spice mix, Greek clover, cummin, sweet pepper, hot pepper, red pepper which are used in production of fenugreeks are shown in Tables III and IV.

B. Water Activity and pH Change of Fenugreeks during Storage

Water activity and pH change of fenugreeks during storage are shown in Table V. pH of first fenugreek increased from 5.10 to 5.12. pH value of second fenugreek was measured as 5.23. During storage, there has been no significant changes in pH values of fenugreeks. Water activity of first fenugreek was decreased from 0.893 to 0.890. Water activity of second fenugreek was measured as 0.903.

TABLE V
WATER ACTIVITY AND PH VALUES OF FENUGREEKS ON 0TH AND 15TH DAYS

	0th Day		15th Day	
	Fenugreek 1	Fenugreek 2	Fenugreek 1	Fenugreek 2
pH	5.10	5.23	5.12	-
Water activity	0.893	0.903	0.890	-

C. Microbial Change of Fenugreeks during Storage

In Table VI, microbial loads of fenugreek pastes are shown. Microbial loads of fenugreek pastes purchased from local market are shown in Table VII. In fenugreek paste, TMAB number decreased and yeast, mould, spore and coliform number increased during storage. Among purchased fenugreek pastes, although mould was not detected in manufactured fenugreeks, it was detected in high quantity in fenugreek paste which was produced at spice-store.

D. MIC and Agar Diffusion Methods to Determine Antifungal and Antibacterial Effect

1. Agar Diffusion Method

In this study, antimicrobial effect of fenugreek and greek clover on tested microorganisms was determined. Obtained agar diffusion results are shown in Table VIII. Fenugreek paste did not inhibit growth of *E. coli* and *S. aureus* at three different concentrations. *B. subtilis* growth inhibited as the fenugreek paste concentration increased. Fenugreek paste did not show inhibitory effect on *C. rugosa* growth but it was determined that *D. hansenii* growth was sensitive to fenugreek paste. Among moulds, it was detected that *Mucor* spp. showed resistance to fenugreek, mycelium of *Mucor* spp. showed medium sensitivity to fenugreek. As much as fenugreek concentration was increased, mycelium of *A. parasiticus*

showed more sensitivity. As a result, it was determined that fenugreek inhibited growth of mould mycelium.

TABLE VI
MICROBIAL LOADS OF FENUGREEKS ON 0TH AND 15TH DAYS

	0th Day		15th Day	
	Fenugreek 1	Fenugreek 2	Fenugreek 1	Fenugreek 2
TAMB*	6.65	5.79	5.79	-
Yeast*	4.47	5.20	5.20	-
Mould*	3.49	4.74	4.74	-
Spore*	5.64	4.47	6.5	-
Coliform**	4.30	24.00	9.30	-
*E. coli***	4.30	0.36	0.74	-

* log CFU/mL, ** MPU/g

TABLE VII
MICROBIAL LOADS OF PURCHASED FENUGREEKS

F	TAMB*	Yeast*	Mould *	Spore *	Coliform**	*E. coli* **
MF 1	7.92	4.60	N.O.	N.O.	0.36	0.36
MF 2	6.93	5.85	N.O.	6.08	0.36	2.30
SS	5.34	5.20	5.70	5.41	0.30	<0.30

F: Fenugreek, MF 1: Manufactured fenugreek paste 1, MF 2: Manufactured fenugreek paste 2, SS: Spice-store fenugreek paste * log CFU/mL, ** MPU/g, N.O. Not Obtained

TABLE VIII
AGAR DIFFUSION RESULTS OF FENUGREEK

	Concentration (mm)		
Microorganisms	8%	20%	29%
E. coli	NO	NO	NO
S. aureus	NO	NO	NO
B. subtilis	8	11	14
C. rugosa	NE	NE	NE
D. hansenii	15.6	18.3	20
Mucor	7	6.7	8.3
Mycelium	12.3	13.3	13.6
A. parasiticus	7.6	8	12.3
Miseller	18	21.3	22.3

NG: No growth, NE: Not effected

Greek clover at 8%, 12% and 16% concentrations did not inhibited growth of test microorganisms in agar diffusion method. Inhibition zones and inhibition activities of antimicrobials were evaluated according to Table IX.

TABLE IX
EVALUATION OF INHIBITION ZONES [17]

Zone diameter	Inhibition rate
0	-
<10	(+)
10-14	(++)
15-19	(+++)
>20	(++++)

(-) Strong Resistant; (+) Resistant; (++) Moderate resistant; (+++) Slight resistant; (++++) Not resistant

According to the results, it was determined that due to low concentrations of greek clover, it did not inhibited growth of microorganisms although low concentrations of fenugreek inhibited growth. This result indicated that antimicrobial effect of fenugreek paste was based on spices in fenugreek paste, except for greek clover.

2. MIC Method

In this study, there was not an inhibitory effect of greek clover concentrations on bacterial and yeast cultures with MIC method. *Mucor* spp. and *Aspergillus parasiticus* growth was inhibited at 12% and 8% greek clover concentrations, respectively.

IV. CONCLUSION

Among fenugreeks which were produced in this study, mould growth was seen was observed on first fenugreek by the 30th day and on second fenugreek by the 15th day.Mould load of spices that mixed in first fenugreek was higher than spices of second fenugreek. Besides, water activity of first fenugreek was higher than second fenugreek.

According to the results obtained, fenugreek inhibited growth of all microorganisms used in this study but greek clover inhibited only mould growth. It was determined that moulds were not resistant to greek clover even at low concentrations. Regarding these results, it appears that fenugreek contributes to the microbial quality of pastrami through covering it and cutting off contact with the external environment. It was determined that higher concentrations of greek clover could be used to observe antimicrobial effect on yeast and bacterial cultures.

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