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The Effect of Addition *Thymus vulgaris* on the Chemical and Microbial Properties of Cupcake Product

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This research aimed to study the chemical and microbial properties of the cupcake product after adding thyme extract. Samples were prepared by adding thyme extract (1,2,3,4%) the samples were stored for 90 days at refrigerator temperature. The moisture content of the samples to which thyme extract was added increased compared to the control. The moisture content increased in conjunction with the increase in the concentration of thyme extract, reaching its maximum at a concentration of 4%. The acidity in the cupcake samples fortified with thyme extract also increased in conjunction with the increase in the concentration of thyme extract, reaching 5.41 at a concentration of 4% of thyme extract after 90 days of storage, while the control sample became unfit for consumption. The addition of thyme extract also led to an increase in the value of the total content of phenols, which was accompanied by a decrease in the general count of bacteria as well as yeasts and fungi in the samples fortified with thyme compared to the control during the storage period. The control became unfit for human consumption after 90 days, while the samples fortified

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with thyme remained fit for human consumption. As for the degree of sensory acceptance of the cupcake samples, the degree of sensory acceptance of these samples decreased compared to the control or remained close to the degree of sensory acceptance of the control. The 1% achieved the highest sensory acceptance score in terms of color, shape, odor, taste and texture, while the 1% achieved the highest acceptance in terms of mouth feel.

Keywords: Thyme extract; cupcake; storage; bacteria total count; mold and yeasts; total phenol.

1. INTRODUCTION

In the food industry, artificial preservatives are used to preserve food, but this causes concern among consumers, especially with regard to the health safety of food [1]. The negative effects associated with the consumption of artificial preservatives for long periods of time cannot be ignored. Therefore, the current trend is focused on producing foods free of industrial chemical additives, As a result, there has been increased interest in natural antimicrobials as alternatives to conventional antimicrobials and thus the use of natural preservatives can extend shelf life [2].

In recent years, there has been a focus on medicinal plants for the purpose of using them as food additives. The extent of their safety as food additives has been studied on the one hand, and on the other hand, their effect on increasing the shelf life of food products on the other hand [3,4].

The chemical composition of essential oils varies greatly depending on the aromatic plants from which they are extracted, often ranging from a simple mixture of terpenes to a complex mixture of terpenes and is often credited with the continuity and survival of plants [5].

Thyme (*Thymus vulgaris* L.), an aromatic plant of the *Lamiaceae* family [6], Thyme is native to southern Europe, and is found throughout the world. It is an economically important plant with various use [7]. Thyme has shown many antimicrobial, antiviral, anticancer and antioxidant properties [8]. Many studies have shown that thyme essential oil, in addition to various thyme extracts, are increasingly used in the pharmaceutical industries in addition to cosmetics, and are also used in the food industries for the purpose of giving a desirable flavor to the food in addition to enriching the food in terms of antimicrobial and antioxidant properties, which makes it a functional food [9,10]. Previous studies showed that a few Thymus species could be proposed as very interesting natural resources with antioxidant activity such as *T. toseviivar. Tosevii (Kicevo), T. toseviivar. Degenii (Kitka), T. toseviivar. Longifrons (Kitka) T. toseviissp* [11].

Moreover, the antioxidant property of thyme make its helpful for food safety [12]. The most important compounds found in thyme essential oil are Eugenol, thymol and carvacrol, which are known for their antioxidant properties [13].

Cake is one of the most important bakery products. There are many types of cakes available in the market. Cake can be defined as a baked product obtained by preparing dough using the following ingredients: flour, sugar, oil, eggs, baking soda, water and sometimes milk and baked at a temperature of 180 C^{o} [14].

There are many factors that affect the quality of the cake during storage, the most important of which are: the type of fat used, storage conditions, and the temperature used during the baking process. This in turn affects the oxidation of the fats in the cake. The process of oxidation of fats produces many compounds, the most important of which are peroxides, ketones, hydrocarbons, and acids. This causes the appearance of rancidity in the cake. Auto-oxidation and lipolysis are responsible for off-flavors in lipid-containing food products [15].

The use of medicinal plants has become widespread in order to increase the shelf life of food products such as cakes and others. Methanol extract of sage leaves and clove buds was added at a concentration of 100, 200 and 300 ppm to the weight of butter used in cake production. The resulting cake was stored at room temperature for 8 weeks. The results showed that increasing the concentration of natural antioxidants led to longer stability of butter and delayed rancidity compared to the control group [16].

The essential oil extracted from thyme was also used to increase the shelf life of the cake. Adding the essential oil increased the shelf life by 30 days without using synthetic antioxidants, in addition to a decrease in the MIC value in the

samples to which the essential oil was added compared to the control [17].

Given the richness of thyme in antioxidants, in addition to the antimicrobial properties of this plant, and given the lack of local studies on the possibility of using thyme extract in food, this research aimed to:

- Study some chemical and microbial indicators of cupcakes before and after adding thyme aqueous extracts in different concentrations (1,2,3,4%)
- Study the effect of adding thyme extract during storage of cupcakes for three months in terms of chemical composition and content of biologically active compounds.

2. MATERIALS AND METHODS

2.1 Plant Material

Thymus vulgaris samples were collected in Syria, Representative homogeneous samples of each population were collected during the balsamic time, corresponding to the flowering stage. The plant samples were spread in thin layers on sheets of paper on wooden tables raised off the ground in a shaded place with an air duct. The plants were constantly turned over to ensure that the lower layers did not rot. The drying process lasted for about a week, during which the humidity decreased to 10%. The dried leaves were ground in a laboratory grinder to a diameter of 425 microns. Then the samples were placed in opaque glass containers.

2.2 Alcoholic Extract

Alcoholic extract of thyme prepared according to Al-Shawi et al [18] with some modification, by adding 100 g of thyme to 500 ml of 100% ethyl alcohol and mixing well. The sample was placed in a vertical shaker at 30°C for 24 hours after which the extract was filtered using (Whatman No.1) filter paper. The filtrate then was concentrated by using rotary vacuum evaporator at 40°C to dispose of the solvent, then left at laboratory temperature until it gets dry and viscous, weighed and placed in dark sealed bottles and kept in the refrigerator until use. Ethyl alcohol extraction was used because of the higher extraction rate in this method compared to aqueous extraction.

2.3 Cupcake Ingredients

Wheat flour, sugar, eggs (10 g fat /100 g egg), milk (3.3 % fat), corn oil, shortening (99.9 % fat), baking powder and vanilla.

2.4 Preparation of the Cakes

1 Kg of cake was prepared by mixing (75 g) shortening, (80 ml) oil, (266 g) sugar and 3 g vanilla for 3 min. at medium speed then 2 min. at high speed. Then whole eggs (3 eggs around 195 g) were added one after one at medium speed. After that flour (220 g) which mixed previously with baking powder (5 g) was added in five steps alternately with milk (160 ml) started and ended with flour.

Cupcake with herbs are prepared by the following technology: flour and herbs (1-2-3-4%) are mixed and stirred until total homogenization [19]. while control cake was prepared without any addition. The cake dough was filled in small cupcakes and baked at (165 °C) for 11 min. The baked cakes leave to be cooled and then covered with nylon films and stored at room temperature for 90 days. The Samples of all analysis were examined at 1st, 30th, 60th, and 90th days of storage [20].

2.5 Chemical Analysis

The moisture content % and pH value of cake samples were determined according to AOAC [21]; AOAC [22].

2.6 Preparation of Total Phenols Extract

Total phenols were extracted according to the method [23]. Where 1 g was taken from the studied sample, and placed in a polyethylene tube (50 ml), 30 ml of absolute ethanol was added, Then mix well at room temperature using a magnetic stirrer at maximum speed for an hour, then centrifuge the sample At maximum speed (3000 rpm), And take the clear liquid for analysis. Total phenolics were determined by the Folin Ciocalteu method as reported [24] 0.5 ml of the previous alcoholic extract was taken in a 10 ml volumetric flask, 3 ml of distilled water and 0.2 ml of Folin Ciocalteu reagent were added, stirred and shaken for 2 minutes using a vortex at room temperature, 4 ml of sodium carbonate (7%) was added and the volume was completed using distilled water, the samples were left for 2 hours at room temperature, centrifuged and the optical absorbance was measured at a wavelength of

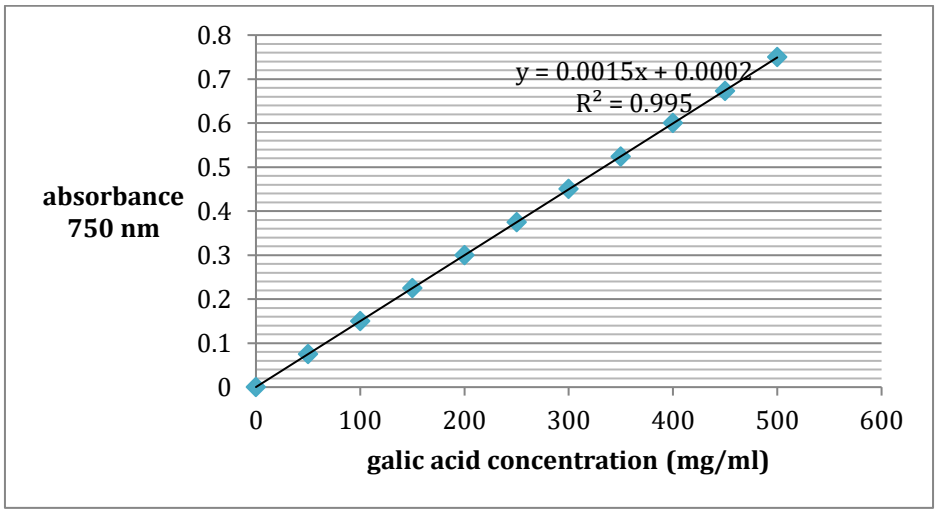

Fig. 1. A standard series of gallic acid

750 nm. Gallic acid was used as standard. The total phenolic content was calculated from the calibration curve that was in the range of 0–600 mg/mL (R2=0.995) and the results were expressed as mg of galic acid equivalent per g dry weight (mg of GAE/100 g DW).

2.7 Microbiological Tests

The microbiological study included the general enumeration of microorganisms, yeast and molds counts for cupcake samples, at time zero and after storage, where 1 g of samples were taken under sterile conditions and placed in a test tube containing 9 ml of distilled and sterile water for each and were homogenized for 90 seconds, and 1 ml was taken from it using a standard pipette, placed in a Petri dish and the sterile medium was poured over it using medium and the samples were incubated after solidification at 25 °C for 72 hours to study yeasts and molds using PDA. To study the general enumeration, the samples were incubated at 37 °C for 48 hours using (Nutrient Agar) and incubated at 36 °C for 48 hours to study coliform bacteria using (V.R.B) Violet Red Bile Agar medium [25].

2.8 Sensory Analysis

The sensory analysis of cake samples obtained in a lighted and ventilated environment by a group of 20 people (students and teachers) from the Food Science Department, Faculty Of Agriculture Engenering, Tishreen University. Sensory evaluation of cakes treated with different levels of thyme and control. The coded samples were randomly submitted to the panelists as well as a glass of water in order to drink after each tasted sample to neutralize the mouths of the panelists. Sensory parameters, including shape, color, odor, taste, texture, and mouth feel, were evaluated through 5 point hedonic evaluation scale, where 1 = dislike extremely and 5 = like extremely [26].

2.9 Statistical Evaluation

The data were statistically analyzed using SPSS program (Version 24). The significant differences between experimental results were determined using (Dancun test) and considered significant when $P<0.05$. Data were expressed as means ± standard deviation of three replicates.

3. RESULTS AND DISCUSSION

3.1 Study of Chemical Indicators of Thyme-Fortified Cupcake Product

Table 1 shows the results of the chemical indicators of the cupcake product with thyme added at four concentrations (1,2,3,4%) in addition to the control sample, immediately after manufacturing and during storage periods.

Significant differences were shown in moisture content ($p < 0.05$), where cupcake enriched with thyme differed from the control sample. which can be explained by the fact that powdered thyme may has greater water holding capacity than wheat flour. Similarly [27] reported a significant increase in moisture content with the addition of 3,5 and 10% of chamomaile extract in cupcake composition. Dashti et al [28] reported that with addition of 0.05, 0.1, 0.2 (ml/100g) essential oil of thyme moisture content showed significantly lower rates.

Table 1. The moisture content in cupcake product

Sample	The moisture content %			
	Zero time	30 days	60 days	90 days
Control	16.78±0.078^{d}	16.83±0.023^{e}	17.61±0.012^{d}	-
1%	16.95±0.12^{d}	17.05±0.041^{d}	17.62±0.025^{d}	18.02±0.13^{d}
2%	17.3±0.23^{c}	17.51±0.052^{c}	18.1±0.036^{c}	18.63±0.095^{c}
3%	18.2±0.081^{b}	18.6±0.071^{b}	18.97±0.083^{b}	19.4±0.065^{b}
4%	18.7±0.077^{a}	19.2±0.034^{a}	19.65±0.026^{a}	20.04±0.077^{a}

(-): *Indicates that the sample has rotted and spoiled and has not been tested. Mean values not followed with the same letter in a column are significantly different at p < 0.05*

Table 2. The pH value in cupcake product

Sample	pH value			
	Zero time	30 days	60 days	90 days
Control	6.91±0.14^{a}	7.3±0.09^{a}	8.1±0.12^{a}	-
1%	6.8±0.013^{a}	6.4±0.16^{b}	6.23±0.081^{b}	6.09±0.054^{a}
2%	6.51±0.08^{b}	6.21±0.063^{bc}	6.04±0.071^{c}	5.8±0.069^{b}
3%	6.02±0.09^{c}	5.9±0.11^{cd}	5.73±0.063^{d}	5.62±0.071^{c}
4%	6±0.034^{c}	5.82±0.091^{d}	5.64±0.074^{d}	5.41±0.13^{d}

(-): *Indicates that the sample has rotted and spoiled and has not been tested. Mean values not followed with the same letter in a column are significantly different at p < 0.05*

Table 3. The total phenolic content in cupcake product

Sample	Total phenolic content (mg galic acid equivalent /100g)			
	Zero time	30 days	60 days	90 days
Control	1.42±0.032^{e}	1.23±0.033^{e}	1.18±0.037^{e}	-
1%	4.9±0.012^{d}	4.85±0.042^{d}	4.62±0.045^{d}	4.41±0.018^{d}
2%	5.4±0.042^{c}	5.31±0.035^{c}	5.09±0.038^{c}	4.93±0.027^{c}
3%	6.1±0.014^{b}	5.9±0.053^{b}	5.72±0.047^{b}	5.64±0.029^{b}
4%	6.9±0.016^{a}	6.7±0.044^{a}	6.51±0.028^{a}	6.42±0.021^{a}

(-): *Indicates that the sample has rotted and spoiled and has not been tested. Mean values not followed with the same letter in a column are significantly different at p < 0.05*

Table 2 shows the results of the pH value of the cupcake product with thyme added at four concentrations (1,2,3,4%) in addition to the control sample, immediately after manufacturing and during storage periods.

Only the cupcake samples with thyme extracts showed significant differences from the control cupcake samples. This can be explained by the fact that thyme has a high content of organic acids , which has influenced the increase in the overall acidity of the cupcake. Studies have indicated that thyme contains ursolic acid and oleic acid [19].

Total phenolics results for fortified cupcake product with thyme: The Total phenolics of cupcake samples with thyme extracts comparing with control sample was presented as variations of The Total phenolics in Table 3.

Concerning the total phenolics content the data in Table 3 showed that addition of thyme to cake samples led to higher the total phenolics content than control sample. The presence of phenolic compounds in thyme, in addition to its antimicrobial properties, suggests that it could be used as an alternative to the use of synthetic antioxidants, as their addition reduces the oxidation of lipids that cause unpleasant sensory characteristics in the product during storage [10]. In thyme it contains about 5.0 % tannins, resins, flavonoids, ursolic and oleic acid. It contains useful constituents and anti-oxidants [19]. These results are in good agreement with data obtained by [29] Thyme powders were added to sunflower oil at ratio of 0.5%, 1% and 1.5%, and the frying period were estimated for 2 h at 250 ± 1 °C. The oil samples collected intervals were at 0.5, 1, 1.5, and 2 h and the potatoes were fried in each time. The antioxidant activity of thyme powders was 93.05 %, estimated using DPPH root scanning

methods. Karoui et al [30] revealed that extracts of thyme have antioxidant activity due to their containing phenolic acids, such as rosmarinic, carnosic, and cinnamic. They also contain flavonoids,such as luteolin and apigenin. Various herbs, spices, and natural extracts from selected herbs are stable for oxidation due to the presence of natural phenolic compounds [31]. The results of this study are consistent with what was found by Dashti et al., [28] that the antioxidant effect of thyme essential oil was shown by the lower peroxide value and TBARS levels found in thyme-treaded nuggets.

Effect of thyme on the general count of microorganisms of cupcake samples studied during the period of refrigerated storage.

The antibacterial and antifungal activity exhibited by Thymus species has already been demonstrated [32].

As seen from Table 5 it is clear that the mold and yeasts ratio (sample / control) of all samples were increased during storage. After 30 days there was a significant difference between mold ratio of samples containing thyme and control.

The effect of thyme on the number of coliform bacteria in the cupcake samples studied during the refrigerated storage period: The results showed that there were no coliform bacteria growths in the control samples and the samples enriched with thyme before and after storage for 3 months, and they were in accordance with the Syrian standard specification No.2007/2179.

Sensory evaluation of cake sample at the beginning of storage period (zero time) at room temperature.

Regarding sensory evaluation of cake after baking (at zero time) the results in Table 6 declared that, thyme quantity influences on all indicators, color was gradually decreased. At the same time addition of thyme extraction to cake caused significant different in color compared to control. This finding may be related to thyme extraction is green, As for the taste, it was found that the sensory acceptance of the samples enriched with thyme extract was higher than the control, The mouth feel for the thyme-infused samples remained pleasant to the consumer compared to the control and was higher at the concentration 2%.

Table 4. Effect of thyme on the general count of microorganisms of cupcake samples studied during the period of refrigerated storage

Sample	General Census Test (CFU/g)			
	Zero time	**30 days**	**60 days**	**90 days**
Control	10×10^{3a}	23×10^{3a}	40×10^{3a}	-
1%	9×10^{3b}	10×10^{3b}	19×10^{3b}	22×10^{3a}
2%	7×10^{3c}	9×10^{3c}	16×10^{3c}	19×10^{3b}
3%	5×10^{3d}	8×10^{3d}	12×10^{3d}	18×10^{3c}
4%	2×10^{3e}	5×10^{3e}	9×10^{3e}	11×10^{3d}

***(-):** Indicates that the sample has rotted and spoiled and has not been tested. Mean values not followed with the same letter in a column are significantly different at $p < 0.05$*

Table 5. The effect of adding thyme on the count of yeasts and mold in cupcake samples during the period of refrigerated storage

Sample	the count of yeasts and mold in cupcake samples			
	Zero time	**30 days**	**60 days**	**90 days**
Control	0	15×10^{3a}	31×10^{3a}	-
1%	0	7×10^{3b}	14×10^{3b}	18×10^{3a}
2%	0	5×10^{3c}	11×10^{3c}	15×10^{3b}
3%	0	4×10^{3d}	10×10^{3d}	14×10^{3c}
4%	0	2×10^{3e}	7×10^{3e}	10×10^{3d}

***(-):** Indicates that the sample has rotted and spoiled and has not been tested. Mean values not followed with the same letter in a column are significantly different at $p < 0.05$*

Table 6. Sensory evaluation of cake sample at the beginning of storage period (zero time) at room temperature

Sample	Color	Shape	Odor	Taste	Texture	Mouth feel
Control	9.5±0.32^{a}	9.3±0.22^{a}	9.4±0.09^{a}	9.5±0.23^{a}	9.4±0.08^{b}	9.2±0.07^{b}
1%	9.3±0.11^{ab}	9±0.13^{ab}	9.1±0.10^{b}	9.5±0.32^{a}	9.5±0.07^{a}	9.3±0.12^{a}
2%	9.1±0.14^{b}	8.9±0.23^{b}	9.1±0.08^{b}	9.5±0.16^{a}	9.5±0.11^{a}	9.4±0.10^{a}
3%	9.0±0.21^{c}	8.7±0.15^{c}	8.9±0.12^{bc}	9.3±0.25^{b}	9.6±0.09^{a}	9.1±0.09^{b}
4%	9.0±0.15^{c}	8.6±0.24^{c}	8.8±0.13^{c}	9.0±0.17^{c}	9.6±0.12^{a}	9.0±0.08^{c}

Mean values not followed with the same letter in a column are significantly different at $p < 0.05$

4. CONCLUSION

As a conclusion, It was observed in this study that thyme was stable during baking and can be considered as efficient natural antioxidants in cupcake production during three months of storage**.** A significant increase in the total count of microorganisms, yeasts and fungi during the storage period for the control sample, and a significant decrease in the total count of microorganisms, yeasts and fungi for cupcake samples enriched with thyme with an increase in the percentage of the extract. In addition to a significant increase in the total phenolic content of the thyme-fortified samples.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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