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## ANTIBACTERIAL ACTIVITY OF SOME NATURAL PRESERVATIVE MATERIALS AND THEIR EFFECTS ON CHARACTERISTICS OF YOGHURT

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### ABSTRACT

This research aimed to study the antibacterial activity of some natural preservative materials (rosemary, garlic, onion, black cumin, thyme, parsley, celery essential oils, Kombucha, propolis ethanolic extract 20% and propolis water extract 20%) compared with the antibacterial activity of some chemical preservatives which may be used in dairy products and their effects on characteristics of yoghurt. The antibacterial activity of these preservative materials was studied on some spoilage and pathogenic bacteria (*Bacillus subtilis* subsp *spizizenii* ATCC 6633, *Escherichia coli* ATCC 14169, *Streptococcus pyogenes* ATCC 21060 and *Staphylococcus aureus* ATCC 29737), through, the inhibition zone and the minimum inhibitory concentration (MIC). The results indicate that the inhibition zone for thyme recorded the highest level ranging from 16 mm for *Str. pyogenes* to 24 mm for *Staph. aureus* followed by celery and rosemary. While, the inhibition zone of propolis water extraction (20%) and onion essential oil recorded the lowest. The effect of using some natural preservative materials in yoghurt manufacture on the keeping quality, sensory evaluation, chemical composition, microbiological analysis and rheological properties of the obtained product was evaluated. The results cleared that the Kombucha (3 & 4%), ethanolic extraction of propolis (0.1%), black cumin essential oil (0.1%) and parsley essential oil (0.05 & 0.1%) can produce yoghurt with good organoleptic, rheological properties and prolonged shelf life for 28 days.

### INTRODUCTION

One of the most important trends in the food industry today is the application of natural food additives. On the other hand, many naturally occurring compounds found in plants have been shown to possess antimicrobial functions and serve as a source of antimicrobial agents against food borne pathogenic microorganisms. Herbal spices have been added to food since ancient times, not only as flavoring agents, but also as folk medicine and food preservatives. In addition to imparting characteristic flavors, certain spices and herbs prolong the shelf life of foods *via* their bacteriostatic or bactericidal activity; also preventing rancidity through their antioxidant activity. Essential oils and their components are becoming increasingly popular as natural antimicrobial agents to be used for a wide variety of purposes, including food preservation, complementary medicine and natural therapeutics.

At present, essential oils are used by the flavouring industry for flavour enhancement and for their antioxidant effect, while the potential use of these oils as natural antimicrobial agents has been less explored (Cosentino *et al.*, 2003). In recent years, there has been a constant search for alternative and efficient agents for food preservation aiming a partial or total replacement of antimicrobial chemical additives and hence reduce the risk of bacteria and fungi in food and play important role for enhancing shelf-life of foods and controlling food pathogenic.

Dairy and dairy products considered to be the most likely foods overall the world. Fermented milks also considered to be most public dairy products. Yoghurt is one of the major likely fermented milks. Although yoghurt has many desirable properties, it is still prone to deterioration, especially at ambient temperature, within a matter of days.

There are so many ways for prolonging the keeping quality of yoghurt. Nevertheless, yoghurt become impalatable within 1-2 weeks, for this reason an alternative preservation processes such as adding of either herbs or essential oils for more prolonging shelf life of such dairy products. One of the most accepted ways to extend the shelf-life of sensible products the use of bio-preservatives (Burt, 2004 and Draughon, 2004).

Therefore, the present investigation aims to study the following objectives:

- 1- The antibacterial activity of some natural preservatives against some pathogenic and spoilage bacteria compared with the antibacterial activity of some chemical preservatives used in the dairy field.
- 2- Producing yoghurt contains some natural antibacterial and flavouring agents and studying the chemical, microbial and sensory qualities along the refrigeration storage periods.

## MATERIALS AND METHODS

### 1. Materials

#### 1.1 Milk:

Fresh mixed cows and buffalos' milk (1:1) was obtained from the herd of Faculty of Agriculture, Moshtohor, Benha University. The chemical composition of milk was ~ 3.2% fat, 3.4% protein, 5% lactose and 0.7% ash.

#### 1.2. Yoghurt starter:

Dried yoghurt starter culture consists of *Lactobacillus delbreuckii* subsp. *bulgaricus* and *Streptococcus thermophilus* was obtained from Chr. Hansen, Copenhagen, Denmark. The obtained starter culture activated and added at a rate of 2g/100g for all the treatments.

#### 1.3. Spoilage and pathogenic bacterial strains:

Spoilage and pathogenic bacterial strains *i.e.* (*Bacillus subtilis* subsp. *spizizenii* ATCC 6633, *E. coli* ATCC 14169, *Streptococcus pyogenes* ATCC 21060 and *Staphylococcus aureus* ATCC 29737) were obtained from

Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

**1.4. Natural preservatives:**

- 1.4.1. Rosemary, parsley, black cumin and celery essential oils were obtained from Kato Aromatic Company, Giza, Egypt.
- 1.4.2. Garlic and onion essential oils were obtained from Al-Neenaea Company for drying food products, Menofia Governorate, Egypt.
- 1.4.3. Propolis was purchased from local market which collected from the hybrid honey bee colonies at Qualiobia Governorate, Egypt. Stored separately in the freezer until used.
- 1.4.4. Kombucha solution was prepared at Food Science Department, Faculty of Agriculture, Moshtohor, Benha University, Egypt.

**1.5. Chemical preservatives:**

Sodium nitrate, sodium nitrite, potassium nitrate, potassium nitrite and hydrogen peroxide were obtained from El-Nasr pharmaceutical CO. Adwic, E0018111, Egypt.

- 1.6. Standard disc 0.6 mm diameter was obtained from El-Nasr pharmaceutical CO. Adwic, E0018111, Egypt.

**2. Methods:**

**2.1. Extraction of propolis:**

Extraction was prepared according to the method of (Tosi *et al.*, 2007)

**2.2. Preparation of essential oils emulsion:**

Essential oils emulsions were prepared according to the method described by (Ismail and Pierson, 1990).

**2.3. Estimation of antibacterial activity:**

Antibacterial activity was measured using agar diffusion method (Kirbey-Bauer method) as described by (Tosi *et al.*, 2007). Minimum inhibitory concentration (MIC) was determined by the microdilution agar plate method of (Moreira *et al.*, 2005).

**2.4. Manufacture of yoghurt:**

Yoghurt was manufactured according to the method described by Tamime (1978). The fresh mixed milk 1:1 cow's: buffalo's was divided into 8 portions, 5 kg each. The 1<sup>st</sup> portion was served as yoghurt control, the other 7 portions were used for manufacture of yoghurt with different types of natural preservatives *i.e* Kombucha at a rate of 3% T<sub>1</sub>, Kombucha at a rate of 4% T<sub>2</sub>, ethanolic extraction of propolis at a rate of 0.1% T<sub>3</sub>, black cumin essential oil at a rate of 0.1% T<sub>4</sub>, parsley essential oil at a rate of 0.05% T<sub>5</sub>, parsley essential oil at a rate of 0.1% T<sub>6</sub> and garlic essential oil at a rate of 0.1% T<sub>7</sub>, respectively. Each portion was heat treated up to ~ 85°C for 30 min, cooled to 42°C, inoculated with 2 g/100 g (w/w) of starter culture, dispensed into plastic cups (120 g each) fitted with press-on-lids and incubated at 40 – 42°C till the pH reached 4.6 the time required for coagulation of each treatment was recorded.

The produced yoghurt was stored at ~5°C and analyzed for its properties *i.e.* (chemical, microbiological, penetrometer reading, curd syneresis and sensory).

### 3. Methods of analysis:

#### 3.1. Chemical analysis of yoghurt:

- 3.1.1. Protein, fat, and total solids of milk and yoghurt were determined according to the methods of International Dairy Federation (IDF), 1993, 1991a and b, respectively.
- 3.1.2. Titratable acidity of yoghurt treatments was determined according to the methodology of AOAC (1990).
- 3.1.3. Total volatile fatty acid contents (TVFA) were determined by the direct distillation method as described by Kosikowski (1997).
- 3.1.4. Acetaldehyde content was determined according to the method described by Lees and Jago (1969).

#### 3.2. Microbiological examination:

- 3.2.1. Lactic acid bacteria (LAB) was enumerated according to Elliker *et al.* (1956).
- 3.2.2. Coliform bacterial count was determined by plating suitable dilution on violet red bile agar (VRBA) medium as suggested by APHA (1992).
- 3.2.3. Yeasts & moulds count was determined according to IDF (1990).

#### 3.3. Physical properties:

##### 3.3.1. Penetrometer reading:

Penetrometer reading was measured using the penetrometer supplied by Setamatic MKVI controller. The results were expressed as penetrometer reading (0.1 mm/s) according to Dixon and Parekh (1980).

##### 3.3.2. Curd syneresis:

Curd syneresis was determined according to the method of Dave and Shah (1998). Curd syneresis is calculated as the percent weight of the separated whey during the determination time (120 min).

##### 3.4. Sensory evaluation:

Organoleptic evaluation was done by 10 experienced Food Scientists Staff at Food Science Department, Moshtohor, Faculty of Agriculture, Benha University, according to the methodology of IDF (1995).

##### 3.5. Statistical analysis:

Statistical analysis for the obtained data was carried out according to the method described by Clarke and Kempson (1997).

## RESULTS AND DISCUSSION

### Antibacterial activity

#### A- Inhibition zone:

The data obtained in Table (1), indicate the inhibitory effect of the natural preservatives (rosemary, garlic, onion, black cumin, thyme, parsley, celery essential oils, Kombucha, propolis ethanolic extract 20% and water extract 20%), and chemical preservatives (H<sub>2</sub>O<sub>2</sub> 250 ppm, sodium nitrate 200 ppm, potassium nitrate 200 ppm, sodium nitrite 200 ppm and potassium nitrite 200 ppm) on the

tested bacteria (*B. subtilis*, *E. coli*, *Str. pyogenes* and *Staph. aureus*). The diameter of inhibition zone proportionally lead to the antibacterial effect of the added preservatives.

**Table (1). Inhibition zones (mm) of natural and chemical preservatives on some spoilage and pathogenic bacteria.**

Preservative materials	Tested bacterial strains			
	<i>B. subtilis</i>	<i>E. coli</i>	<i>Str. pyogenes</i>	<i>Staph. aureus</i>
<b>1-Natural essential oils or preservatives</b>				
Rosemary	15	21	15	20
Garlic *	14	NI	22	11
Onion *	12	NI	NI	7
Black cumin	NI	NI	14	15
Thyme	22	22	16	24
Parsley	13	NI	NI	NI
Celery	21	16	21	21
Kombucha extract	18	21	NI	16
Ethanol extraction of propolis (20%)	15	NI	20	11
Water extraction of propolis (20%)	11	NI	NI	8
<b>2- Chemical preservatives:</b>				
Hydrogen peroxide (250 ppm)	8	10	NI	NI
Sodium nitrate (200 ppm)	NI	NI	NI	NI
Sodium nitrite (200 ppm)	NI	NI	NI	NI
Potassium nitrate (200 ppm)	NI	NI	NI	NI
Potassium nitrite (200 ppm)	NI	NI	NI	NI

\*Concentration of garlic and onion essential oils is 10% of the stock oil.

NI:No Inhibition

Whereas, Garlic and onion essential oils were diluted with Tween 80 to 10% concentration.

The results indicate that the inhibition zone for thyme recorded the highest level followed by celery and rosemary on all tested bacteria, while, the inhibition zone of propolis water extraction 20% and onion essential oil recorded the lowest levels. These results are in agreement with Smith-Palmer *et al.* (1998), Vardar-Unlü *et al.* (2003), Mišić *et al.* (2008) and Snoussi *et al.* (2008) who reported that the antimicrobial activity test with fractions of the essential oils showed that the activity was mainly observed in those fractions containing thymol. Also Imelouane *et al.* (2009) evaluated the essential oil of thyme for its antibacterial activities against Gram positive and Gram negative pathogenic bacteria *i.e.* *Staph. aureus*, *Streptococcus sp.*, *Pantoea sp.*, and *E. coli*. El-Gayyar *et al.* (2001) reported similar results with respect to rosemary on *E. coli* which showed inhibition zone ranging from 23 to 30 mm. Also the inhibition diameters of rosemary essential oil were 15, 21, 15 and 20 mm for *B. subtilis*, *E. coli*, *Str. pyogenes* and *Staph. aureus*, respectively. The inhibition action for rosemary essential oil is due to the main compounds present in this oil *i.e.* 1,8-cineole,  $\alpha$  pinene, camphor... *etc.* which have been their antimicrobial effects (Viljoen *et al.*, 2003 and Fu *et al.*, 2007).