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Original Article

Effect of thyme (*Thymus vulgaris* L.) essential oil on the quality parameters of stirred yoghurt

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Abstract

Aim

Stirred yoghurt was supplemented with thyme essential oil in an attempt to enhance its quality.

Materials and methods

Thyme (*Thymus vulgaris* L.) essential oil concentrations (0, 2, 4, 6, 8, and 10 µl/ml) were added to the prepared stirred yoghurt and then examined for microbiological count and mycotoxin quality during storage intervals following the Egyptian standards.

Results

Samples contained ≥ 6 µl/ml oil showed significant increase ($p<0.05$) of total viable bacterial count and lactic acid bacteria, whereas mold and yeast count was decreased. Pathogenic bacteria including *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli*, and coliform group were not detected in any of the samples, which complied with the standards. Moreover, aflatoxin M1 content was reduced by time in direct proportion to the thyme essential oil concentration.

Conclusion

The bioactive components in thyme act as a natural preservative in stirred yoghurt throughout the storage interval when the yogurt is fortified with hydro-distilled thyme essential oil.

Keywords: Stirred yoghurt, thyme essential oil, Microbiology

Introduction

Stirred yoghurt is a type of fermented dairy product that is incubated in tanks and then poured in packages [1]. Its health benefits are attributing to lactic acid bacterial count and metabolites, besides the high calcium content [2]. It is produced by converting lactose to lactic acid via *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* [3]. These strains exhibit symbiotic relationship by producing growth-stimulation substance in mutual favorable interaction [4]. The resultant lactic acid confers its characteristic taste, flavor, and texture [5].

Fermented dairy products are vulnerable to microbial contamination in which molds and yeasts are the major agents of spoilage owing to the selective environment provided by the low pH [6]. Moreover, various studies have established the statistical evaluation of bacteria in yoghurt [5–7]. The Egyptian standards for fermented milks coined the allowable microbial counts as follows: total viable bacterial count (TVBC) (>10^6 CFU/g), starter culture bacteria (>10^7 CFU/g), and coliform group (should not exceed 10^1 CFU/g), whereas pathogens including true fecal coliform, *Escherichia coli*, *Salmonella spp*, *Staphylococcus aureus* coagulase positive, and *Listeria monocytogenes* should not be detected [8].

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Aflatoxin M1 (AFM1) is found in milk and dairy products from lactating animals fed with aflatoxin B1 (AFB1)-contaminated feedstuffs, where AFB1 is biotransformed under the influence of cytochrome p450 oxidase to AFM1 [9]. The Egyptian standards for certain contaminations in foodstuffs (ES: 7136) [10] set the maximum level of AFM1 limit less than or equal to 50 ng/kg. It was in detectable amount in 59 of 60 yoghurt samples studied in Iran [11].

Thyme (*Thymus vulgaris* L.) belonging to *Lamiaceae* family is a popular spice that contains volatile oils. Thymol and carvacrol are the main components of thyme [12]. Its essential oil is an ideal food additive instead of synthetic ones. It has a potential food-preservative ability because of its antimicrobial activity against food-spoilage bacteria and mycotoxin-producing fungi [13]. Moreover, 1% thyme essential oil possesses a great inhibitory effect against *L. monocytogenes* (to <1 $\log_{10}$ CFU/ml within 10 days) more than *E. coli* 0157: H7 [14]. The addition of plant extract, that is thyme, in yoghurt production modifies the reduction and acidification activities of starters, which changes the fermenting time as well as product quality [15]. Better sensory properties of drink yoghurt fortified with thyme essential oil when compared with untreated samples were observed [16]. It inhibits a number of food-borne spoilage and pathogenic bacterial and fungal strains [17].

The objective of this study is to investigate the microbiological, mycotoxin, and sensory quality of stirred yoghurt fortified with thyme essential oil throughout refrigeration storage time.

Materials and methods

Plant material

Thyme (*T. vulgaris* L.) was collected from Horticulture Department, Faculty of Agriculture, Zagazig University, during 2019.

Production of essential oil

The volatile oil was produced by hydro-distillation of 75 g dry leaves using Clevenger-type apparatus for 3 h. The yielded oil was kept in dark vial, dehydrated with anhydrous sodium sulfate, and then stored at 4 ± 1°C for later use [18].

Production of stirred yoghurt

Fresh cow milk (3% fat) was procured from Food Science Department, Faculty of Agriculture, Zagazig University, and was heated to 90°C/10 min, then cooled to 42°C. It was then inoculated with Direct Vat Set yoghurt starter culture (YC-X11) from Chr. Hansen's Lab. Denmark, which contained *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* at 30°C/12–15 h to reach pH 4.5–4.6. This fermented coagulum was cooled to 4 ± 1°C, stirred manually up and down using stainless-steel bored disk to form control stirred yoghurt and filled in sterile capped glass jars [19]. Treatments were prepared by mixing the resultant yoghurt drink with 0.001% Tween-80 and 0.5% NaCl, after which 2, 4, 6, 8, and 10 µl/ml concentrations of thyme essential oil were added to form T1, T2, T3, T4, and T5, respectively [16]. Control and treated samples were stored for 21 days at 4 ± 1°C and analyzed every 3 days.

Measurement of pH

The pH value was measured using digital pH meter (ORION; Orion Research Inc., Cambridge MA, USA).

Microbial analysis

Double serial dilutions up to 10^{10} of control and treated samples during storage were prepared, and then the microbial population was determined using standard methods include the media (from Oxoid and Difco) and incubation conditions as follows [20]:

(1) TVBC [21]: plate count agar at 35°C/48 ± 2 h.
(2) Mold and yeast count [22]: Sabouraud D-glucose agar at 25 ± 1°C/5 days.
(3) *S. aureus* [23]: Baird-Parker's medium (37°C/24 and 48 h) and then coagulase production using Brain Heart Infusion Broth (37°C/1–24 h) and rabbit plasma antiserum.
(4) *Bacillus cereus* [24]: Polymyxin Pyruvate Egg Yolk Mannitol Bromothymol Blue Agar Base (PPEMBA) supplemented with polymyxin B solution at 30°C/18–48 h.
(5) Lactic acid bacteria [25]: MRS medium at 30°C/72 h.
(6) Coliform group [26]: MacConkey's broth (30°C/48 h) and then confirmation medium brilliant green bile broth contained in Durham's tube at 30–37°C/24 h.
(7) *E. coli* [27]: EMB (37°C/24 h), tryptone water (37°C/24 h)+indole reagent, MRVP (37°C/5 days)+methyl red solution and (37°C/48 h)+α-naphthol solution, and Simmon Citrate Agar (37°C/48 h).
(8) *L. monocytogenes* [28]:
    (a) Primary enrichment using Half-Fraser Broth contained half concentration of acriflavine and nalidixic acid at 30°C/24 h.
    (b) Secondary enrichment using Fraser broth at 37°C/24 h.
    (c) Plating out using Listeria agar at 37°C/48 h and Oxford medium at 35°C/48 h.
(9) *Salmonella spp* [29]:
    (a) Nonselective enrichment stage: lactose broth at 37°C/24 h.
    (b) Selective enrichment stage: selenite cystine broth and tetrathionate brilliant green broth at 43°C/24 h.
    (c) Differential stage: bismuth sulfate agar and brilliant green agar at 37°C/24 and 48 h.
    (d) Biochemical screening: triple sugar iron agar and lysine iron agar at 37°C/24 h.

Mycotoxin analysis

Samples were subjected to commercial quantitative competitive direct enzyme-linked immunosorbent assay (CD-ELISA) using RIDASCREEN AFM1 kit (Art. No 1121) from R-Biopharm following the manufacturer's instructions [30]. The resultant microplate was read for the absorbance at 450 nm by MRX microwell reader (Dynatech Laboratories, Guernsey, Channel Islands, Great Britain) using software version 1.2 to observe concentrations in ng/kg (ppt).

Statistical analysis

All results were recorded in triplicates and subjected to two-way analysis of variance and then expressed in figures, where data were plotted in multiple line graphs using SPSS software (IBM-SPSS, 20; Chicago, IL, USA) [31].

Results and discussion

It can be seen in Fig. 1 that there was a significant reduction ($P < 0.05$) of pH value in treated samples when compared with the control stirred yoghurt samples. The pH value was 4.6 in the fresh prepared samples (zero time storage) and then decreased by time to be 3.9–4.4 at the end of storage period (21 days). The data indicated that the higher the thyme essential oil concentration, the lesser the acidity (higher pH value), which may be attributed to the lactic acid starter activity. These results are in agreement with those reported about the quality of treated stirred yoghurt mixes [19]. This finding may be explained by the inhibitory effect of thyme essential oil [32]. There were no significant difference between groups ($P>0.05$), which are in line with the previous studies [33]. The increase in lactic acid decreases the pH is because of fermentation of lactose to form lactic acid by the functioning of the starter cultures [5]. These results indicate that the decrease in the pH value with the cold storage was a result of further fermentation of lactose to lactic acid [34]. This pH value provides selective media for contaminants and spoilage microorganisms [35]. It may be explain the decrease of pH by lactic acid bacterial pertinacious metabolic activity, which exhibit inhibition of other bacterial populations. Pathogenic acid-resistant and tolerant bacteria contribute to virulence, survival, and pathogenicity, which cause hazards [36].

S. aureus, *B. cereus*, fecal coliform, *E. coli*, *L. monocytogenes*, and *Salmonella* spp. were not detected in any of the studied samples either in control or thyme essential oil-treated stirred yoghurt during the storage intervals. These findings agreed with those coined by the Egyptian standards [8].

The absence of the aforementioned food-borne pathogens is a result of proper production and preservation methods, which demonstrate good quality [5].

Stirred yoghurt is highly vulnerable to microbial contamination and hence is perishable; therefore, microbial assessment is necessary [6].

It is clearly evident in Fig. 2 that TVBC during storage interval increased in control and T1 samples, with nonsignificant difference ($P>0.05$), followed by T2, with significant difference, whereas, T3, T4, and T5 showed significant decrease ($P<0.05$). The highest TVBC ranged between 6.9×10^7 and 4.1×10^9 CFU/g in control sample and between 4.7×10^7 and 6.1×10^4 CFU/g in T5 with the highest thyme essential oil concentration (10 µl/ml).

Thyme essential oil causes thickening, roughening, and eventual rupture of bacterial cell wall as well as alterations in the structure and function of cell membrane by affecting the ability of constituents to penetrate and consequent lack of cytoplasm [17].

The antibacterial effect of thyme essential oil is associated with the presence of some phenolic components such as carvacrol, thymol, and other hydrocarbons. The carvacrol can disrupt the cytoplasmic membrane, leading to bactericidal effect [37].

The thymol binds to the bacterial membrane proteins (hydrogen bonding and hydrophobic bonding), which change its permeability. It also decreases intracellular adenosine triphosphate and increase extracellular adenosine triphosphate, which disrupt plasma membrane function [38].

Fig. 3 illustrates that the population of lactic acid bacteria was in agreement with the Egyptian acceptable limits [8], which were ≥107 CFU/g at the beginning of the storage interval (zero time), increased to be the highest bacterial count at the sixth storage day then decreased to be the lowest at the 21st day of storage.

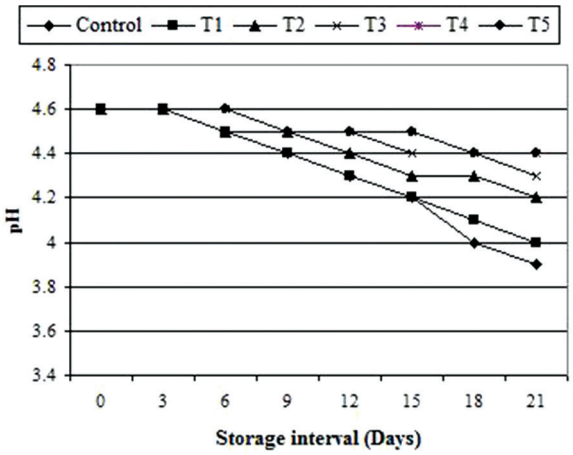

Figure 1: Prevalence of pH in stirred yoghurt treated with thyme essential oil during storage. Significant reduction was observed between control and treatments ($P<0.05$).

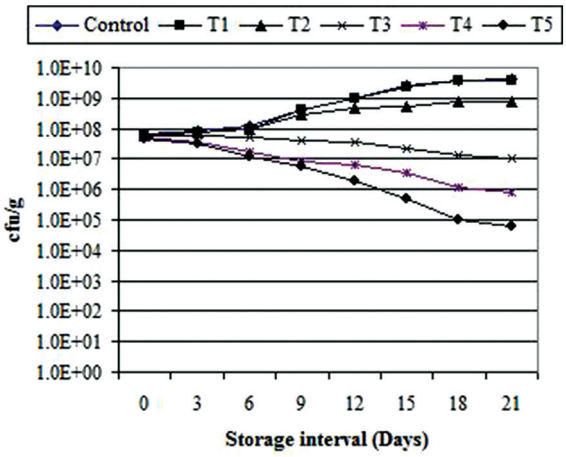

Figure 2: Prevalence of TVBC in stirred yoghurt treated with thyme essential oil during storage (in logarithmic scale). Significant difference between treatments were observed ($P<0.05$). TVBC, total viable bacterial count.

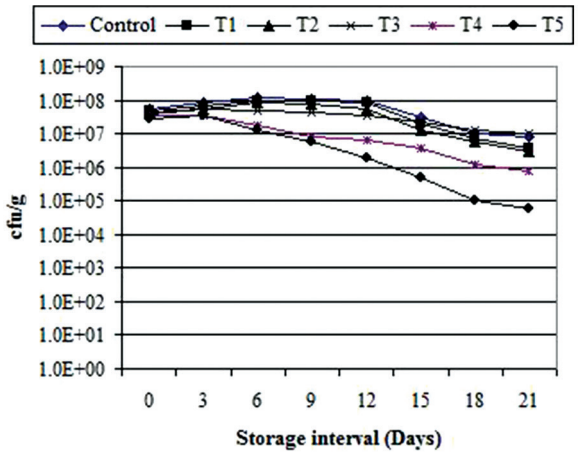

Figure 3: Prevalence of lactic acid bacteria in stirred yoghurt treated with thyme essential oil during storage (in logarithmic scale). Significant difference between treatments were observed ($P<0.05$).

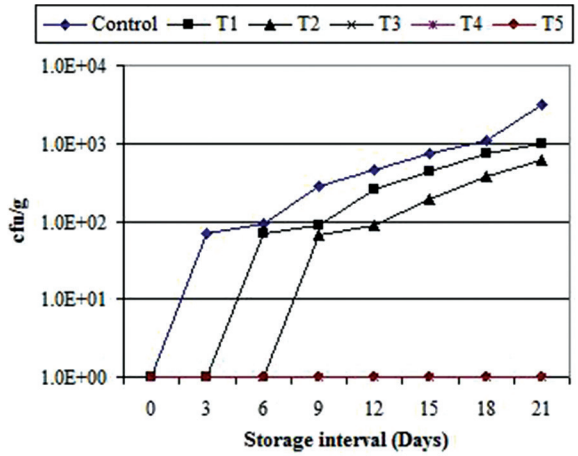

Figure 4: Prevalence of mold and yeast count in stirred yoghurt treated with thyme essential oil during storage (in logarithmic scale). Significant difference between treatments were observed ($P<0.05$).

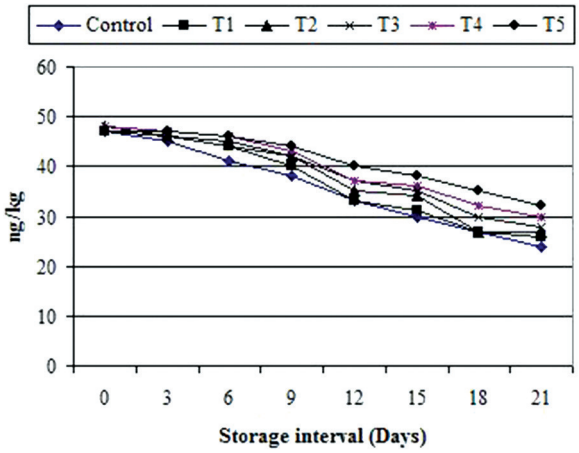

Figure 5: Prevalence of aflatoxin M1 in stirred yoghurt treated with thyme essential oil during storage. Significant difference between treatments were observed ($P<0.05$).

Lactic acid bacteria are indigenous bacteria in yoghurt. They create an unfavorable condition for the growth of pathogens and spoilage organisms because of the lactose fermentation and further pH reduction, in addition to producing bacteriocin [39].

Low pH provides selective media for molds and yeast growth, which are the major cause of yoghurt spoilage [6].

As shown in Fig. 4, no mold and yeast count was found in control or treated samples; the counts ranged between 7×10^1 CFU/g in the third storage day of control samples and 6.5×10^1 CFU/g in the ninth storage day of T2, whereas no detectable count was found in T3, T4, and T5.

The mold and yeast contamination may be owing to the packaging, storage, and transportation process, and also their ability to survive and metabolize in low pH [40]. They decrease the acidity, which favors the putrefactive bacteria [41].

Fig. 5 shows that AFM1 in all of the samples were within the acceptable limits stated by the Egyptian standards [10], which were less than 50 ng/kg. AFM1 content was the highest in zero time ranged between 47 and 48 ng/kg, whereas the lowest in the end of storage interval (21 days), ranged between 24 and 32 ng/kg. A decrease of AFM1 was observed throughout storage time, whereas the higher the thyme essential oil concentration, the higher the mycotoxin content, with significant difference ($P<0.05$) between treatments. The addition of thyme essential oil causes increase in AFM1 content.

The reduction of AFM1 in yoghurt is because of the physical binding of the bacterial cell wall or its components (contaminants and lactic acid bacteria starter strains) and milk casein to the toxin [9].

Conclusion

Thyme essential oil acts as a natural preservative when added to stirred yoghurt in selected concentration. It inhibits the microbial contaminants and lactic acid bacteria starter, whereas had increased effect on the AFM1 content between the treatments correlated to the microbial load.

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Conflicts of interest

There are no conflicts of interest.

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