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STUDY OF ANTIBACTERIAL EFFECTS OF *TEUCRIUM POLIUM* ESSENTIAL OIL ON *BACILLUS CEREUS* IN CULTURAL LABORATORY AND COMMERCIAL SOUP

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ABSTRACT

According to the increasing use of processed and semi-processed food products and the raise in the awareness about the disadvantages of food additives in the products, consumer preferences has increased in the matter of using natural food additives. Considering that the essential oils of medicinal plants had the attributes that increasing shelf life as the antibacterial effect, tending to use this essential oils in food products has increased. Teucrium polium is the one of this essential oils , which its antibacterial effectson Bacillus cereus in medium and industrial soupare examined in this study. The Obtained essential oil from Clevenger system is injected to GC-MS. In this study the commercial barley soup was used as a food model. Sensory evaluation caused by adding the essential oil from Teucrium polium to barley soup was evaluated using sensory acceptance test. The results of this study showed, the samples with higher concentrations of essential oils, had lower bacteria and samples related to farther days had higher bacteria growth. The effect of time on the regression was also significant ($p<0.05$). Acceptance of adding essential oil from Teucrium polium to barley soup decreased significantly ($p<0.01$). The results also confirmed significant and notable role of essential oil on reducing microbial load of food models and increase of the quality and the safety of the final products..

1. Introduction

With the increase of urban population, tourism, immigration, a variety of food with different components, improve technology in the food industry, changes in food consumption culture and approach to food consumption, food preparation, and finally international trade in food, overburdened the more food illness in the present age, so that about 30 percent of people in developed countries at least once a year to develop food-borne diseases (Burt, 2004).Food storage methods which maintain the quality and extend the shelf life of food because of improved production, supply and trading is important. Human familiar to kept foods by different methods such as the use of heating, cooling, drying and salting long time ago, but in order to reduce or eliminate pathogenic microbial agents and also prevent food spoilage new method is more in need,therefore one of these methods is the use of essential oils as antimicrobial additives in food.Essential oils have antibacterial effect, which mainly is due to the phenolics (Burt, 2004).In latest reports ofvalid scientific sources, antimicrobial properties offered diverse mix of herbs, spices, fruits, vegetables, leaves, bark and found animal,However, still many have not been scientifically studied natural ingredients (Cowan, 1999). In recent years, due to the harmful effects of synthetic and chemical food preservatives, consumers and producers of food prefer to use of natural preservatives such as essential oils which increase the shelf life of food (due to its antimicrobial properties) of a multiplicity of holders harmful chemicals are safe (Cutter, 2000; Akhondzadeh *et al*., 2007).

Preservatives are used to limit the growth and microbial activity in pharmaceutical products, food and cosmetics and by interfering with cell membranes, enzymes or genetic structure of microorganisms have a preventive effect. To apply the essential oils as chemical preservatives in food, investigate their antibacterial activities alone and in combination with other factors affecting the growth of microorganisms in food and nutrition is essential in laboratory models (Darabpour *et al*., 2010). Because essential oils are often used for some food as taste modifiers, protective properties and some of their antimicrobial can also encourage their use for this purpose. Essential oils, commonly called volatile or ethereal oils, are obtained through various distillation (Akin *et al*., 2010).Soil, harvest, plant age and climatic conditions affect the type and amount of the compounds in the oil. The studies also have shown that coincides with the time of flowering, have a stronger antimicrobial activity of essential oils is extracted from plants (Darabpour et al., 2010). Mainly phenolic compounds are responsible for the antimicrobial activity of essential oils (Burt, 2004).The higher amount of composition of the oil causesthe more anti-microbial properties.These materialsinclude eugenol, carvacrol and thymol (Akin *et al*., 2010).

Mary peas is a herb belonging to the mint family, plateau, a height of 10 to 30 cm, with a white cottony appearance, usually in areas poor in nutrients and organic matter, rocky areas and sand dunes in Europe, the Mediterranean region, North Africa and south west Asia, including Iran, especially Khorasan province, for example, different areas of the north , west, south, and Central and Mountain arid Tehran and Alborz region have dispersion.Teucrium genus include about 340 species of annuals and perennial herbaceous species, of which 12 are endemic to Iran Scientific studies have shown that this herb has antioxidant effects, antipyretic and antimicrobial and antispasmodic effect(Zare *et al*., 2011). It has been reported that ethanol extract of this herb is also antibacterial activity against gram positive and gram negative microorganisms of the show itself (Darabpour *et al*., 2010). 10 combined terpenoids such as coderol, linalool Gaol and beta pinene have been found in the oil (Akin *et al*., 2010).

1 to 20 percent of all outbreaks of food poisoning in the world is due to Bacillus cereus (Gilbert and Kramer, 1998).At least from 1906 Bacillus cereus is known to cause food poisoning (Mulet-Powell *et al*., 1998).The bacteria produces extracellular material such as lesitinase C and beta hemolysin, which are important in the bactreria identification.The enterotoxin produced by the bacteria causes diarrhea and nausea, and can induce the syndrome of

diarrhea and vomiting syndrome.In 1950, Bacillus cereus known as causes food poisoning (Rahimifard *et al*., 2007).The bacteria in raw and processed meat, vegetables, rice, cereals and milk and milk-based soups has the ability of proliferation and toxin formation (Gilbert and Kramer, 1998).

2.Materials and methods

View the treatment and study duration is specified in Table 1.

In order to extract the essential oil of TP, the plant was completely milled and dried using a Clevenger apparatus for 3 hours, volatile oil was extracted by water distillation.After dehydrationby dry sodium sulfate, essential oils were stored in dark glass containers and in the refrigerator until the anibacterial properties and the components were identified.The prepared sample extract was injected into gas chromatography connected to mass spectrograph and mass spectra compounds were obtained (Rahimifard *et al*., 2007).

Table 1. Time and the evaluated treatments profile

Number	Sample's name	Added essential oil content (ppm(	Time (Day)
1	Soup 1(Witness)	0	1-5
2	Soup 2	625	1-5
3	Soup 3	1250	1-5
4	Soup 4	2500	1-5
5	Soup 5	5000	1-5

In order to determine the minimum inhibitory concentration of essential oils Micro Broth Dilution Method 2 was used in the study.In this experiment, 96 wells of microplates were used.To increase the solubility and uniform development of oil in the medium, dimethyl sulfoxide, 5% as emulsifiers and for stabilizers agar - agar was used as the 0.05 percent (Akhondzadeh *et al*., 2007).Concentration of essential oil samples were studied for serial dilution.In short, sterile BHI broth in each 80 ml well, 100 ml studied oil dilution was added and eventually 20 ml bacterial culture (containing approximately cfu / ml 106 × 5 of the studied bacteria) were added per well(Akhondzadeh *et al*., 2007).

In each experiment, positive control which contained no oil wells was containing sterile BHI broth along with the studied bacteria and negative control which contained the well containing sterile BHI broth without bacteria was used.After adding the desired concentration of essential oils and bacteria, microplate were incubated at a temperature of approximately 37 °C for 24 hours (Akhondzadeh *et al*., 2007).

After the incubation period for determining the minimum inhibitory concentration, microplate wells werestudied by determining the amount of cloudiness using the naked eye.Finally, theminimal concentrations in which create the inhibition zone or lack of certain opacity compared with the control group was designated as MIC (Akhondzadeh *et al*., 2007).

Barley soup was used according to the manufacturer after the purchase, preparation and meal as models.Autoclavable samples in flasks of 100 ml volume of distribution (50 ml per flask barley soup) and were sterilized.After adding oil at a concentration of 2500 ppm MIC, two lower concentrations of 625 and 1250 ppm and a higher concentration of 5000 ppm, the correct amount of bacteria under sterile conditions into flasks were inoculated. The final number of bacteria in each sample was Cfu / ml 103, which was confirmed by surface culture (Moosavy *et al*., 2008).

Table 2. Treatments table

Treatment	Day	Average essential oil concentrations PPM
Witness	1	0
1	1	625
2	1	1250
3	1	2500
4	1	5000
Witness	2	0
5	2	625
6	2	1250
7	2	2500
8	2	5000
Witness	3	0
9	3	625
10	3	1250
11	3	2500
12	3	5000
Witness	5	0
13	5	625
14	5	1250
15	5	2500
16	5	5000

In order to assess the sensory characteristics of samples generated from sensory acceptance test was used. In order to do so, the prepared barley porridge was devided into seven parts (including 1000 ml flask with a volume of 500 ml barley porridge), and oil concentrations were added to each flask.Sensory evaluation was conducted by the assessment team 7 seater. Panel members made the sensory evaluation of the essential oil-containing barley soup using the intuitive 9 point scale.

The score of 9 was very high, score 8 washigh, score 7 was good, score 6 was relatively good, score 5 was so so, score 4 was relatively bad, score 3 was bad, score 2 was very bad, and the score 1was super bad, for organoleptic characteristics were evaluated in terms (Meilgaard *et al*., 1991).

Data analysis was performed using response surface method optimal design.Statistical data analysis and charting software was design expert editing 8.To change the relationship between the dependent variables Pearson correlation coefficient and SPSS statistical software version 20 was used.The behavior was Cubic model or Grade 3. Due to the abnormal data analysis of variance in normal mode, it was normalizedusing logarithmic transfer function instead.

3. Results and discussions

According on the analysis made on Kalpureh, 23 volatile compounds were identified in the essential oil, which are shown in table 3 (analysis results using chromatography connected to mass spectrograph- the relevant mass spectrums identified in GC/MC is also available in the appendix).

Table 3. Oils agronomic criteria analysis using the chromatography connected to mass spectrograph

Combine name	Quatz index (K)	Percent
Alpha-Pinene	939	16.2
Beta-Pinene	979	7.1
Myrcene	991	4
Limonene	1029	5.2
Alpha-Campholenal	1126	1.1
Verbenol<trans>	1145	6.3
Camphor	1146	2.1

Menthone	1153	1.3
Mentha-1,5-dien-8-ol <para->	1170	3.2
Myrtenal	1196	1.5
Verbenone	1205	0.75
Pulegone	1237	2.9
Bornyl acetate	1289	2.8
Carvacrol	1299	8
Caryophyllene<(E)->	1419	3.7
Germacrene D	1485	3.5
Bicyclogermacrene	1500	1.9
Spathulenol	1578	10.6
Caryophyllene oxide	1583	5.7
Unknown	1680	3.1
Beta-Eudesmol	1654	5.7
Valerianol	1658	1.6
Cryptomerione	1725	1.7

In this study there were 16 treatments, which are shown in table 4, along with the results of microbial tests on the related samples.

Table 4. Microbial test results (count Bacillus cereus)

Treatment	**Day**	**Average essential oil concentrations) PPM (**	**Average number of Bacillus cereus (cfu / ml)**
Witness	1	0	21700
1	1	625	19000
2	1	1250	13000
3	1	2500	10000
4	1	5000	5000
Witness	2	0	7000
5	2	625	5000
6	2	1250	4500
7	2	2500	4000
8	2	5000	3000
Witness	3	0	3400
9	3	625	2000
10	3	1250	1200
11	3	2500	1100
12	3	5000	1000
Witness	5	0	2000
13	5	625	600
14	5	1250	500
15	5	2500	400
16	5	5000	100

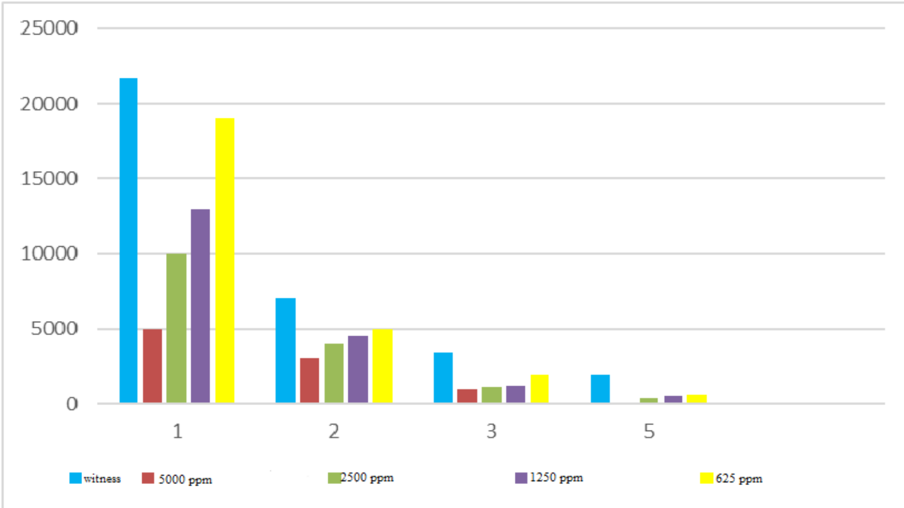

Figure 1. Bar chart of the day and the average number of B. cereus in different concentrations

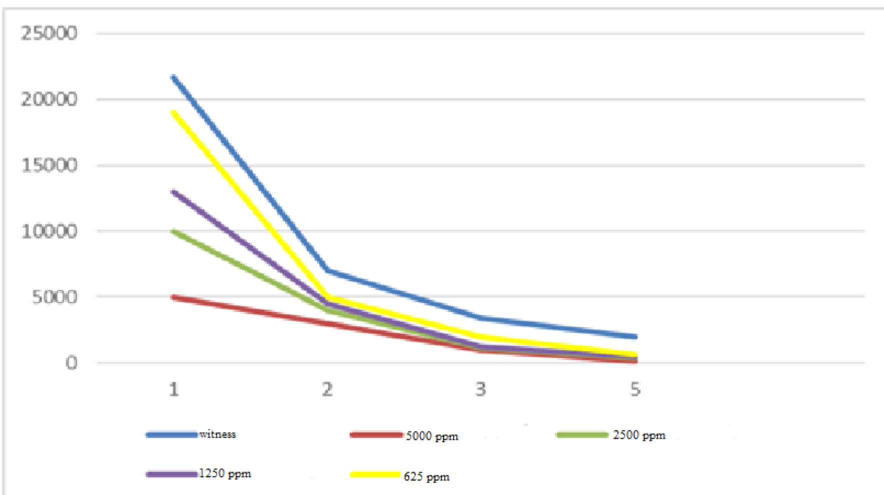

Figure 2. - Line graph B. cereus per day and the average number of different concentrations

The highest concentration was 5000 ppm causing the lowest bacterial count, which was 100 CFU/ml. The highest and lowest bacterial count belonged to the first and last day respectively, as the antimicrobial effect of the essential oil increased by time and in also amplified by increased concentration, as the 5-day-stored sample with the concentration of 5000 ppm caused the lowest bacterial count. Therefore, the two factors of the essential oil concentration and the storage period was effective on the survival rate of Bacillus cereus, leading to meaningful changes during the test.

In order to evaluate the sensory characteristics of adding the essential oil of Klapureh to barley soup, sensory acceptance test number 4 was used. Sensory evaluation was made by a 7-member group. The group members used a 9-numbered scale in order to make a sensory evaluation about the essential oil-containing soup. The analysis of the sensory test was done by Friedman test.

Meaningfulness in smell : P=0.002, therefore ranking in 0.01 is meaningful.

Meaningfulness in taste : P= 0.000, therefore ranking in 0.01 is meaningful.

Meaningfulness in color : P=0.4, therefore the ranking is not meaningful. Increase in concentration and time causes the correlation to increase by 24000 and then decrease, demonstrating the meaningfulness of the time effect.

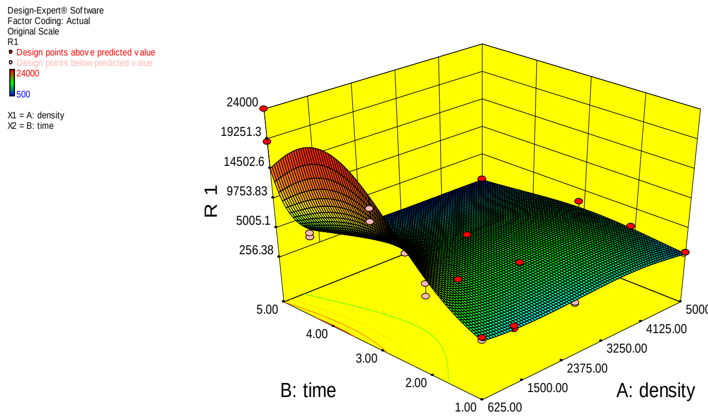

Figure 3. The combined effect of time and concentration on solidarity

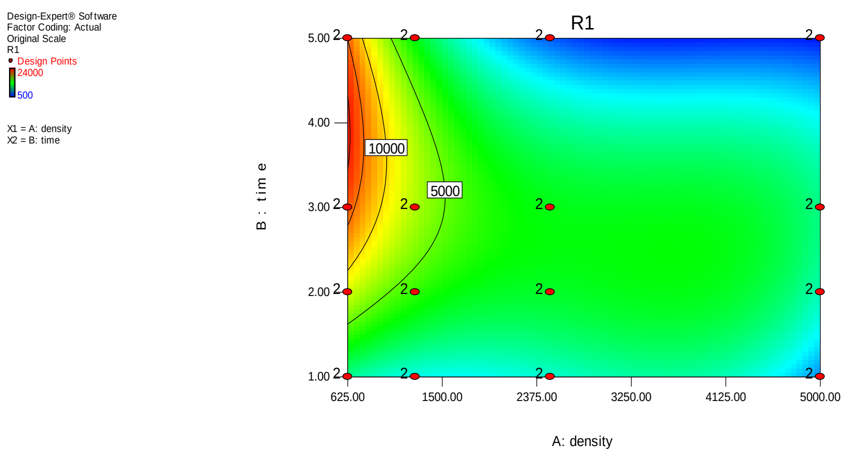

Figure 4. The combined effect of time and concentration on solidarity

According to the data of the analysis of One-way ANOVA, the effect of concentration wasn't meaningful. The applied modelling approach was Stepwise Regression.

Based on the results of Mahmoudi *et al* (2012), *L. casei* populations were not inhibited by low concentrations of the T. polium EO. However, increases in the EO concentrations lead to decreases in bacterial counts ($P < 0.05$) (Mahmoudi *et al*., 2010).

Zare et al (2011) results indicate that this essential oil has a high potential of antibacterial effect and resazurin can be used as a good growth indicator for different bacterial pathogens. Therefore, it can be suggested to purify and evaluate the active substances of this essential oil for future application as antibacterial agent and food preservative to combat pathogenic and toxigenic microorganisms (Zare *et al*., 2011).

4.Conclusions

The results of the present study showed that the commercial barley soup can cause increase in the food safety in refrigerator temperature. Therefore, the joint effect of Kalpureh essential oil and other bacteristat agents (such as natural preservatives) can cause enhancement in the usage of various essential oils in the food industry. Due to the results of this study, the samples containing higher concentration of the essential oil had less bacteria, and the samples of the nest days had more bacteria.

The effect of time on the regression was also meaningful. This fact demonstrates the meaningful and significant effect of the essential oil in decreasing the bacterial count of the food samples and the increase in quality and safety of the final product. However, the bitter taste of the essential oil caused low sensory acceptance.

Therefore, in case of using this essential oil in the food industry as a natural preservative, methods must be taken into consideration in order to eliminate the bitter taste such as microencapsulation of the essential oil.

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