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Estimation of biological properties in Processed White Soft Cheese supported With Thymus Vulgaris seed oil Extract

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Abstract:

Thyme herb (*Thymus Vulgaris* is widely known as a good source of bioactive compounds. Therefore, this study aimed to use the aqueous thyme seed extract in white soft cheese processing and to evaluate the total phenolic content, antioxidant, microbial content and sensory attributes of the resulting cheese. Dried thyme seeds were extracted with water to obtain an aqueous extract. Prior to white cheese processing, different concentrations (2, 4 and 6 %, v/v) of ATSE (aqueous thyme seed extract were added to the milk. White soft cheese containing ATSE had significantly higher phenolic compounds than the control cheese and the highest phenolic content (91.0 mg GAE / 100 g cheese) was recorded in white soft cheese samples containing 4% of ATSE. The results of the microbial analysis showed that the microbial growth in white soft cheese decrease with increasing of thyme extract concentration and no microbial growth was shown in white soft cheeses containing 4% of thyme extract. The sensory evaluation revealed that the addition of thyme extract had a high significant effect on the overall acceptance of all types of cheese manufactured.

Keywords: Thyme; white soft cheese; phenolic compounds; microbial content; sensory proprieties.

INTRODUCTION:

Thyme (Thymus vulgaris L.) is a herbaceous plant belonging to the Lamiaceae family and It is local to Europe and adaptable to a wide range of environmental conditions (1). Thyme is an aromatic medicinal plant of increasing economic importance in Europe, Asia, North Africa and America. Essential oil of thyme has been reported as one of the top 10 essential oils (2), the medicinal properties of the plant, of its different parts extracts, especially the essential oils, has been carefully studied, and recorded many industrial mainly as food and cosmetic additive and medical applications (3). Its oil was reported to have antimicrobial activity (bacteria & fungi) (4), Antispasmodic (5) as well as antioxidant (6) activities were also reported for the alcoholic extract of the plant .The genus Thymus (thyme) consists of about 215 species of herbaceous perennials and subshrubs that are well adapted to hot and dry climates. This is one of the most widely used genera in medicine , Thyme herb (Thyme herba) is produced from the dried leaves and flowering tops of T. vulgaris, and it contains tannins, flavonoids, triterpene compounds, and up to 2.5 % of essential oils , The main components are thymol and p-cymene, The major phenolic components in thyme extracts, especially thymol and carvacrol, present higher antioxidant activity than the well-known BHT (butylated hydroxytoluene) and αtocopherol antioxidants ,while other thyme species also contain carvacrol, a-terpinyl acetate, and cis-myrtanol (7). A previous study shown that the addition of Thyme essential oil to traditional margarine was effective against *Escherichia Coli* and *Staphylococcus aureus* (8). The study of addition thyme seed extract to white soft cheese processing has not yet been fully investigated. As white soft cheese is consumed widely in most countries, the objective of this research would be proofed of the influence of usage of thyme seed as an antimicrobial food additive in white cheese processing. Some plant extracts have been shown antimicrobials as well as antioxidants in food systems. Thyme is one among the potential herbs for extracting natural antioxidants. Thyme leaves are used as spice/ additive to flavor a wide range of food and beverage products. However, antioxidant potential of thyme was not yet well fully studied and exploited properly to improve the shelf-stability of food products.

MATERIALS AND METHODS:

Materials

Fresh cow's milk collected from karbala city Dried thyme seeds were purchased from a local market in karbala, Iraq. Folin-Ciocalteu reagent was purchased from Sigma-Aldrich (St. Louis, Mo., U.S.A.). Sodium carbonate was purchased from Merck (Darmstadt, Germany). Nutrient ager and peptone water were purchased from Sigma-Aldrich.

Preparation of aqueous thyme seeds extract (ATSE):

Dried thyme seed was grounded into powder by using laboratory grinder. The powder was used as the raw material for the following experiments to prepare crude the extract., 1 g of thyme seed powder was added to 10 ml of distilled water at 1:10 (solids: solvent). The mixture was stirred on a hot plate magnetic stirrer for 15 min at 100 °C. After that, the extract was filtered through Whatman No 1 filter paper and centrifuged for 10 min to produce the clear aqueous extract. The supernatant was stored at – 20 °C until it used.

Determination of total phenolic compounds

Total phenolic compounds of thyme seed extract and white soft cheese samples were evaluated according to the method reported by [9]. Room temperature mixture was carried out at a test tube by mixing 100 µl of the sample with 10 ml of distilled water and 0.5 ml of Folin-Ciocalteau reagent. The mixture kept standing for 3 min before addition of 1.5 ml of sodium carbonate (20%) and 6.9 of distilled water. After that, the mixtures were stirred and incubated at 40 °C for 30 min. The optical density at 765 nm was measured for the samples using a spectrophotometer (UV-1100 Spectrophotometer, Gallic acid was used as a standard and the results of thyme seed extract were stated as mg Gallic acid equivalents per gram of sample (mg GAE / g crude material). For white cheese samples, the results were stated as mg Gallic acid equivalents / 100 gram of cheese sample (mg GAE / 100 g).

Processing of White soft cheese :(WSC)

The milk was putt into a sterile cheese maker container and the temperature increased up to 90 C for 2 sec. After cooling down the milk to 45 C2 ,1 , and 3% (v/v %) concentrations of ATSE were added to it. Than 0.004 % of microbial Rennet was added to the milk. After 30 min incubation at 37 C, clots were transferred into the sterile mesh for removing water and incubated for 2 h 37 C. After completion of the dehydration steps, cutting and molding of clots was done. Cheeses were putt into sterile containers and stored at 4°C for 2 days for the following steps.

Determination of antimicrobial activity:

1 g from the core of each cheese sample was aseptically cut and placed into 9 ml of 0.1% buffered peptone water and homogenized with a homogenizer. The resulting were serially diluted with peptone water (0.1%) and the diluted samples were used for the microbial assay. Plate count agar was used for the

evaluated of total plate counts using the pour plate method. Then, the plates were incubated at 37 °C for 24 h and counted. The results counts in this research were estimation as colony forming units (CFU) per g of cheese sample.

Determination of Sensory properties

The sensory testes were done into five steps evaluating taste, color, texture, , flavor and overall acceptance. The test was submitted to choose judges with to evaluate the basic tastes of cheese-manufactured samples. The cheese samples used in the texture tests were cut to make suitable cubes (2.5 cm size). This step aimed at measure main cheese texture characteristics (surface roughness, surface moisture, elasticity, and hardness). Applicants were recruited among people living in the of karalla city; most of them were employed at University of karbala (10 potential chooses). All the choses were briefed on the scope of the sensory evaluation technique and the procedures of each test; previous results were presented and discussed before running any new test.

Thyme as anti-oxidant in the manufacture of cheese &meat:

The warm alcohol extract of Thyme seed used, which contains the highest concentration of volatile oils, flavonoids, and phenols. The samples tested by adding extract at 100 mg per kg of mixing cheese and meat under test which is not additive by industrial additives were the physical and sensory changes .

Analysis of data:

Data done were analyzed using SPSS software). Analysis of variance (ANOVA) was performed and significant differences between mean values were determined by Duncan's test. The results were expressed as means ± standard deviation. P-values < 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

Composition of milk samples

The composition of milk is recorded in Table 1. Milk content of Fat, SNF, protein , and lactose were 4.23, 7.89, 3.52 and 4.57 % respectively. The general composition of bovine milk from different regions is similar with some differences depends on environmental and genetic factors [8]. Fats content in cheese yield (per kilogram of milk) and firmness, as well as in the color and flavor of dairy products [8].The protein content of bovine milk has increased interest in recent years because an increasing rate of milk is used for manufactured products such as cheese [10].

Table (1) Composition of crude milk used in the current study to prepare white soft cheese

Component	Concentration (%)
Lactose	4.57±0.47
Protein	3.52±0.43
SNF	7.98±0.38
Fat	4.23±3.76

Content of Total phenolic prepared with ATSE

Table 2 showed the total phenolic content of WSC samples prepared with different concentrations (2%, 4%, 6%) of ATSE. As seen in this Table, the total phenolic content of WSC increased significantly ($P < 0.05$) with the addition of ATSE as compared to the control WSC sample (white soft cheese prepared without ATSE). The control WSC had significantly the lowest phenolic content (24.5 mg GAE / 100 g cheese) compared to the WSC samples prepared with different concentrations of ATSE. From WSC samples prepared with different concentrations of ATSE, WSC containing 6% ATSE had significantly the higher phenolic content (90.32 mg GAE / 100 g cheese). These results showed that the addition of ATSE significantly improved the phenolic content of WSC. Similar results were recorded for cheese samples supplemented with different concentrations of another herb extract [11].

Table 2. Content of Total phenolic compounds of WSC samples enhanced with different concentrations ((2%, 4%, 6%) of thyme seed extract

Samples	Content of total phenolic mg GAE/ 100 g cheese
Control	19.98±0.34
WSC+2%ATSE	65.33±0.53
WSC+4%ATSE	83.21±0.49
WSC+6%ATSE	90.32±0.54

Antimicrobial activity of ATSE in WSC

Data recorded the effect of different concentrations of ATSE on the dependent variable according to CFU/g in WSC showed that the overall concentrations of ATSE had a highly significant effect on the growth of total pathogenic bacterial counts ($P < 0.05$). ANOVA test showed that the growth of total bacterial counts in white soft cheese, with concentrations of 2, 4and 6% of ATSE was significantly decreased compared with the control cheese. In the present study, the 6 % thyme seed extract had the strongest antimicrobial activity against the selected bacteria. (12).

Table 3. Total bacterial counts in white soft cheese samples incorporated with different concentrations (2%, 4%, 6%) of thyme seed extract.

Sample	Total bacterial count (cfu/g)
Control	354±0.45
WSC±2%ATSE	298±0.43
WSC±4%ATSE	158±0.39
WSC±6%ATSE	124±0.43

Table (4): Antioxidant activity of thyme seed evaluated by Rancimat on meat and butter:

Samples	Antioxidant activity
vitamin E	34.76±0.43
(α-tocopherol)	22.75±0.54
Meat ±thyme extract	10.54±0.34
butter± thyme extract	18.54±0.37

The result showed higher induction period of the meat and butter with the thyme extract added, compared with the control implies the better antioxidant activity of thyme extracts. Thyme antioxidant was effective in keeping the stability of the meat and butter for an extended time when treated samples were exposed to the accelerated condition in rancidity. But, thyme extract has low antioxidant activity compared to vitamin E (α-tocopherol) applied at 0.05% concentration as a control treatment.(13).

Determination of Sensory properties of white soft cheese prepared with ATSE

Table 5 showed the results for the sensory-recorded evaluation of white soft cheese samples prepared with different concentrations (2%, 4%, 6%) of ATSE. In this research, the parameters selected to test were taste, texture, color, flavor and overall acceptability. The sensory analysis reveals that there is no significant effect of ATSE addition on sensory attributes of white soft cheese. The white soft cheese prepared with different concentrations of ATSE was not significantly differed from the control cheese, except for the flavor. The white soft cheese prepared with 4% and 6% of ATSE had higher scores of flavor and was more acceptable as compared to the control cheese. However, increasing ATSE concentration did not significantly affect flavor scores of the cheese produced. In general, few significant differences were observed in the overall acceptability of all types of cheese and all were acceptable by panelists. Other findings showed that herbs like oregano and rosemary essential oils demonstrated effect against lipid oxidation and fermentation in flavored cheese prepared with cream cheese base [14].

Table 5. Sensory degree of white soft cheese samples enhanced with different concentrations (2%, 4%, 6%) of thyme seed extract.

Sensory properties	Score degree range	Control	WSC with 2% ATSE	WSC with 4% ATSE	WSC with 6% ATSE
Color	1-10	6.71±0.54	6.81±0.58	8.43±0.61	9.21±0.67
Taste	1-10	6.45±0.43	7.45±0.33	8.32±0.64	8.54±0.54
Texture	1-10	7.65±0.42	7.35±0.47	8.90±0.73	8.65±0.61
Flavor	1-10	8.43±0.65	8.95±0.61	9.43±0.67	9.67±0.54
Over all acceptability	1-10	7.43±0.54	7.43±0.58	8.93±0.76	8.43±0.59

REFERENCES:

1- **Begrow**, F., J. Engelbertz, B. Feistel, R. Lehnfeld, K. Bauer, E. J. Verspohl. 2010. Impact of thymol in thyme extracts on their antispasmodic action and ciliary clearance. Planta Med. 76(04): 311-318.

2- **Cetin**, B., S. Cakmakci, R.Cakmakci. 2011. The investigation of antimicrobial activity of thyme and oregano essential oils. Turk. J. Agric. For., 35: 145-154.

3- **De Peters**., E. J. & Cant, J. P. 1992. Nutritional Factors Influencing the Nitrogen Composition of Bovine Milk: A Review1, Journal of Dairy Science, 75(8), 2043-2070.

4- **El-Din**, H. M. F. ., Chita, E. I. ., Badran, S. M. AGad . A. S.., and El-Said, M. M. 2010. Manufacture of low fat UF-soft cheese supplemented with rosemary extract (as natural antioxidant), Journal of American Science, 6 (10), , 570-579.

5- **Fayad**, N.K. Omar Hamad Shahab AL- Obaidi, Taghreed H. Al-Noor, Mohammed Oday Ezzat. 2013. Water And Alcohol Extraction Of Thyme Plant (Thymus Vulgaris) And Activity study Against Bacteria, Tumors And Used As Anti-Oxidant In Margarine ; Innovative Systems Design and Engineering, Vol.4, No.1,pp:41-52.

6- **Joon** K, and Shibamoto T. 2009. Antioxidant Assays for Plant and Food Components. Journal of Agriculture and Food Chemistry, 57:1655-1666.

7- **Olmedo**, R. H. ., Nepote, V. And Grosso N.R. 2013. Preservation of sensory and chemical properties in flavoured cheese prepared with cream cheese base using oregano and rosemary essential oils, LWT-Food Science and Technology, 53(2), 409-417.

8- **Palmquist**, D. L., Beaulieu A. D. & Barbano, D. M. 1993. Feed and animal factors influencing milk fat composition1, Journal of dairy science, 76(6), 1753-1771.

9- **Pereira**, V. P. ., Knor, F. J. ., Vellosa., J. C. R. And F. L. Beltrame. 2014. Determination of phenolic compounds and antioxidant activity of green, black and white teas of Camellia sinensis (L.). Kuntze, Theaceae, Revista Brasileira de Plantas Medicines, 16(3).

10- **Raghdi**, B.H., Z.M. Maki. 2003. Review of common Thyme. J .Med. Plan. 2(7): 1-12.

11- **Ruiz**-Navajas, Y., Viuda-Martos, M., Sandra, E., Perez-Alvarez,J.A and Fernández-López,J. 2012. Chemical characterization and antibacterial activity of Thymus moroderi and Thymus piperella essential oils, two Thymus endemic species from southeast of Spain, Food Control, 27 :294–299.

12- **Sacchetti**, G., S. Maietti, M. Muzzoli, M. Scaglianti, S. Manfredini, M. Radice, R. Bruni. 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chem. 91(4): 621-632.

13- **Sokovi**, M.D. Vukojevi, j., Marin, P.D. ., Brki, D.D. Vajs, V., Van Griensven, L.J.L.D. 2009.Chemical composition of essential oils of Thymus and Mentha species and their antifungal activities, Molecules, 14 238–249.

14- **Stahl**-Biskup, E., F. Sáez. 2002. Thyme: the genus Thymus. London: Taylor & Francis.