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Original Research

# The effect of ajowan oil on the physicochemical, sensory and microbial parameters of yogurt (low-fat with mold)

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**ABSTRACT:**
The purpose of the current research is to investigate the effects of ajowan oil on physicochemical, organoleptic, microbiological and shelf life of set yogurt (low-fat mold yogurt). Factorial test was used to analyze the data in a completely randomized design. SAS (9.1 version) software was used for the statistical analysis. The extraction process was done through water distillation in the Clevenger apparatus. Ajowan oil was added to the produced yogurt *in vitro* in the concentrations of 20, 40, 60, 80 ppm after the starter and before packaging stages. The produced yogurt was evaluated physicochemically, microbiologically and also subjected to organoleptic studies (5 spotted hedonic) within 28 days at specified interval times. The evaluated treatments of the study included oil concentrations (20, 40, 60 and 80 ppm) and time (0, 7, 14, 21 and 28 days). The parameters such as the pH, acidity, Water Holding Capacity (WHC), mold and yeast counting and organoleptic properties of yogurt were evaluated. The analysis of variance showed that the effect of the treatments of concentration, time and their reaction was significant on these properties ($P<0.05$). Also, the addition of ajowan oil had a significant effect on the physicochemical, organoleptic and microbiological properties ($P<0.05$). In general, the results showed that the addition of ajowan oil to the yogurt led to reduced microbial spoilage and controlled the acidity increase and pH decrease of yogurt and decreased the WHC. Ajowan oil (60 ppm) had a favorable impact on organoleptic characteristics (appearance, taste, texture, odor and total acceptance) and it was found that the 60 ppm concentration showed the best result among the used concentrations.

**Keywords:**
Ajowan oil, yogurt, physicochemical properties, organoleptic evaluations.

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## INTRODUCTION

Keeping food healthy and with high quality during production, storage and consumption is the main concern of nutrition and food industry specialists (Tnore *et al.,* 2011). Today, various types of yogurt is produced in the world that could be categorized based on the physical nature (mold, stirred yogurt, etc.), the chemical nature (high-fat, low-fat, etc.), characteristics of flavor (natural and simple, fruity and flavored) and diversity (the addition of vitamins and vegetable oils, heat treatments, etc.) (Tamime and Robinson, 1999). Like many popular dairy products, the consumption of yogurt grew significantly. This product was popular with the general public due to the presence of high amount of calcium, vitamins, minerals and low content of fat and also its effects on health and longevity (Kowalski *et al.,* 2000; Rosemont, 1990). The tendency to consume foods with natural additives has increased due to raising awareness of consumers and concerns about the use of chemical additives and artificial (Ekhtiarzade *et al.,* 2011). Essences are very important for the properties of aroma, flavoring and preventing corruption in the food industry (Ghasemi, 2009 ). The results of most studies showed the beneficial effects of various additives on improving the properties of non-fat yogurts (Amaya-Llando *et al.,* 2008; El-Aziz *et al.,* 2004; Mistry and Hassan, 1992; Sandoval-Castilla *et al.,* 2004). Ajowan with the scientific name (*Trachyspermum copticum*) is a plant from the family of Apiaceae (Najafi, 2011). It grows in Iran, India, Pakistan and Egypt. It grows naturally and is being cultivated in southern Iran especially in Sistan, Baluchestan, Khuzestan and Fars (Rao, 1986). The essence of this plant is known as ajowan oil. This essence is colorless or a brown is color and smells like thyme. Its components are thymol, carvacrol, alpha, beta-pinene, terpinene, para-simon, etc. (Aktug and Karapikar, 1987). Since, thymol and para-simon are the main constituent of this plant, the antimicrobial activity is higher. (Azar *et al.,* 2010). In general, phenolic compounds are the main components of ajowan which have antioxidant and antimicrobial properties and could be used as preservatives in foodstuffs. It also has various applications in medical and pharmaceutical industries (Bown, 1996).

## MATERIALS AND METHODS

### Extraction method

The extraction of was done through distillation by the Clevenger apparatus for 3 hours. After that, the extract was preserved in a dark glassy container in the refrigerator at 4 to 5°C (Shakerian *et al.,* 2012). Extract was injected into the Gas Chromatography/Mass Spectrophotometer (GC/MS) to identify the components of ajowan oil (Adams, 2001).

### Yogurt production with ajowan oil

Healthy and high-quality raw milk free of antibiotics was used for all the treatments. The average composition of the milk used in the yogurt was shown in Table 1. To prepare milk, the percentage of milk-fat was standardized to 1.5 percent and 1.5 percent dry milk was added to milk to standardize the dry matter of the yogurt. It is then pasteurized and homogenized at a temperature of 85-90°C for 5 minutes (Atash *et al.,* 2006). Milk in sterile epithelial containers were divided and cooled to a temperature of 45°C and 2% starter (starter of the Christine Hansen Company) was added in a direct method and mixed completely. Afterwards, ajowan oil was added in the different concentrations of 20, 40, 60 and 80 ppm, with a sterile syringe in a specified size under completely sterile conditions to the containers containing whey milk. It is then packaged and door sealing was performed. Also, the samples were divided in sterile containers for sampling which was previously

**Table 1. Composition of milk for yogurt production**

| Percent | Compound |
|---|---|
| 1.5 | Fat |
| 3.3 | Protein |
| 8. 32 | SNF |
| 14.53 | Acidity |

coded, and then capping was done. After that, all of the samples were transferred to the incubator set at 45°C. After four hours, the acidity of the yogurt reaching to 68 degrees dornik was kept is the incubator and refrigerated at 4°C. Given that fermented products like yogurt are usually consumed between one week and ten days after production, the yogurt samples were stored for four weeks (Shakerian *et al.,* 2012).

**Parameters studied**

**Measuring pH**

To measure pH, calibrated at $20^0$C was used pH meter. Then, the samples were poured into the epithelial containers and the electrode of pH meter was completely immersed into the samples for 40 seconds and the temperature of the samples was about $20^0$C and the pH was read and noted (unknown, 2006).

**Acidity**

10 ml of the sample was poured into a suitable epithelial container (100 or 250 ml) and 5.0 ml of phenolphthalein was added to it and was tittrated with the sodium hydroxide (1 N) till pale pink color appears which was stable for at least five seconds. Acidity was calculated in terms of the percent of the lactic acid by the following formula (unknown, 2006).

Acidity (g/l) =The amount of consumed sodium × 0.9

**Water Holding Capacity**

Water holding capacity was estimated through the method by Sahan *et al.* (2008), 5g of sample was centrifuged at 4500 rpm for 30 minutes. The aqueous phase and sedimentary mass was separated, collected and weighed. WHC was calculated by the following equation:

$$WHC = (1 - \frac{w_t}{w_i}) \times 100$$

in which:

Wt is the sedimentary mass weight (g)

Wi is the original sample weight (g)

**Mold and yeast count**

By serial dilution, 0.1 ml of the sample from $10^{-1}$ dilution was poured onto petri dishes containing sterile SDA medium. It is then incubated at $25^0$C for five days and the colonies were then counted.

**Organoleptic evaluation**

In organoleptic evaluation, four characteristics such as taste, appearance, oral and odor of the samples were tested by using a five point hedonic test in which the highest option rated '5' and the poor one was rated as '1' (Shakerian *et al.,* 2012).

## RESULTS

**pH**

The analysis of variance showed that the effect of the treatments on concentration, time, and their interaction were significant with pH at 5% level ($P<0.05$). The highest and the lowest mean of pH devoted to the concentration of zero at the day 28 and the concentration of 60 at the day 14 respectively. The concentration of zero had the highest average, throughout the experimental period (Table 2-4).

**Acidity**

The analysis of variance showed that the effect of the treatments on concentration, time and their interaction were significant on acidity at 5% level ($P<0.05$). The highest mean of acidity devoted to the concentration of zero at the day 28 and there was the lowest mean in all concentrations at the day 28, and they were placed in one statistical group (Table 2-4).

**Water Holding Capacity**

The analysis of variance showed that the effect of the treatments on concentration at 1% and time and their interaction at 5% level were significant with moisture property ($P<0.05$). The highest and the lowest mean of moisture was devoted to the concentration of zero at the day 0 and the concentration of 80 at the day 28 respectively.

**Mold and yeast count**

The analysis of variance showed that the effect of the treatments on concentration, time and their

**Table 2. Analysis of variance in response to the treatments**

| S. O. V | df | pH | Acidity | WHC | Mold and yeast count |
|---|---|---|---|---|---|
| Concentration | 4 | 2.12* | 0.32* | 304.34** | 14.45** |
| Time | 4 | 2.23* | 0.25* | 187.55* | 15.41** |
| Interaction | 16 | 1.79* | 0.23* | 144.27* | 11.56** |
| Error | 50 | 0.77 | 0.10 | 68.70 | 3.21 |

*and ** show significant effect at 5 and 1% probability level, respectively

S.O.V: Source of Variation; df: degrees of freedom

**Table 3. Means and comparisons of traits in response to treatments**

| Concentration (ppm) | pH | Acidity | WHC | Mold and yeast count |
|---|---|---|---|---|
| 0 | $5.4^{a}$ | $81.8^{c}$ | $84.0^{a}$ | $1.3^{a}$ |
| 20 | $4.5^{c}$ | $82.4^{bc}$ | $82.2^{c}$ | $1.3^{a}$ |
| 40 | $4.3^{c}$ | $83.0^{b}$ | $83.6^{b}$ | $1.1^{b}$ |
| 60 | $4.3^{c}$ | $83.6^{b}$ | $82.2^{c}$ | $1.0^{b}$ |
| 80 | $5.0^{b}$ | $86.2^{a}$ | $82.2^{c}$ | $0.2^{c}$ |
| **Time (days)** | | | | |
| 0 | $4.7^{b}$ | $88.8^{a}$ | $88.8^{a}$ | $0.0^{c}$ |
| 7 | $4.6^{c}$ | $84.6^{b}$ | $88.4^{a}$ | $1.1^{b}$ |
| 14 | $4.6^{b}$ | $84.8^{b}$ | $85.8^{b}$ | $1.1^{b}$ |
| 21 | $4.7^{b}$ | $82.4^{c}$ | $78.4^{c}$ | $1.3^{a}$ |
| 28 | $4.7^{a}$ | $76.4^{d}$ | $72.8^{d}$ | $1.4^{a}$ |

**Table 4. Means and comparisons of traits in response to interaction of treatments**

| Concentration (ppm) | Time (days) | pH | Acidity | WHC | Mold and yeast count |
|---|---|---|---|---|---|
| 0 | 0 | $5.31^{ab}$ | $85^{d}$ | $91^{a}$ | $0^{f}$ |
| | 7 | $5.38^{ab}$ | $82^{de}$ | $88^{ab}$ | $1.39^{cd}$ |
| | 14 | $5.32^{ab}$ | $85^{d}$ | $86^{bc}$ | $1.54^{bc}$ |
| | 21 | $5.44^{ab}$ | $81^{de}$ | $80^{c}$ | $1.74^{ab}$ |
| | 28 | $5.5^{a}$ | $76^{e}$ | $75^{de}$ | $1.92^{a}$ |
| 20 | 0 | $4.62^{c}$ | $87^{cd}$ | $89^{ab}$ | $0^{f}$ |
| | 7 | $4.46^{cd}$ | $83^{de}$ | $87^{bc}$ | $1.48^{bc}$ |
| | 14 | $4.37^{cd}$ | $84^{d}$ | $85^{bc}$ | $1.45^{bc}$ |
| | 21 | $4.45^{cd}$ | $82^{de}$ | $78^{cd}$ | $1.65^{ab}$ |
| | 28 | $4.47^{cd}$ | $76^{e}$ | $72^{e}$ | $1.9^{a}$ |
| 40 | 0 | $4.345^{cd}$ | $88^{bc}$ | $89^{ab}$ | $0^{f}$ |
| | 7 | $4.37^{cd}$ | $84^{d}$ | $90^{a}$ | $1.24^{d}$ |
| | 14 | $4.25^{d}$ | $84^{d}$ | $87^{bc}$ | $1.39^{cd}$ |
| | 21 | $4.24^{d}$ | $84^{d}$ | $78^{cd}$ | $1.38^{cd}$ |
| | 28 | $4.28^{cd}$ | $75^{e}$ | $74^{de}$ | $1.45^{bc}$ |
| 60 | 0 | $4.39^{cd}$ | $91^{ab}$ | $88^{ab}$ | $0^{f}$ |
| | 7 | $4.34^{cd}$ | $84^{d}$ | $88^{ab}$ | $1.15^{d}$ |
| | 14 | $4.23^{d}$ | $85^{cd}$ | $85^{bc}$ | $1.24^{d}$ |
| | 21 | $4.31^{cd}$ | $81^{de}$ | $77^{cd}$ | $1.34^{cd}$ |
| | 28 | $4.38^{cd}$ | $77^{e}$ | $73^{de}$ | $1.3^{cd}$ |
| 80 | 0 | $4.94^{b}$ | $93^{a}$ | $87^{bc}$ | $0^{f}$ |
| | 7 | $4.96^{b}$ | $90^{ab}$ | $89^{ab}$ | $0^{f}$ |
| | 14 | $4.98^{b}$ | $86^{bc}$ | $86^{bc}$ | $0^{f}$ |
| | 21 | $4.85^{b}$ | $84^{d}$ | $79^{cd}$ | $0.5^{e}$ |
| | 28 | $5.05^{b}$ | $78^{e}$ | $70^{e}$ | $0.5^{e}$ |

interaction were significant with mold and yeast count at 1% level ($P<0.01$). The highest and the lowest mean of mold and yeast count were devoted to the concentration of zero at the day 28 and all the concentrations at the day zero respectively (Table 2-4).

**Table 5. Analysis of variance of treatments on organoleptic characteristics**

| S. O. V | df | Appearance | Taste | Texture | Odor | Total acceptance |
|---|---|---|---|---|---|---|
| Concentration | 4 | 8.44* | 12.09* | 6.34* | 0.97* | 1.47* |
| Time | 4 | 8.19* | 13.73* | 8.51** | 1.01* | 1.86* |
| Interaction | 16 | 7.80* | 9.30* | 6.14** | 0.79* | 1.12* |
| Error | 50 | 3.21 | 4.43 | 1.98 | 0.35 | 0.56 |

*and ** show significant effect at 5 and 1% probability level, respectively

S.O.V: Source of Variation; df: degrees of freedom

**Table 6. Means and comparisons of organoleptic traits in responses to treatments**

| Concentration (ppm) | Appearance | Taste | Texture | Odor | Total acceptance |
|---|---|---|---|---|---|
| 0 | $3.8^{bc}$ | $3.4^{b}$ | $3.6^{b}$ | $3.6^{c}$ | $3.6^{c}$ |
| 20 | $3.4^{c}$ | $3.3^{b}$ | $3.7^{b}$ | $3.4^{c}$ | $3.5^{c}$ |
| 40 | $4.5^{a}$ | $4.5^{a}$ | $4.5^{a}$ | $4.3^{b}$ | $4.3^{ab}$ |
| 60 | $4.7^{a}$ | $4.7^{a}$ | $4.5^{a}$ | $4.5^{a}$ | $4.7^{a}$ |
| 80 | $4.4^{b}$ | $3.7^{a}$ | $4.4^{a}$ | $4.2^{b}$ | $4.6^{a}$ |
| **Time (days)** | | | | | |
| 0 | $4.3^{ab}$ | $4.1^{a}$ | $4.6^{a}$ | $3.8^{c}$ | $4.4^{a}$ |
| 7 | $4.2^{b}$ | $4.1^{a}$ | $4.1^{b}$ | $4.2^{a}$ | $4.2^{b}$ |
| 14 | $4.3^{a}$ | $3.8^{c}$ | $4.0^{b}$ | $4.0^{b}$ | $4.1^{b}$ |
| 21 | $4.0^{c}$ | $4.0^{b}$ | $4.0^{b}$ | $4.0^{b}$ | $4.0^{c}$ |
| 28 | $4.0^{c}$ | $3.6^{d}$ | $4.0^{b}$ | $4.0^{b}$ | $4.0^{c}$ |

**Table 7. Means and comparisons of organoleptic traits in response to interaction of treatments**

| Concentration (ppm) | Time (days) | Appearance | Taste | Texture | Odor |
|---|---|---|---|---|---|
| 0 | 0 | $4.0^{c}$ | $4.0^{b}$ | $4.0^{c}$ | 4.0 |
| | 7 | $5.0^{a}$ | $4.0^{b}$ | $4.0^{c}$ | 4.0 |
| | 14 | $4.0^{c}$ | $3.0^{c}$ | $4.0^{c}$ | 4.0 |
| | 21 | $3.0^{e}$ | $3.0^{c}$ | $3.0^{e}$ | 3.0 |
| | 28 | $3.0^{e}$ | $3.0^{c}$ | $3.0^{e}$ | 3.0 |
| 20 | 0 | $4.0^{c}$ | $3.0^{c}$ | $5.0^{a}$ | 3.0 |
| | 7 | $3.0^{e}$ | $3.5^{bc}$ | $3.5^{d}$ | 4.0 |
| | 14 | $3.0^{e}$ | $3.0^{c}$ | $3.0^{e}$ | 3.0 |
| | 21 | $4.0^{c}$ | $4.0^{b}$ | $4.0^{c}$ | 4.0 |
| | 28 | $3.0^{e}$ | $3.0^{c}$ | $3.0^{e}$ | 3.0 |
| 40 | 0 | $5.0^{a}$ | $5.0^{a}$ | $5.0^{a}$ | 4.0 |
| | 7 | $4.7^{ab}$ | $4.7^{ab}$ | $4.7^{ab}$ | 4.3 |
| | 14 | $4.5^{bc}$ | $4.5^{ab}$ | $4.5^{b}$ | 4.5 |
| | 21 | $4.0^{c}$ | $4.0^{b}$ | $4.0^{c}$ | 4.0 |
| | 28 | $4.5^{b}$ | $4.5^{ab}$ | $4.5^{b}$ | 4.5 |
| 60 | 0 | $5.0^{a}$ | $5.0^{a}$ | $4.0^{c}$ | 4.0 |
| | 7 | $5.0^{a}$ | $5.0^{a}$ | $5.0^{a}$ | 5.0 |
| | 14 | $5.0^{a}$ | $5.0^{a}$ | $5.0^{a}$ | 5.0 |
| | 21 | $4.0^{c}$ | $4.0^{b}$ | $4.0^{c}$ | 4.0 |
| | 28 | $4.5^{b}$ | $4.5^{ab}$ | $4.5^{b}$ | 4.5 |
| 80 | 0 | $3.3^{de}$ | $3.7^{bc}$ | $5.0^{a}$ | 4.0 |
| | 7 | $3.5^{d}$ | $3.5^{bc}$ | $3.5^{d}$ | 3.5 |
| | 14 | $5.0^{a}$ | $3.5^{bc}$ | $3.5^{d}$ | 3.5 |
| | 21 | $5.0^{a}$ | $5.0^{a}$ | $5.0^{a}$ | 5.0 |
| | 28 | $5.0^{a}$ | $3.0^{c}$ | $5.0^{a}$ | 5.0 |

**Appearance**

The analysis of variance showed that the effect the treatments on concentration, time and their interaction were significant with appearance at 5% level ($P<0.05$). The highest point of appearance was devoted to the treatment at days 0, 7 and 14, and the highest mean was devoted to the concentration 80 ppm at the day 21 and 28 (Table 5-7)

**Table 8. The analysis result of ajowan by using GC-MAC**

| Percent | Prevent index[2] | Prevent time[1] | Compound name |
|---|---|---|---|
| 63/42 | 1235 | 27/64 | Thymol |
| 0/07 | 852 | 9/41 | Alpha- Thujene |
| 0/06 | 857 | 9/64 | Alpha- Pinene |
| 0/44 | 899 | 11/34 | Sabinene |
| 19/01 | 956 | 14/06 | P- Cymene |
| 0/10 | 958 | 14/12 | Beta- Ocimene X |
| 16/89 | 993 | 15/85 | Gamma- Terpinene |
| 99/99 | | | Total |

**Taste**

The analysis of variance showed that the effect of the treatments on concentration, time and their interaction were significant with taste at 5% level ($P<0.05$). The concentration of ajowan oil decreases the speed of becoming tasteless and the best acceptation was at the day where the concentration was 60 ppm.

**Texture**

The analysis of variance showed that the effect of the treatments on concentration at 5% level and time and their interaction at 1% level were significant with texture ($P<0.01$). The mutual effect of concentration and time implied that decreasing the mean of texture was lower in higher concentrations and using the ajowan oil prevented from decreasing the mean of texture along time. The highest mean of texture was devoted to the concentration of 80 ppm at the day 28 (Table 5-7).

**Odor**

The analysis of variance showed that the effect of the treatments on concentration, time and their interaction were significant with odor at 5% level ($P<0.05$). The lowest mean was devoted to the concentration of 20 ppm at the day zero and the highest mean was devoted to the concentrations of 60 and 80 ppm on all the days.

**Total acceptance**

The analysis of variance showed that the effect of the treatments on concentration, time and their interaction were significant with total acceptance at 5% level ($P<0.05$). The highest mean was devoted to the concentration of 60 ppm at the days 0, 7 and 14, and the highest mean was devoted to the concentration of 80 ppm at the days 21 and 28.

**GC-MS analysis**

GC-MS analysis showed that thymol was in the highest percentage among the constituents and the lowest percentage of constituents was devoted to alpha-pinene (Table 8).

**DISCUSSION**

The current study investigated the effect of ajowan oil on physicochemical, organoleptic and microbiological properties of the yogurt. The studied concentrations were 0, 20, 40, 60 and 80 ppm at 0, 7, 14, 21 and 28 days. The results showed that ajowan oil concentrations had an effect on the studied traits and the effects of these concentrations were different at different times. The obtained results were in line with the results of Burt (2004) .

In the current study, increasing ajowan oil concentrations reduced the pH. In this regard, the result of current study was in line with Khosroshahi *et al.* (2013). They reported that the type of oil (*Angelica* and oregano*)* had no significant effect on pH, but using different concentrations had a different effect on pH. Also, Zadeh (2012) investigated the effect of Shang oil on organoleptic and shelf life of the yogurt. In this study, the Shang oil controlled the increase of acidity and decrease of pH leading to increased shelf life of the yogurt, which was in line with the result of the present study.

Moreover, the result of this study was in line with the result of Shakerian *et al.* (2012) who reported that celery oil had effect on physicochemical property of yogurt and controlled the increasing acidity of the yogurt. The treatments of ajowan oil led to decrease of the moisture in comparison to the control. Hacheso *et al.*

(2014) investigated the effect of *Angelica* oil on microbial property of dough. The results showed that increasing the concentration of oil decreased doughs' molds and yeasts.

## CONCLUSION

The results of present study showed that the addition of ajowan oil had a significant effect on physicochemical, organoleptic and microbial properties of the yogurt. The addition of ajowan oil controlled the acidity of the yogurt and prevented from souring across the time increasing its shelf life and so it could be possible to produce a stable product. Also, the addition of ajowan oil decreased the WHC and increased the yogurts' water. Moreover, the ajowan oil increased the shelf life of the yogurt through decreasing the number of molds and yeasts, so it could be possible to produce a product with more than 21 days of consumption date. The ajowan oil at a concentration of 60 ppm had a favorable impact on organoleptic characteristics (appearance, taste, texture, flavor and overall acceptance) of the yogurt and the concentration of 60 ppm had the best result among the used concentrations.

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