

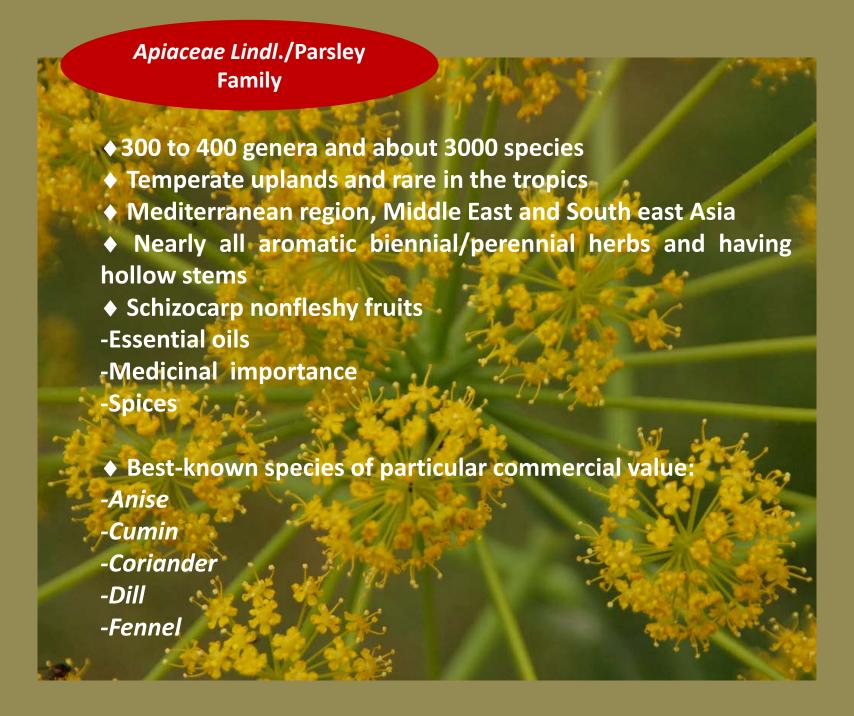
# THE INFLUENCE OF SOME CUMINUM CYMINUM L. ESSENTIAL OIL BIOACTIVE METABOLITES, AS ANTIMICROBIAL AGENTS ON THE QUALITY OF COTTAGE CHEESE

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Michael J of Romania" from Timisoara, Romania



- > Cumin flowering plant in the family Apiaceae
- ➤ Native to Egypt and has been cultivated in the Middle East, India, China and Mediterranean countries for millennia
- ➤ The fruits, also commonly known as ,seeds' are darker in color with a fusiform shape and each one contain only a single seed



## Cuminum cyminum L.

Used in traditional and veterinary medicine

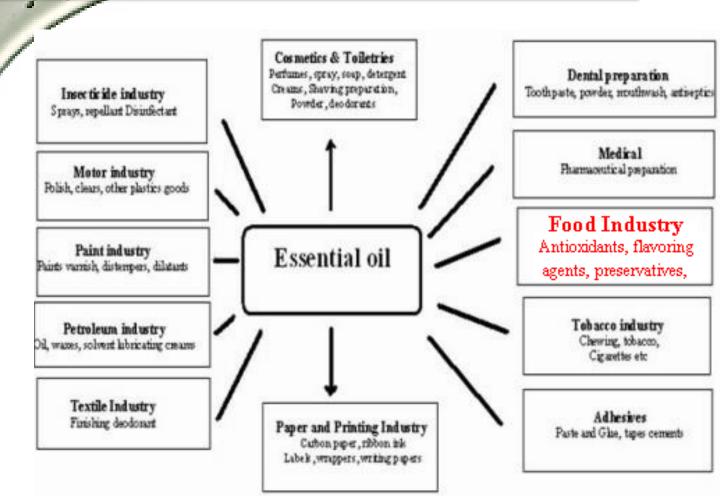
Arabia, India, China and in the countries bordering the Mediterranean Sea – spice condiment in vegetarian and nonvegetarian preparations

Flavoring agent

Pharmacological properties:

- antioxidant activity
- stimulates the secretion of pancreatic enzymes
- antibacterial activity
- antitumor effect
- ameliorates symptoms of diabetes and Parkinson diseases

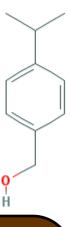
Essential oils are the most important and effective plant secondary metabolites including many type of biological effects.



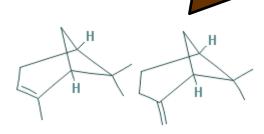
### **ESSENTIAL OIL CONSTITUENTS**

- Terpenes (monoterpenes, sesquiterpenes, and diterpenes) inhibit the accumulation of toxins and help discharge existing toxins from the liver and kidneys (Kang et al., 2014)
  - Sesquiterpenes are antiseptic and anti-inflammatory. They work as a liver and gland stimulant and have the ability to surpass the blood-brain barrier and enter the brain tissue (Boaduo et al., 2014)
- Oxygenated compounds (esters, aldehydes, ketones, alcohols, phenols, and oxides) cause majority of the antibacterial and antifungal action (Carson et al., 2006)
  - ■Aldehydes are highly reactive and in general, they are anti-infectious with a sedative effect on the central nervous system. They can be quite irritating when applied topically (citral being one example), but may have a profound calming effect when inhaled (Chang et al., 2013)
  - Alcohols are commonly recognized for their antiseptic and anti-viral activities. They create an uplifting quality and are regarded as non-toxic. Terpene Alcohols stimulate the immune system, work as a diuretic and a general tonic, and are anti-bacterial as well (Yu et al., 2012; Lee et al., 2005; Reddy et al., 2004)
  - Sesquiterpene Alcohols are anti-inflammatory, anti-bacterial, anti-mycotic, and ulcer-protective
  - ■Phenols are responsible for the fragrance of an oil. They are antiseptic, anti-bacterial, and strongly stimulating but can also be quite caustic to the skin. They contain high levels of oxygenating molecules and have anioxidant properties.
  - **■***Ketones* are sometimes mucolytic and neuro-toxic when isolated from other constituents, however they are helpful with such conditions as dry asthma, colds, flu and dry cough

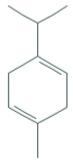




- ☐ Terpenoides: Cuminaldehyde, cuminyl alcohol, in the essential oil
- $\Box$  Terpenes: α- and β- pinene, γ- terpinene, in the essential oil







that are the result of exposure to have acute effects on human health.

Milk is ideal medium for growth of pathogens

Pathogen bacteria







Food safety is defined as "the practical certainty that injury or damage will not result from the food consumed in a reasonable manner".

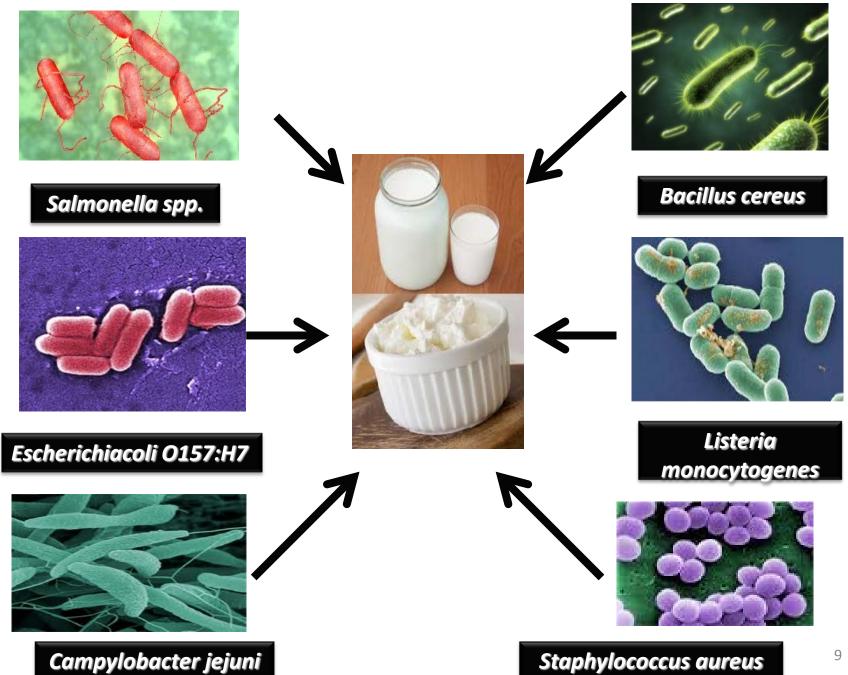
## Food – borne diseases can be caused by certain factors, including:

0	Food from unsafe sources	12%
0	Improper storage temperature	63%
0	Poor personal hygiene	28%
0	<b>Contaminated equipment</b>	23%
0	Inadequate cooking	21%
0	Other things	20%

This total is over 100% because of overlapping.







Staphylococcus aureus





- **❖** Analyses of the cumin essential oil (*Cuminum cyminum L.*) by GC/MS
- **❖Identify the major components**
- **❖**Evaluation the effect of the addition of the oil and its major compound, as a potential antimicrobial agent against some pathogenic bacteria (*Staphylococcus aureus ATCC 6538*, *Escherichia coli ATCC 8739*, and *Salmonella enteritidis PT4*), in cottage cheese, during rippening and storage



**Extraction Procedure: Hydrodistillation** 

Gass chromatography/Mass-spectrometry (GC - MS) Analysis

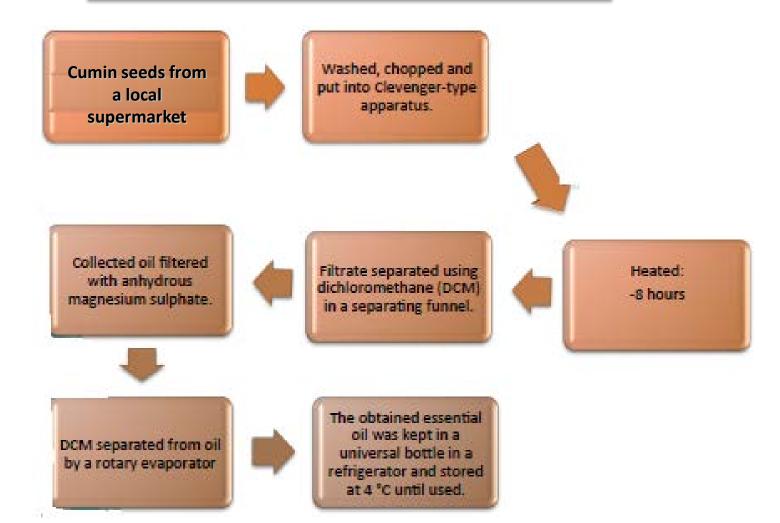
**Preparation of microorganisms** 

Preparation of the cottage cheese

Antibacterial activity: Broth microdilution (MIC)

**Analysis of bacterial membrane integrity** 

## **HYDRODISTILLATION**



## GC-MS ANALYSIS



The essential oil components were identified by comparing their mass spectra (MS) fragmentation pattern and relative retention time with the National Institute of Standards and Technology (NIST) mass spectral database library

(Kamazeri *et al.*, 2012).

Relative percentage of peak area

Area of the peak

Total peak area × 100%

## PREPARATION OF MICROORGANISM

Bacterial strains

- S. aureus (ATCC 6538)
- E. coli (ATCC 8739)
- · Salmonella enteritidis (PT4)

Subculture on Petri dish • 28 g Nutrient agar + 1 L distilled water

Culture in broth • 8 g Nutrient broth + 1 L distilled water

Incubate

• Incubate at 37 °C for 24 hours.

## MANUFACTURE OF THE COTTAGE CHEESE

#### **RAW MILK**

pH-6.55-6.85

#### PASTEURIZATION

65 °C/30 min cooling to 23 °C

 $\downarrow$ 

#### INOCULATION

mesophilic »O« type culture 0.5 % (v/v)/23 °C



#### FERMENTATION

at 22 °C until pH=4.45-4.82



#### CUTTING

curd size = 8-10 mm



#### COOKING

30 min after cutting 55-60 °C/120-180 min



#### WASHING

water ≈ 40 °C/20 min
 water ≈ 15–18 °C/20 min
 water ≈ 1 °C/20 min



#### DRAINING

at ambient temperature, 1.5-3 h

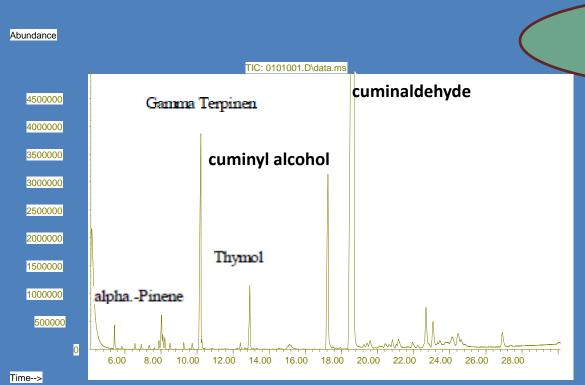


## COTTAGE CHEESE CURD

pH=4.82-5.29

- The parameters for the manufacturing of cheese by the long-set method (4) are presented in Fig. 1.
- Fermentation of 5 L raw milk was performed at 22 °C with 0.5% (v/v) mesophilic starter culture, »O« type (production and without rennet addition.
- Cooking of curd (in water bath) to 55-60 °C was carried out slowly to reach the end point in 120-180 min.
- Before the inoculation with mesophilic culture, the milk was split in 4 equal parts and 3 of them were inoculated each one with tested pathogenic bacteria (10<sup>6</sup> CFU/mL).

In essential oil analysis, identified compounds representing % 94.5 of the oil of cumin. The cuminaldehyde (59.3%) has been found as major component in the essential oil.



The GC/MS analysis of the essential oil from *Cuminum cyminum L seeds* 

- 44 compounds were identified
- Main components being cuminyl alcohol (21.2%), γ-terpinen (17.3%), thymol (6.6%) α-pinene (2.6%), β-pinene (1.1%).

## Minimum Inhibitory Concentration (MIC) of the cumin essential oil and cuminaldehyde

Microorganisms	Essential oil (μL/mL)	Cuminaldehyde (μL/mL)
S. aureus	25.50	30.25
E. coli	75.50	85.50
S. enteritidis	45.50	55.25

## Well diffusion method



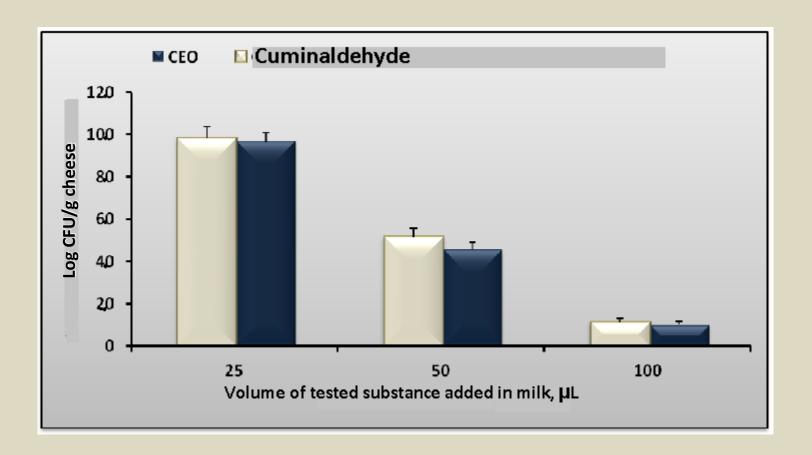


Antibacterial effect of essential oils /cuminaldehyde on S. aureus

Sensitivity to essential oil/cuminaldehyde -mean diameter of the inhibition zone diameter in mm

Microorganisms	Essential oil	Cuminaldehyde
S. aureus	31.2 ±0.21	25.3 ±0.11
E. coli	19.5 ±0.39	17.2 ±0.16
S. enteritidis	25.3 ±0.31	21.2 ±0.21

Effect of cumin essential oil/cuminaldehyde supplementation on bacterial colonization of Cottage cheese after 21 days of storage



## Conclusions



The investigations on antimicrobial activity of cumin essential oils against foodborne bacteria confirmed the potential of this plant volatile oils to be used in Cottage cheese conservation as alternative to chemical preservatives. Gram-negative bacteria

E. coli and S. enteritidis, were less sensitive than Gram-positive bacteria, S. Aureus, but the difference in susceptibility were not that evident

Essential oil of cumin mainly consists of oxygenated monoterpenes, 78.3-89.5%,

Future studies are warranted to investigate the synergic effect of cuminaldehyde against drug resistance bacteria



