Combined Effects of Modified Atmosphere Packaging and Thymol for Prolonging the Shelf Life of Caprese Salad

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ABSTRACT

The aim of this study was to investigate the influence of packaging atmosphere and a thymol dipping solution on the shelf life of caprese salad. Caprese salad was prepared with sliced tomatoes and mozzarella cheese. The samples were pretreated by dipping in a 400-ppm thymol solution and then packaged under air or under a modified atmosphere (65% N₂, 30% CO₂, and 5% O₂). Changes in natural microflora of mozzarella and tomato, the O₂ and CO₂ in the head space, pH, and sensory characteristics were monitored during storage. The combination of the thymol dip and modified atmosphere decreased the coliform populations in caprese salad from 5.65 to 4.23 log CFU·g⁻¹ and extended the shelf life from 3.77 to 12 days. A decrease in the concentration of Pseudomonadaceae from 7.03 to 5.09 log CFU·g⁻¹ was observed, and the lag phase was prolonged to approximately 3 days. The combination of the modified atmosphere and thymol did not affect the growth kinetics of lactic acid bacteria and enterococci, thus preserving the function of mozzarella cheese in the salad.

Despite modern improvements in food hygiene and production, food safety remains an important public issue (31). An estimated 30% of people in western countries suffer foodborne illnesses (31). Therefore, new methods in combination with existing methods, as stated by the hurdle principle (22), are needed to reduce or eliminate foodborne pathogens. In industrialized countries, there is a trend toward green consumerism (30), i.e., consumers desire fewer synthetic food additives and products with a smaller impact on the environment. The World Health Organization has recommended decreased consumption of salt to reduce the incidence of cardiovascular diseases (32). A reduction in the concentration of salt in food may make it necessary for other additives to be used to maintain the safety of foods. One group of possible additives are essential oils and their active compounds (6).

Essential oils are aromatic oily liquids obtained from plant material (6). Their antimicrobial activity against spoilage microorganisms, pathogens, yeasts, and molds has been well documented (15, 25, 28); however, increased interest in green consumerism has lead to a renewal of scientific interest in these substances (6).

Most of the antimicrobial activity of essential oils from spices and culinary herbs appears to be associated with phenolic compounds (3). Thymol (5-methyl-2-(1-methyl-ethyl)phenol) is the major component in thyme (10 to 64%) and oregano (trace to 64%) (7). Its antimicrobial activity against a wide range of microorganisms is well documented in both model systems (12) and foods, alone (6) or in combination with bifidobacteria (1) and NaCl electrolyzed solutions (23).

According to the Annex II of Council Regulation 2377/90, the Committee of Experts on Flavoring Substances of the European Council registered thymol in the flavorings foodstuff list. An upper limit for inclusion of thymol in food as a flavoring agent was established at 50 and 10 mg·kg⁻¹ in beverages, with these usage levels expected to be nontoxic for humans (12).

Consumers are requiring more convenience foods, i.e., foods that are minimally processed and easy to prepare. Convenience foods or tertiary processed foods are products designed to save consumers time in the kitchen and to reduce costs due to microbial spoilage. These foods require minimum preparation, typically just heating, and are packaged for a long shelf life with little loss of flavor and nutrients over time. In a study by Consumer Watch (8), convenience was associated with reducing the input required from consumers in shopping, preparation, cooking, or cleaning after a meal. According to the Institute of Grocery Distribution (16), convenience foods are increasingly based around “meal solutions”; the aim is “to make consumers’ lives easier when choosing and preparing meals.” Despite a lack of consensus about the definition of convenience foods, the basic element of these products is that they can minimize preparation, cooking, and clean-up time (11).

Caprese salad is a traditional Italian dish, and like other fourth tier products (i.e., minimally processed and ready-to-eat foods), it is sold as a minimally processed, ready-to-use convenience food. It contains tomatoes, cow or water buffalo mozzarella cheese slices, oregano, extra-virgin olive oil, basil, and wall rocket (arugula, Eruca sativa). Despite several benefits, including simplicity and quickness of preparation, the very short shelf life (1 to 2 days) of caprese salad hampers its presence in canteens and local markets.

The microbial safety and sensory and nutritional quality of foods usually are based on the application of com-
bined preservative elements, i.e., hurdles (22). In western
countries, hurdle technology is particularly useful for min-
imal processing products (22) and for enhancing the sta-
bulity and safety of perishable foods (20). Although chilling
is the most important and sometimes the only hurdle for
refrigerated foods, thermal abuse during distribution can
lead to microbial spoilage (22). Therefore, additional hur-
dles should be incorporated into chilled foods as safe-
guards, using an approach called invisible technology (21).

Another field of major interest in food microbiology is
predictive modeling. Mathematical models such as the
modified Gompertz equation, Richards, Stannard, Schnute
and Logistic (33), Ratkowsky (26), and the dynamic model
of Baranyi and Roberts (4) now are used to estimate the
microbial shelf life of foods, isolate critical points in the
production and distribution process, and provide insight
into how environmental variables affect the behavior of
pathogenic and spoilage bacteria. Microbial growth curves
are sigmoidal, reflecting the lag, log (exponential growth
pathogenic and spoilage bacteria. Microbial growth curves
into how environmental variables affect the behavior of
pathogenic and spoilage bacteria. Microbial growth curves
are sigmoidal, reflecting the lag, log (exponential growth
rate), and stationary phases (33). The Gompertz equation
was developed originally for nonmicrobiology uses, but
Zwietering and coworkers (33) gave a physical meaning to
its parameters; therefore, this model can be used to explain
and quantify the duration of the lag phase (λ), the ex-
ponential growth rate (μmax), and the increase in cell load
during the stationary phase (A). The shelf life or sanitary
risk of a food can be evaluated by using the kinetic param-
eter of the Gompertz equation (9, 10, 29).

In this study, the possibility of prolonging the shelf life
of caprese salad, a typical Italian convenience food, was
evaluated using modified atmosphere packaging in combi-
nation with thyme, the active compound of oregano, which
is traditionally added to this dish. Shelf life and stability
times were calculated by using kinetic parameters of spoil-
ing microflora and sensory scores to determine the optimal
storage conditions for caprese salad.

MATERIALS AND METHODS

Caprese preparation. Cow mozzarella cheese slices (50 g
each; 55% moisture, 24% fat, and 1.2 fat:protein ratio) and Pa-
chino variety tomatoes were purchased from a local market
in Apulia, a region in southern Italy. The calyx was removed from
the tomatoes, the tomatoes were washed, and tomatoes and cheese
were cut into 10-g slices. Before packaging, cheese slices were
dipped into a 400-ppm thymol solution (ICN Biomedical, Aurora,
Ohio) for 2 min. Sliced mozzarella in control samples was dipped
into sterile distilled water.

Five slices of cheese and six slices of tomato were packed
gether as a sample in high-barrier plastic bags (nylon-polyeth-
ylene, 102 μm; Tecnovac, San Paolo D’Argon, Bergamo, Italy)
measuring 170 by 250 mm with properties specified by the man-
ufacturer as follows: CO2 and O2 permeability of 3.26 × 10–19
and 9.23 × 10–19 mol m m–2 s–1 Pa–1, respectively, and a water
vapor transmission rate of 1.62 × 10–10 kg m–2 s–1 Pa–1. The samples
were packaged in air (control atmosphere) and in a modified at-
mosphere (MA; 65% N2, 30% CO2, and 5% O2), stored at 4°C
for 12 days, and removed periodically for analysis.

Microbiological analyses. Ten grams of sliced mozzarella
and tomato were separately diluted with 90 ml of sterile saline
solution (0.9% NaCl), homogenized in a stomacher bag for 1 min
(Stomacher Lab Blender 400, PBI International, Milan, Italy), and
decimally diluted to determine the microbial counts. Populations
of lactic acid bacteria, yeasts, and molds were determined in sliced
tomatoes, and populations of mesophilic and psychrotrophic bac-
teria, lactic acid bacteria, total coliforms, enterococci, and Pseu-
domonadaceae were determined in the mozzarella cheese. The
media (Oxoid, Milan, Italy) and conditions were as follows. Plate
count agar was incubated at 5°C for 1 week or at 32°C for 48 h
to isolate psychrotrophic and mesophilic bacteria, respectively.
deMan Rogosa Sharpe agar was modified by adding 0.17 g liter–1
cycloheximide (Sigma-Aldrich, Milan, Italy) after autoclaving and
incubated at 30°C for 4 days under anaerobic conditions for iso-
lation of lactic acid bacteria. Violet red bile agar was incubated
at 37°C for 18 to 24 h for isolation of total coliforms. Slanetz
Bartley medium was incubated at 37°C for 48 h for isolation of
terococci. Pseudomonas agar base was modified by adding Pseudomonas
CPC selective supplement after autoclaving and then incubated at 30°C for 48 h for isolation of Pseudomonas spp.

Evaluation of head space gas. The concentrations of O2 and
CO2 in the head space were determined with a Gas Analyser
Checkmate 9900 O2/CO2 (PBI-Dansensor, Ringsted, Denmark).
The data were expressed as volume/volume.

Determination of organoleptic parameters during storage.
A simple organoleptic evaluation of overall appearance, odor, and
firmness was performed by five untrained panelists (researchers at
the Department of Food Science, Faculty of Agricultural Science,
Foggia University). Samples were given a three digit code, and a
5-point hedonic scale was used, where 5 represented attributes
most liked, 2 represented attributes at an unacceptable border, and
0 represented attributes most disliked.

Data modeling and calculation of shelf life. Two different
batches of caprese salad were analyzed. These analyses were
performed in triplicate, and the experimental data are the average
of all repetitions and are accompanied by the standard deviation.

Cell population data and sensory scores were modeled ac-
cording to the Gompertz equation, as modified by Zwietering et
al. (33), with the option Non-linear Regression of the software
Statistica for Windows (Tulsa, Okla.). The kinetic parameters and
data from chemophysical analyses were submitted to a one-way
analysis of variance and to Duncan’s test (significance set at P <
0.05).

The shelf life of caprese salad was evaluated based on total
coliforms and Pseudomonadaceae kinetics; the critical values
were set to 1 × 105 and 5 × 105 CFU g–1 for coliforms and
Pseudomonadaceae, respectively, as reported by Italian law (2)
and Bishop and White (5).

Shelf life was calculated with the following equation:

\[
\text{shelf life} = \frac{A}{e^{\mu_{\text{max}}} + \lambda - \frac{\ln \left( L_u - \frac{\lambda}{A} \right)}{e^{\mu_{\text{max}}}}}
\]
TABLE 1. Gompertz parameters, shelf life, and stability time for total coliforms in mozzarella cheese

<table>
<thead>
<tr>
<th>Sample type</th>
<th>(k) (log CFU·g(^{-1}))</th>
<th>(A) (log CFU·g(^{-1}))</th>
<th>(\mu_{\text{max}}) (Δ log CFU·g(^{-1})·day(^{-1}))</th>
<th>(\lambda) (days)</th>
<th>(R^2)</th>
<th>Shelf life (days)(^d)</th>
<th>Stability time (days)(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.13 ± 0.04 A</td>
<td>4.52 ± 0.63 A</td>
<td>1.19 ± 0.09 A</td>
<td>0.53 ± 0.10 A</td>
<td>0.997</td>
<td>3.77</td>
<td>1.93</td>
</tr>
<tr>
<td>Dipped</td>
<td>1.27 ± 0.23 A</td>
<td>4.41 ± 0.37 A</td>
<td>1.61 ± 0.08 A</td>
<td>0.97 ± 0.06 B</td>
<td>0.997</td>
<td>4.53</td>
<td>1.98</td>
</tr>
<tr>
<td>MA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.24 ± 0.14 A</td>
<td>4.11 ± 0.21 A</td>
<td>1.32 ± 0.27 A</td>
<td>0.81 ± 0.03 c</td>
<td>0.999</td>
<td>4.72</td>
<td>1.95</td>
</tr>
<tr>
<td>Dipped</td>
<td>1.33 ± 0.03 A</td>
<td>2.90 ± 0.05 B</td>
<td>3.10 ± 0.69 B</td>
<td>4.59 ± 0.09 d</td>
<td>0.999</td>
<td>&gt;12</td>
<td>4.93</td>
</tr>
</tbody>
</table>

\(a\) CA, air; MA, modified atmosphere (65% N\(_2\), 30% CO\(_2\), and 5% O\(_2\)). Cheese slices were dipped in 400 ppm thymol for 2 min.

\(b\) \(k\), initial cell load; \(A\), maximum increase of the cell load attained at the stationary phase; \(\mu_{\text{max}}\), maximal growth rate; \(\lambda\), lag phase.

Values are mean ± standard error. Within a column, means with the same letters are not significantly different (one-way ANOVA, Duncan’s test; \(P > 0.05\)).

\(c\) Regression coefficient.

\(d\) The critical value was set at \(1 \times 10^5\) CFU·g\(^{-1}\).

\(e\) Time corresponding to the maximum of the second time derivative of the Gompertz function.

RESULTS

Gompertz parameters and \(R^2\) values for total coliforms in mozzarella cheese are reported in Table 1. MA plus the thymol dip significantly decreased the coliform population in the stationary phase from 4.52 to 2.90 log CFU·g\(^{-1}\) and prolonged the lag phase (from 0.53 to 4.59 days). As detailed earlier, shelf life was calculated based on coliform kinetic parameters. In air, the shelf life of caprese salad was 3.77 days (control sample) but increased to 4.53 days when the thymol dip was used; however, the stability time was unaffected by the addition of thymol. When used alone, MA did not affect shelf life; however, shelf life and stability time were significantly affected when MA was used in combination with the thymol dipping solution.

Thymol did not affect the number or log growth rate of Pseudomonadaceae (Table 2). In contrast, MA packaging decreased maximum populations by about 2 log CFU·g\(^{-1}\), reduced the growth rate, and increased the lag phase from 1.30 to 2.40 days. The combination of MA and thymol treatment increased the lag phase up to 4.24 days. The shelf life of caprese salad samples, estimated on the basis of Pseudomonadaceae growth kinetics, was about 5 days. Based on shelf life alone, it was not possible to identify an interactive effect between MA and thymol because in MA samples Pseudomonas concentrations did not attain the critical value (5.70 log CFU·g\(^{-1}\)). However, stability time was a useful parameter for evaluating the influence of thymol treatment. For coliforms in air, treatment with thymol was a useful parameter for evaluating the influence of thymol treatment.

TABLE 2. Gompertz parameters, shelf life, and stability time for Pseudomonadaceae in mozzarella cheese

<table>
<thead>
<tr>
<th>Sample type</th>
<th>(k) (log CFU·g(^{-1}))</th>
<th>(A) (log CFU·g(^{-1}))</th>
<th>(\mu_{\text{max}}) (Δ log CFU·g(^{-1})·day(^{-1}))</th>
<th>(\lambda) (days)</th>
<th>(R^2)</th>
<th>Shelf life (days)(^d)</th>
<th>Stability time (days)(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.95 ± 0.09 A</td>
<td>5.18 ± 0.17 A</td>
<td>1.02 ± 0.18 A</td>
<td>1.30 ± 0.18 A</td>
<td>0.999</td>
<td>5.27</td>
<td>3.17</td>
</tr>
<tr>
<td>Dipped</td>
<td>1.92 ± 0.18 A</td>
<td>5.14 ± 0.37 A</td>
<td>1.02 ± 0.11 A</td>
<td>1.39 ± 0.09 B</td>
<td>0.998</td>
<td>5.43</td>
<td>3.24</td>
</tr>
<tr>
<td>MA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.99 ± 0.06 A</td>
<td>3.37 ± 0.28 A</td>
<td>0.57 ± 0.07 B</td>
<td>2.40 ± 0.12 B</td>
<td>0.999</td>
<td>&gt;12</td>
<td>4.57</td>
</tr>
<tr>
<td>Dipped</td>
<td>1.98 ± 0.15 A</td>
<td>3.11 ± 0.30 B</td>
<td>0.70 ± 0.13 B</td>
<td>4.24 ± 0.15 c</td>
<td>0.993</td>
<td>&gt;12</td>
<td>5.87</td>
</tr>
</tbody>
</table>

\(a\) CA, air; MA, modified atmosphere (65% N\(_2\), 30% CO\(_2\), and 5% O\(_2\)). Cheese slices were dipped in 400 ppm thymol for 2 min.

\(b\) \(k\), initial cell load; \(A\), maximum increase of the cell load attained at the stationary phase; \(\mu_{\text{max}}\), maximal growth rate; \(\lambda\), lag phase.

Values are mean ± standard error. Within a column, means with the same letters are not significantly different (one-way ANOVA, Duncan’s test; \(P > 0.05\)).

\(c\) Regression coefficient.

\(d\) The critical value was set at \(1 \times 10^5\) CFU·g\(^{-1}\).

\(e\) Time corresponding to the maximum of the second time derivative of the Gompertz function.
mol did not significantly affect stability time, but when combined with MA thymol treatment increased the stability time by about 1.3 days.

Figure 1 shows the growth of lactic acid bacteria in mozzarella cheese. MA and thymol treatment did not inhibit these microorganisms, as observed for the spoilage microflora. Similar results were obtained for mesophilic bacteria (data not shown).

Enterococci populations (Fig. 2) were not affected by the combination of MA and thymol. Numbers of enterococci increased from 3.5 to approximately 5.0 log CFU·g⁻¹, but there were no significant differences among the samples.

Psychrotrophic bacteria were inhibited by the low pressure of O₂ in the head space of MA samples. Populations were significantly lower in MA samples after 5 days of storage (Fig. 3).

Microbes on the tomato slices were mainly lactic acid bacteria (Fig. 4). No significant differences were detected between samples packaged in air and in the MA. A slight increase (not significant) in parameter A (0.6 to 0.7 log CFU·g⁻¹) was observed in thymol-dipped samples that were packaged in MA.

Yeast and molds were always below the level of detection (2 log CFU·g⁻¹). The pH of mozzarella cheese (pH 6.5) and tomato slices (pH 3.9) was not influenced by thymol treatment or MA (data not shown).

Figure 5A and 5B show the evolution of O₂ and CO₂ in the head space of caprese samples. In the bags packaged in air, O₂ concentrations decreased significantly in both the control and thymol-dipped samples during storage, whereas a slight decrease was observed for samples packaged in air.
MA. A significant increase in the CO2 concentration was observed for samples packaged in air.

The organoleptic observations regarding firmness and general appearance (Table 3) were not affected by MA and thymol treatment and confirmed the microbiological data. The break point was at 3 to 5 days. MA increased odor stability times by approximately 2 days.

**DISCUSSION**

Coliforms are an indication of poor sanitary conditions, but according to Italian law (2) they are the designated test microorganisms used to evaluate the shelf life of mozzarella cheese and to determine whether the product is safe for human consumption. Gas formation in retail mozzarella cheese has been associated with coliform populations of approximately $10^7$ log CFU·g⁻¹ and with the growth of *Klebsiella pneumoniae* (24). Spoilage by psychrotrophic *Pseudomonas* spp. is a field of great interest for the dairy industry (13) because these organisms are responsible for surface discoloration, off-odors, off-flavors, and bitterness due to the activity of their thermostable lipolytic and proteolytic enzymes.

Microbial safety and stability and sensory and nutritional quality of foods are usually enhanced the application of combined preservative factors (22). For traditional foods, inherent empirical hurdles are often sufficient, whereas for novel products (19) such caprese salad the hurdles are carefully selected and applied.

Food preservation implies creating a hostile environment for microorganisms that will inhibit their growth or shorten their survival (22) and disrupt microbial homeostasis. Ten years ago Leistner (19) introduced the concept of multitarget preservation as an ambitious goal for a gentle but more effective means of prolonging shelf life. Different hurdles could act synergistically (22) if they simultaneously affected different targets (e.g., cell membrane, DNA, and enzyme system) and disturbed microbial homeostasis, making repair of homeostasis and activation of stress shock proteins more difficult (19).

The well-known mode of action of thymol is to disintegrate the outer membrane of gram-negative bacteria, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to ATP (6). Helander et al. (14) reported that the presence of magnesium chloride did not alter the effectiveness of thymol, suggesting a mechanism other than the chelation of cations in the outer membrane. Juven et al. (18) examined the antimicrobial activity of thymol against *Salmonella Typhimurium* and *Staphylococcus aureus* and hypothesized that thymol binds to membrane proteins hydrophobically and changes the permeability characteristics of the membrane. These authors found that thymol was more inhibitory at low pH because of its hydrophobicity and ability to bind more efficiently to the hydrophobic areas of proteins and to dissolve in the lipid phase (18).

Our findings confirmed the results of Altieri and co-workers (1), who reported that a 400 ppm thymol dipping solution combined with MA reduced *Pseudomonas* populations by 2 to 3 log CFU·g⁻¹ on fresh cod fillets, whereas a slight decrease was observed in samples packed in air. They reported also that thymol was not able to inhibit total

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**TABLE 3. Stability time of sensory scores for caprese salad samples packaged in air (CA) and in a modified atmosphere (MA; 65% N₂, 30% CO₂, and 5% O₂) and stored at 4°C**

<table>
<thead>
<tr>
<th>Sample type&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Odor</th>
<th>Texture</th>
<th>General appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.09</td>
<td>5.06</td>
<td>4.11</td>
</tr>
<tr>
<td>Dipped</td>
<td>3.11</td>
<td>5.07</td>
<td>4.13</td>
</tr>
<tr>
<td>MA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.00</td>
<td>4.99</td>
<td>4.05</td>
</tr>
<tr>
<td>Dipped</td>
<td>4.97</td>
<td>5.00</td>
<td>4.11</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mozzarella slices were dipped in 400 ppm thymol for 2 min.

<sup>b</sup>Time corresponding to the maximum of the second derivative of the Gompertz equation.
coliforms. We cannot compare our data with their results because they used a lower critical value (2 log CFU·g⁻¹), as recommended by the international trade rules for fish (17).

The low concentration of O₂ in the head space is an important hurdle for Pseudomonadaceae, as indicated by the decrease in parameter A and the log growth rate (μₘₚₐₓ). Thymol reinforced this inhibition by prolonging the lag phase and slightly increasing the stability time.

The hypothesis was that thymol and MA act synergistically against coliforms. Although a decrease in the maximum population and a prolonged lag phase and microbiological shelf life were observed in thymol-dipped samples packaged in MA, this hypothesis needs further investigation.

The inhibition of Pseudomonadaceae and coliforms in mozzarella cheese significantly increased the shelf life of caprese salad. Microbial populations in tomatoes, mainly lactic acid bacteria, cannot influence the quality of the product if it is kept refrigerated. Thymol treatment combined with MA did not affect the growth of lactic acid bacteria, thus preserving the functionality of the mozzarella cheese in the salad.

Odor was the most significant sensory property affecting the organoleptic quality of samples packaged in air, probably because of volatile compounds produced by microbial metabolism. The increased stability time of MA samples could be related to the inhibition of Pseudomonadaceae, thus confirming the importance of these organisms in the spoilage of caprese salad.

To our knowledge, no other published data are available regarding the shelf life of caprese salad; this work is the first investigation of the shelf life of this typical Mediterranean product. Currently, caprese salad usually is prepared at home and consumed 1 to 2 h after preparation. Our results indicate that it is possible to consume this product within 4 to 5 days of preparation when two or more hurdles are in effect (i.e., refrigerated storage, MA, and use of natural inhibitory compounds). Thymol did not affect the sensory characteristics of caprese salad because oregano is a traditional component of this dish. Further efforts are needed to identify the effects of other natural bioactive substances in different MAs and at abusive temperatures.

REFERENCES