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Effect of Adding Essential Oils of *Nigella sativa* and Thyme on the Yogurt Flavor

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ABSTRACT

Background and Objective: Essential oil extracts have attracted much attention due to their bioactive properties, including antimicrobial activities. The study focused on the effect of using the essential oils of *Nigella sativa* and thyme on the sensory properties of laboratory made yogurt. **Materials and Methods:** It was produced from cow's milk during its storage period at a temperature of 5±2°C for periods of 0, 3 and 7 days. Samples were placed in plastic containers and incubated at a temperature of 42±2°C until complete coagulation within 4 hrs and the pH reached 4.6. Then, it is transferred to the refrigerator for cooling and storage at a temperature of 5±2°C, after which the necessary sensory tests are carried out for periods of 0, 3 and 7 days. **Results:** The results showed that the yogurt sample with thyme oil was superior compared to other samples in terms of sensory properties, which included color, flavor, texture and bitterness during the different storage periods. The results showed that there were significant differences in the average values of the studied sensory characteristics between the samples, especially the flavor characteristic of the samples by adding oils compared to the control sample as a result of its richness in biologically active compounds present in its oils. **Conclusion:** This type can enrich a variety of dairy products. The market also represents a healthier alternative for the consumer.

KEYWORDS

Yogurt, *Nigella sativa*, thyme, essential oils, flavor

INTRODUCTION

In the current decade, scientific research has drawn attention to the impact of essential oil extracts and brought them into the research as the leading trend of their versatility. It has become an attractive factor in food systems specifically designed for its benefits, in addition to increasing consumer demand and their need for safe and effective natural health products. Its applications in the food, pharmaceutical and agricultural industries have been enhanced due to technological progress and scientific innovation. They are considered evidence to support the pivotal links between its active components and human health.

The increase in pathogens transmitted through processed foods is a real source of concern and to find alternatives to the use of industrial food preservatives due to their relationship to human health

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determinants, especially when foods, including dairy products, are contaminated with pathogenic contaminants. In recent years, attention has been directed towards extracting bioactive ingredients from natural sources.

Essential oil extracts have attracted much attention due to their bioactive properties, including antimicrobial activities. These components must be effective against those organisms that are likely to be present and grow in food in particular and which cause its poisoning. Therefore, foods are treated with extracts of essential oils of some herbal plants, as they aim to find biologically active natural preservatives to preserve foods as well as prolong their storage life[1,2].

Popescu[3] has indicated that spices and the effective compounds they contain are safe to use, in addition to the wide spectrum of natural compounds they provide, which constitute an alternative to industrial antioxidants and protect and improve their properties and biological activities. Thus, attention has turned to fortifying processed foods with extracts of medicinal herbal plants, which are an important source for obtaining effective compounds such as phenolic, terpene and sulfhydryl compounds, which are used as natural food additives to prevent food spoilage. In addition, the production of many compounds such as peroxides, ketones and aldehydes that generate undesirable odors and accompany changes in sensory characteristics[4].

It has been traditionally used as a food preservative or as a spice additive to give aroma and flavor to foods, as well as being widely used as a medicinal plant. Most of the therapeutic properties, such as anti-inflammatory, antimicrobial and antioxidant, are due to the biologically active components in the essential oil, mainly phenolic compounds, especially thymoquinone (30-48%). It has many biochemical and physical properties and is characterized by the removal of free radicals, in addition to its oil containing more than 30% fixed oil and 0.4-0.45% volatile oil[5].

Thyme is a medicinal herbal plant belonging to the Lamiaceae family. It is grown in the Mediterranean region, North Africa and many regions of Asia. Various studies have been conducted to reveal the pharmacological activities of thyme. The therapeutic potential is due to containing effective compounds such as flavonoids, thymol, carvacrol, eugenol and phenols, which have the ability to inhibit harmful microbes. It is also considered a rich source of iron, calcium, manganese and vitamin K, as well as antioxidant properties, as it works to remove free radicals and inhibit hydroxyl peroxide[6].

Abd El-Hack *et al*.[7] have explained that the antioxidant activity of thymol is due to the presence of phenolic compounds, especially the hydroxyl group. It donates hydrogen to the intermediate radicals, which are produced during the first step in fat oxidation, as it works to slow down their oxidation by inhibiting and suppressing the activity of free radicals and thus delaying the formation of peroxides and hydroperoxides. In addition, Tohidi *et al*.[8] study showed that thyme oil was analyzed by gas chromatography-mass spectrometry (GC-MS). The results showed that thyme oil is a mixture of more than 38 different compounds. It contains phenols, including (12.4-79.74%) thymol, which is considered a major active compound as it exerts its antimicrobial activity by binding the membrane to proteins and changing the permeability of the membrane. In addition to its ability to rupture the cytoplasmic membrane of gram-negative bacteria, leading to the leakage of lipopolysaccharide and (4.37-42.14%) carvacrol, as well as p-cymene (0.8-12.86%) and geraniol (0.3-22.44 %).

Milk is one of the basic sources of food for humans and a vital source for the progress and prosperity of people because it possesses the ingredients that make it self-sufficient because it contains carbohydrates, protein, fat, lactose, vitamins, as well as mineral salts. Yogurt is known as a fermented milk product with a soft texture and a mild acidic taste. It is characterized by its desired flavor. It is obtained by pasteurizing

milk and fermenting it with lactic acid bacteria *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. It is considered one of the main milk products for the sustainability of life[9,10].

Pinto *et al.*[11] have explained that the method of food preservation is one of the most important critical processes that control the quality of food and the extent of its acceptance by the consumer after it is manufactured or prepared. There is no doubt that the consumer is affected by the surrounding environment, whether air, water, or food. Therefore, it was found that adding essential oils to medicinal plants is considered one of the most important ways to preserve foods, especially dairy products such as yogurt, in terms of quality and preserving sensory properties, in addition to the chemical changes that may occur, especially since it is a product rich in unsaturated fatty acids such as omega-3. Thus, this study aimed to investigate the possibility of adding *Nigella sativa* and thyme oils to yogurt and the effect of this addition on its flavor properties during different periods of cold storage.

MATERIALS AND METHODS

Study materials: The study was applied at Wasit University, College of Agriculture for the period 20/2/2023 to 15/12/2023. *Nigella sativa* and thyme plants were obtained from local markets in the Kut City, Wasit Province, Iraq. They were dried at room temperature and then ground. Samples weighing 10 g were taken from them. The oils were extracted using a Soxhlet device, according to Kamel *et al.*[12], while cow's milk was prepared from an animal breeder in the Al-Battar area, Kut City, Wasit Province, Iraq.

Yogurt manufacturing: Yogurt is made according to the method recommended by Anuyahong *et al.*[13] where the milk is pasteurized at a temperature of 85°C for 5 min, then cooled to 45°C. After cooling, the bacterial strains *Streptococcus Salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* prepared for the manufacture of prepared yogurt were added. They were obtained from the French company (Danisco) by adding them directly to the product. The product is divided into 2 halves, the first half is left unadded (control sample) and the second is divided into two parts, one to which *Nigella sativa* oil is added and the other to which thyme oil is added at a rate of 0.5%, respectively.

It is placed in plastic containers and incubated at a temperature of 42±2°C until complete coagulation within 4 hrs and the pH reaches 4.6. Then, it is transferred to the refrigerator for cooling and storage at a temperature of 5±2°C, after which the necessary sensory tests are carried out for periods of 0, 3 and 7 days.

Sensory evaluation: The method Anuyahong *et al.*[13] was followed in conducting sensory tests for manufactured yogurt samples by professors and students at the College of Agriculture, University of Wasit using an evaluation form with some modifications, as it included the characteristics of flavor, smell, texture, color and bitterness and they were given. Each characteristic has a score from (1-5).

Statistical analysis: The data were analyzed using SPSS program (2014). The results were compared using least significant differences at 0.05 level.

RESULTS AND DISCUSSION

The results showed that there were significant differences at the probability level ($p<0.05$) in the average values of the studied sensory characteristics between the samples, especially the flavor characteristic of the samples by adding oils compared to the control sample as a result of its richness in biologically active compounds present in its oils, as it remained acceptable until the end of the storage period (Table 1). The results agreed with Benguedouar *et al.*[14] which found that yogurt containing thyme extract had better sensory properties than regular yogurt. The study explained that the presence of this extract improves the

Table 1: Milk structure that used for making yogurt

Chemical structure of milk						
Moisture (%)	Protein (%)	Fat (%)	Lactose (%)	Ashes (%)	PH	Percolation acidity (%)
86.77	3.23	4.58	4.75	0.67	6.5	0.17

Table 2: Sensory characteristics of yogurt samples manufactured by adding essential oils during a storage period

		Storage period (days)		
Samples	Sensory characteristics	0	3	7
Control	Flavor	4	3	2
	Texture	3	3	2
	Color	5	5	3
	Bitterns	4	2	2
	Total	16	13	9
Nigella sativa	Flavor	4	3	3
	Texture	4	3	2
	Color	5	4	4
	Bitterns	4	3	2
	Total	17	13	11
Thyme	Flavor	5	4	4
	Texture	4	3	3
	Color	5	5	3
	Bitterns	4	4	3
	Total	18	16	13

sensory properties of the product through the development of flavor compounds represented by acetaldehyde, acetoin and diacetyl, during different storage periods. In addition, the development of acidity is due to the increase and activity of lactic acid bacteria and their enzymes that decompose fat and protein into short-chain peptides that contribute to maintaining the preservation of the product flavor (Table 2).

This result was supported by de Oliveira Neves and de Oliveira[15] and Wińska *et al.*[16] which concluded that when making cheese and butter, flavor defects may occur resulting from the decomposition of fats before or after manufacturing into non-desirable compounds. However, yogurt is less susceptible to these defects due to technological factors such as the development of acidity, low storage temperature, as well as short shelf life, as the development of acidity is linked to increased microbial activity, accompanied by the accumulation of lactic acid[17].

Helal and Tagliazucchi[18] have explained that adding herbal extracts improves the characteristics of yogurt, especially the flavor through synergy with the bacteria present in it. It increases proteolytic activity during fermentation and is characterized by high contents of biologically active peptides. It enhances acidity and improves antioxidant activities by increasing the total phenolic content compared to traditional yogurt, offering a new range of yogurt with the potential to offer healthy multi-functional properties to consumers.

Kalaitsidis *et al.*[19] indicate that the activity of antimicrobials has been increased and can be affected by the production of antimicrobial peptides and organic acids. Park *et al.*[20] found that the addition of 2% red ginseng extract increased the activity and capacity of oxygen radical uptake values (DPPH) and DNA damage, pathological diseases caused by hydrogen peroxide. Therefore, this study shows that the addition of red ginseng can efficiently support the antioxidant and antitoxic effects of dairy products and thus increase the shelf life of products.

Ghalem and Zouaoui[21] have mentioned that during the manufacture of laboratory yogurt by adding rosemary oil extract at ratios of 0.14, 0.21, 0.29 and 0.36 g/L, the assessors gave the maximum levels of

taste, flavor and texture to the yogurt supplemented with 0.14 g/L of essential oil. It also improved the reduction of pH values, lactose and dry matter, but increased the content of acidity, proteins, fats and ash. In general, the storage time of 21 days had no effect on the sensory properties of the manufactured product. However, Kumar *et al*.[22] found that adding mint leaf powder in proportions of 2, 4 and 6% to yogurt, as the best proportion was 2%, to increase the shelf life to 10 days at a temperature of 5°C, to optimal in all sensory characteristics.

Zaky *et al*.[23] have explained that adding oil extracts of caraway and dill plants at a rate of 2 µm/100 mL of milk to yogurt made from buffalo milk enhanced the antioxidant activity and improved the quality of the manufactured yogurt and sensory properties such as taste and smell. Its shelf life was 28 days compared to the control yogurt. The reason for that was the fact that the essential oils of the above two plants are natural, economical and medicinal components in addition to being healthy.

In addition, Christaki *et al*.[24] and Jrad *et al*.[25] have found that the sensory properties of fermented milk products can be significantly improved all over the world as a result of technological development as well as the manufacture of new products with different ingredients and flavors by adding essential oils of medicinal plants such as thyme, as its trait is considered a very important trait that affects consumer preference. Al-Otaibi and El-Demerdash[26] added three essential oils of thyme, marjoram and sage plants to yogurt at concentrations of 0.2, 0.5 and 1.0 ppm, as no fungi, coliform, or spore-forming bacteria were detected in all treatments. They stated that adding 0.2 ppm of the above oils can increase their shelf life. The reason was the mechanism of action of the oils, as they restrict harmful microbial growth, in addition to keeping the product at an appropriate temperature of 5°C during the entire storage period of 21 days, as it was acceptable from the perspective of product quality, with flavor and appearance without revealing any pathogenic organisms.

Das *et al*.[27] have indicated that essential oils can increase oxidative stress in pathological microbial cells through metabolic interference, disruption of the cell wall and depletion of ATP, which leads to their damage and thus their programmed death. They have shown that adding aloe oil in different proportions (100, 150 and 200 mg/mL) increased the total solids and volatile fatty acids, antioxidant activity, tocopherol and the positive effect against pathogenic organisms such as Gram-positive and negative, yeast and fungal strains with an increasing percentage of oil used.

Abou Ayana and Gamal El Deen *et al*.[28] studied the effect of adding 0.3% cinnamon oil to laboratory-made yogurt, which led to an increase in its shelf life of up to 24 days at a temperature of 6°C and gave it an appropriate taste and flavor, free from any microbial spoilage. The results obtained indicate that essential oils have a stimulating effect on lactic acid bacteria by promoting their growth and acid production and this may be due to the high nutritional composition of the oil such as essential fatty acids and vitamins.

Mehdizadeh *et al*.[29] explained that during the laboratory enriched with dill at a concentration of 100 ppm at 5°C for 21 days, the maximum degree of sensory indicators such as color and texture was obtained for the experimental sample without any indicators of damage microorganisms and with acceptable numbers of organisms. However, it found that when using dill extract to manufacture yogurt, showed that the percentage of inhibitory activity and the amount of total phenolic compounds increased with the increase in the amount of extract added to the product.

Aziz and Karboune[30] have confirmed that concerns regarding the safety and quality of food products have prompted food manufacturers to search for natural alternatives and move away from artificial additives, especially essential oils. This is because they contain phenolic compounds, which are primarily responsible for antimicrobial properties as well as antioxidants, as they remove free radicals and prevent the formation

of new ones, thus prolonging the shelf life of the products. Mohammed *et al*.[31] showed that *N. sativa* oil is characterized by a high level of phenolic compounds, as these compounds possess strong activities, to which most of the inhibitory effects on harmful microbes are attributed.

Bettaieb *et al*.[32] found that thymol causes disruption of cell membranes and interacts with membrane proteins and intracellular components, leading to the release of K^+ and ATP, causing a change in permeability. The results from studies on the effect of fortification of foods by adding different concentrations of essential oils and varying the diversity of microorganisms, ranging from partial inhibition to complete inhibition.

Tizghadam *et al*.[33] showed that the essential oils of aloe vera and thyme plants contain 60-74 and 45% carvacrol, respectively, as a main component, as it inhibits a wide range of positive bacteria through interaction with bacterial membranes and changing the permeability. As for negative bacteria, it works on disruption of the outer membrane, release of lipopolysaccharide and enhanced permeability from the cytoplasmic membrane to ATP. The results of Ehsani *et al*.[34] showed that cheese samples fortified with black seed oil (1%) obtained higher scores than control cheese in all the sensory properties studied (flavor, texture, smell, color and general acceptance) during 45 days of storage. It has been shown that the components of the essential oil used are more effective because they have antimicrobial activity to prevent the growth of harmful microbes and thus increase the acceptability and quality of the product.

Adris *et al*.[35] and EL-Kholy *et al*.[36] have indicated that thyme oil extract gave the maximum antioxidant activity at a concentration of (300-400 ppm). The study attributed the increase in this activity to the presence of phenolic compounds, mainly thymol and their ability to remove free radicals and inhibit fat oxidation, as their inhibition is of utmost importance for products, as well as the removal of peroxyl radicals and reactive oxygen species, including pyroxene hydroxyl radicals.

Al-Rimawi *et al*.[37] have found through the results obtained that adding the essential oils of cinnamon and eucalyptus plants at a rate of 600 µL/kg can increase the shelf life of the brick up to 6 weeks at a temperature of 5±1°C with acceptable taste, flavor and texture. It led to a decrease in the numbers of bacteria, yeasts and molds due to the presence of various compounds such as polyphenolics and monoterpenes that affect the growth of disease-causing microorganisms, especially gram-positive ones. Ye *et al*.[38] have confirmed that the most important factor and the main determinant of choosing yogurt by consumers is flavor, especially acceptance and preference, especially acetaldehyde. It imparts a wonderful taste and is an attractive nutritional component that is considered the most important in yogurt.

CONCLUSION

The study concludes that the maximum score for the sensory indicators for the yogurt sample to which thyme oil was added is due to the effect of the oil on the growth and activity of bacteria, which may allow for maintaining the desired flavor in this research. Developing methods for extracting it in an ideal and environmentally friendly manner is of great importance for evaluating natural sources in an appropriate manner and their impact on human health. It must be taken into consideration as it represents a new approach and an effective impact on its development, in addition to improving its nutritional and therapeutic value. Therefore, this type can enrich a variety of dairy products. The market also represents a healthier alternative for the consumer.

SIGNIFICANCE STATEMENT

The yogurt sample with thyme oil was superior compared to other samples in terms of sensory properties, which included color, flavor, texture and bitterness during the different storage periods. The results showed that there were significant differences in the average values of the studied sensory characteristics

between the samples, especially the flavor characteristic of the samples by adding oils compared to the control sample as a result of its richness in biologically active compounds present in its oils. The study explained that the presence of this extract improves the sensory properties of the product through the development of flavor compounds represented by acetaldehyde, acetoin and diacetyl, during different storage periods. In addition, the development of acidity is due to the increase and activity of lactic acid bacteria and their enzymes that decompose fat and protein into short-chain peptides that contribute to maintaining the preservation of the product flavor.

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