Effects of *Allium ascalonicum* essential oil on histamine formation in feta cheese

*Accepted 8th October, 2013*

**ABSTRACT**

Some food-fermenting Lactic Acid Bacteria (LAB) can produce Biogenic Amines (BAs). The consumption of food containing large amounts of these amines can have toxicological consequences. This research evaluated the simultaneous effect of *Allium ascalonicum* essential oil (EO) on formation of histamine in feta cheese. *A. ascalonicum* EO was obtained by hydro distillation and analyzed using gas chromatography (GC/MS). The effect of EO at different concentrations (300, 600, 900 and 1200 ppm, respectively) on histamine formation in feta cheese during ripening period (60 days) was evaluated by Ion-Exchange Chromatography (IEC). The GC-MS analysis showed that oregano sulfide compounds formed the most significant part of the EO (80.3%). Data analysis demonstrated that ripening time and different concentrations of EO were significantly affected histamine content in feta cheese (P<0.05). The content of histamine increases by increasing ripening time, so that the highest level of histamine (11.26 ppm) was reported in control sample at the end of the ripening period. Histamine levels were lower in cheese samples containing EO as compared with control at all stages and histamine formation in cheese samples were affected by EO concentration; its level decreased with increasing EO concentration (P<0.05). Cheese sample containing 1200 ppm EO had the lowest content of histamine at the end of the ripening period. Nevertheless, cheese contacting low concentrations of EO was the most appropriate treatment in sensory assessment. Improved knowledge of the factors involved in the synthesis and accumulation of BA should lead to a reduction in their incidence in foods.

**Key words:** Histamine, *Allium ascalonicum* essential oil, feta cheese.

**INTRODUCTION**

Food-fermenting Lactic Acid Bacteria (LAB) are generally considered non-toxic and non-pathogenic. Some species of LAB, however, can produce Biogenic Amines (BAs) (Spano et al., 2010). BAs are low molecular weight organic bases that mainly generated by the enzymatic decarboxylation of amino acids by microorganisms (Stratton et al., 1991; Spano et al., 2010).

BAs such as tyramine, histamine, putrescine, cadaverine and tryptamine have been found in a wide range of foods, including dairy products especially cheese and they can occasionally accumulate at high concentrations (Standra et al., 2000; Flick and Granata, 2004). The presence of BAs in foods has traditionally been used as an indicator of undesired microbial activity indicates the deterioration of food products. Their toxicity has led to the general agreement that they should not be allowed to accumulate in food (Standra et al., 2000; Flick and Granata, 2004; Linares et al., 2010).

Herbal essential oils are aromatic oil liquids extracted from various parts of plants and are used as flavoring...
agents in foods (Burt, 2004). *Allium ascalonicum* is a plant originally from west Asian areas, growing also in Iran. In genus, *Alliums* more than 500 species have been identified and the most important species existing in this genus includes garlic, onion and leek, which have long being used as spice and drugs (Amin and Kapadnis, 2005).

Research has shown the effect of plant-derived volatile oils on growth and viability of some lactic acid bacteria. Kivanc et al. (1991) showed that EOs of *Mentha longifolia* and *Cuminum cyminum* in low concentrations led to stimulation of growth and acid production; at high concentrations, they prevented *Lactobacillus plantarum* growth (Kivanc et al., 1991).

In a study conducted by Simsek et al. (2007), essential oils of spearmint, thyme and garlic had no inhibitory effects on growth and durability of lactic acid bacteria present in Ayran (Simsek et al., 2007). Agboola and Tesic (2002) showed that lactic acid bacterial count in all cheese samples prepared with various spices (spearmint, green lime and tomato skin) was not significantly different during ripening period (Agboola and Tesic, 2002).

Feta cheese is one type of white brined cheese being produced from cow milk without dry salting of the clot and with ripening period of 40 to 90 days in brine (Khosrowshahi et al., 2006). Feta cheese requires maturation to develop the required sensory properties; in warm climates, it is necessary to preserve cheese in brine. The specific characteristics of brine cheese develop in salted water and chemical, physical and sensorial properties of this type of cheese are controlled by processing and environmental conditions (Azarnia et al., 1997; Khosrowshahi et al., 2006).

BAs accumulation in foods requires the availability of precursors (that is, amino acids), the presence of microorganisms with amino acid decarboxylases and favorable conditions including; temperature, pH, salt and water content for their growth and decarboxylating activity (Fernandez et al., 2007; Arena et al., 2008).

BAs production by LAB in fermented foods may be controlled by fermentation practices and factors involved in food fermentation processes. Due to the use of natural preservatives derived from plant sources to improve organoleptic properties and increase shelf life of food products, therefore, in this study, the effect of different concentrations of *A. ascalonicum* EO on the formation of histamine in the feta cheese during the ripening period were studied by ion-exchange chromatography and the cheese sensory quality assessed.

**MATERIALS AND METHODS**

**Extraction of *A. ascalonicum* EO**

After collection, *A. ascalonicum* was verified in botanical group of Tehran Medicinal Herbs Research Centre of Tehran University of Medical Science. Dried parts of plant were completely ground and essential oil was extracted through distillation by water for 3 h using a Cleveenger unit. The EO was put in amber colour glass containers and stored at 4°C after dehydration with dry sodium sulphate until further evaluations.

The EO was analyzed by gas chromatography according to the method of Mahmoudi et al. (2012). To confirm the results, EO was also analyzed by gas chromatography mass spectrometry (Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass-selective detector; Agilent Technologies, Berkshire, UK) with the same capillary column and analytical conditions as aforementioned. The MS was run in electron-ionization mode with ionization energy of 70 eV.

**Feta cheese production**

Cheese starter of chr. Hansel R 704 (mesophilic starter including *Lactobacillus lactic* subsp. Of *cremoris* and *Lacteous lactic* subsp. *Acetelactis* obtained from Christian Hans company Denmark) was used. In order to prepare cheese, cow milk was prepared and pasteurized in 72°C for 15 s; before beginning various steps of cheese production, temperature of pasteurized milk was downgraded to 35°C and 5 L of milk was poured into each of the sterile containers specific to cheese production.

﻿

Start at 5.0% v/v was added to milk samples simultaneously followed by the addition of calcium chloride at 0.20% w/v after 30 min. When milk pH reached 5.6, microbial rennet solved in distilled water (Meito; Sangyo Co., Nagoya, Japan) at 0.001% w/v was added to milk. At the same time, various concentrations (0, 300, 600, 900, 1200 ppm) of *A. ascalonicum* EO were added to the samples. To provide better efficiency for microbial rennet, milk temperature was kept near 35°C during clot formation. After 1 h, the formed clot was cut and put into brine 20% w/v for 8 h. Then, cheese samples were transferred to sterile brine 18%, kept in 12 to 14°C for 15 days and after the first maturity period, samples were kept in 4°C for 45 days to reach the final ripening period (Mahmoudi et al., 2012).

**Histamine content analysis**

**Standard histamine preparation**

Histamine dihydrochloride was obtained from Merck, Darmstadt, Germany. Stock solutions contained 1 mg of the respective amine per 1 mL. The calibration mixtures were prepared from equal volumes of the stock solutions and diluted with dosage buffer to obtain the concentration of 50 μg of amine per 1 mL. The solution was kept cold and protected from light by an opaque foil.
Table 1. Acceptance test (nine-point scale).

<table>
<thead>
<tr>
<th>Rating of scale</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Properties</td>
<td>Dislike extremely</td>
<td>Dislike very much</td>
<td>Dislike moderately</td>
<td>Dislike slightly</td>
<td>Neither like nor dislike</td>
<td>Like slightly</td>
<td>Like moderately</td>
<td>Like much</td>
<td>Like extremely</td>
</tr>
</tbody>
</table>

**Instruments**

Single-column automatic amino acid analyzer 334T (Mikrotechna, Prague, Czech Republic) with the TZ 4100 recorder (Ingos Ltd., raha, Czech Republic) and the IC-26 integrator (Analitik, Mikhailovgrad, Bulgaria) were used.

**Conditions of chromatography**

The separation of histamine was carried out on a column with the OSTION LG ANB ion exchanger (Spolek pro chemickou a hutni vyrobu, Ustı nad Labem, Czech Republic). The temperature of the column was maintained at 6°C and the flow of buffers and ninhydrin reagent at 14 mL/h and 12 mL/h, respectively. No pre-column for the removal of ammonia from buffers was used in the flow-through system of the analyzer. The amines were detected spectro-photometrically at 520 nm and the 100 mV signal of the recorder was integrated for quantitative determination. The dosage was 200 µl per column.

**Sample processing and ion exchange chromatography**

Preparation of cheese samples and performance of ion exchange chromatography method were conducted according to Standara et al. (2000).

**Sensory properties**

The sensory effects of adding of *A. ascalonicum* EO feta cheese was evaluated using an acceptance test and were equally divided into seven parts of 20 g each and placed on white plates coded with three digit random numbers. The sensory evaluation was performed by a panel of seven judges consisting of the scientific staff of the Department of Food Hygiene, Faculty of Veterinary Medicine, University of Tabriz, experienced in the sensory analysis of food. Each panelist evaluated the samples by rating them using a 9-point scale for various characteristics such as (appearance) color, odor and flavor (Table 1) (Meilgaard et al., 1999).

**Statistical analyses**

All experiments performed in triplicate and all statistical analyses performed using SPSS software. Significant results were considered at P<0.05.

**RESULTS**

**Chemical analysis of the *A. ascalonicum* EO**

The EO was extracted by the hydro-distillation of the dried aerial parts of *A. ascalonicum* and yield of the EO was 0.5%. The main components found as Dialyl disulphide (20.0 %) and Trisulfide, methyl 2-propenyl (18.1%), Trisulfide, di-2-propenyl (15.3%), Disulfide, methyl 1-propenyl (11.25) and Dimethyl trisulfide (7.1%). Predominantly, the oil was rich in oregano sulfide compounds.

**Organoleptic properties**

The mean acceptability scores of the cheese samples containing different concentrations of EO are shown in Figure 1. A sample without EO was used as the control group. The results of this study revealed that EO had significant (P<0.05) effect on the organoleptic properties of the cheese samples. Control sample and then, cheese sample containing 300 and 600 ppm of EO was the most preferred samples (P<0.05).

**Histamine content in various feta cheese treatments**

The mean and standard deviation of histamine content in all types of feta cheese samples are shown in Table 2. Data analysis showed that ripening time and different concentrations of EO were significantly affecting histamine content (P<0.05). The content of histamine increases by increasing ripening time, such that the highest level of histamine (11.26 ppm) was measured in the control sample at the end of ripening period (60 day). Histamine levels were lower in cheese samples containing different concentration of EO as compared with the control sample at all stages of the measurement, also, with increasing EO concentration, the level of histamine was reduced (P<0.05), such that feta cheese containing 1200 ppm EO had the lowest content of histamine at the end of the ripening period (4.25 ppm).

**DISCUSSION**

Dairy products (in particular cheese) can accumulate high levels of BAs. In the raw material (milk), polyamines are the
Figure 1. Mean ratings for the acceptability of feta cheese formulated with different concentrations of *A. ascalonicum* EO (sample A, cheese with no additive; sample B, cheese with 300 ppm EO; sample C, cheese with 600 ppm EO; sample D, cheese with 900 ppm EO; sample E, cheese with 1200 ppm EO).

Table 2. Histamine content (ppm) in various treatments of feta cheese during ripening period.

<table>
<thead>
<tr>
<th>Cheese samples</th>
<th>30 days</th>
<th>45 days</th>
<th>60 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.38±0.25a</td>
<td>8.21±0.38a</td>
<td>11.26±0.41a</td>
</tr>
<tr>
<td>B</td>
<td>4.83±0.31a</td>
<td>7.03±0.29b</td>
<td>10.05±0.36b</td>
</tr>
<tr>
<td>C</td>
<td>3.70±0.12b</td>
<td>6.15±0.20b</td>
<td>9.21±0.23b</td>
</tr>
<tr>
<td>D</td>
<td>3.41±0.34b</td>
<td>5.06±0.32c</td>
<td>7.11±0.12c</td>
</tr>
<tr>
<td>E</td>
<td>2.16±0.22c</td>
<td>3.81±0.22c</td>
<td>4.25±0.10d</td>
</tr>
</tbody>
</table>

The mean values followed by the same letter in the column are non-significantly different (P<0.05); sample A, cheese with no additive; sample B, cheese with 300 ppm EO; sample C, cheese with 600 ppm EO; sample D, cheese with 900 ppm EO; sample E, cheese with 1200 ppm EO.

most abundant. However, in the final product, tyramine, histamine, putrescine, and cadaverine and, at lower concentrations, b-phenylethylamine and tryptamine are all detected. The BA content of different types of cheese varies; indeed, it can also vary within the same type of cheese and even between different sections of the same cheese (Novella-Rodriguez et al., 2003).

The main BA producers in cheese are gram-positive bacteria, with LAB being the main histamine and tyramine producers. The genera *Enterococcus*, *Lactobacillus*, *Leuconostoc* and *Streptococcus* include some strains that have been described as BA producers. These can be present in milk microbiota or introduced through contamination before, during or after the processing of dairy products. LAB producing Bas may even form part of the starters or adjunct cultures. Several authors have reported the presence of tyrosine and histamine decarboxylase activity in strains from various starter cultures (Linares et al., 2010).

There are numerous benefits of herbal essential oils, and therefore, the use of herbal extracts and essential oils as biological preservatives may revolutionize the food industry (Burt, 2004). According to various reports on the antibacterial activity of plant extracts and essential oils against lactic acid bacteria, in the present study, the effect of different concentrations of *A. ascalonicum* EO on histamine formation during ripening of feta cheese were assessed.

The results showed that the levels of histamine in cheese samples containing *A. ascalonicum* EO was significantly
lower as compared to the control sample (Table 2). In our previous study, the antibacterial effect of *A. ascalonicum* EO against *Escherichia coli* in different steps of the ripening Iranian White Brined cheese was quite evident, also, the lower concentration (750 ppm) of this EO maintained the highest acceptable range (Ehsani and Mahmoudi, 2012).

The health related and antimicrobial effects of plants in *Allium* species are believed to depend substantially on four diallyl sulfides including diallyl monosulfide (DMS), diallyl disulfide (DDS), diallyl trisulfide (DTS) and diallyl tetrasulfide (DTTS) which are the sulfur containing compounds found in the plants (Rattanachaikunsopon and Phumkhachorn, 2009).

In the present study, oregano sulfide compounds also formed the most significant part of the EO (Table 1). Thus, the presence of considerable amounts of oregano sulfur compounds having effects on cell membrane disrupting its function and also interfering with metabolic activities suggest the high antimicrobial potential on *A. ascalonium* EO (Tsao and Yin, 2001).

The formation and accumulation of histamine is influenced directly by bacterial activity, pH, salt concentration and indirectly by water availability, storage temperature and ripening time (Martuscelli, 2005). Differences in histamine contents among the various treatment feta cheeses in the present research could be dependent on the effects of *A. ascalonium* EO on LAB activity during different ripening period.

The results of the determination of histamine in feta cheese by ion-exchange chromatography showed the highest concentration being recorded for control cheese and the lowest concentration related to feta cheese containing 1200 ppm *A. ascalonium* EO at the all stage measurement (Table 2).

The results of our previous study showed that significant differences in histamine contents among the various cheeses produced in Iran, with the highest mean content of 42.6 mg/100 g for Kope cheese, and the lowest mean level of 4.99 mg/100 g in feta cheese.

Differences in histamine contents among the various types of cheese in the present research could be dependent on the different ripening time, storage periods and preparation and distribution conditions for each cheese, feta and Lighvan cheese types have more hygienic preparation and are mostly prepared in factories and the storage periods (including transportation from the cheese factory, commercial distribution, purchase and eventual consumption) of these cheese types are shorter (Ehsani et al., 2012).

The acceptable level of histamine in cheese was reported as 100 ppm (Durlu-Ozkaya, 2002). The concentration of histamine in all cheese samples analyzed in the present study was lower than the acceptable limit. Cheese organoleptic assessment demonstrated that low concentration *A. ascalonicum* EO maintained the highest acceptable range (Figure 1). Therefore, the use of low concentrations of this EO along with other methods can be very helpful in reducing and controlling the production of histamine in feta cheese.

**Conclusion**

In the present study, histamine content in various treatment feta cheeses was relatively low; it seems that the characteristic features of feta cheese especially with adding of *A. ascalonicum* EO did not create an environment favorable for biogenic amines accumulation. Knowledge of the metabolic pathways involved in BA production and the factors affecting BA accumulation in food may also be useful in suggesting possible means of reducing BA contents. Undoubtedly, future studies are needed in order to evaluate the application of natural preservatives such as EO and extract from plants on reducing or inhibition of BAs formation.

**REFERENCES**


Cite this article as:

Submit your manuscript at http://www.academiapublishing.org/ajfr