International Journal of
Dairy Science

ISSN 1811-9743

Academic
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www.academicjournals.com

OPEN ACCESS International Journal of Dairy Science

ISSN 1811-9743
DOI: 10.3923/ijds.2021.48.57

Research Article
Acidophilus Labneh Milk Flavored with *Thymus vulgaris* and *Nigella sativa*: A New Functional Dairy Product

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Abstract

Background and Objective: There is growing interest in natural ingredients of spices as *Thymus vulgaris* (*T. vulgaris*) and *Nigella sativa* (*N. sativa*) that can be used as flavors, natural antimicrobial agents and preservative in food and dairy industry. The study aimed to prepare a new functional dairy product that contained *T. vulgaris* and *N. sativa* as oils and *Lactobacillus acidophilus* as probiotic bacteria. **Materials and Methods:** Oils of *T. vulgaris* and *N. sativa* were chemically analyzed by (Gas chromatography) GC system. The antimicrobial activity of *T. vulgaris* and *N. sativa* oils bases in single and in combination was evaluated against different foodborne microorganisms and tested for minimal inhibitory concentrations (MICs). *T. vulgaris* and *N. sativa* were incorporated (in single 25 µg each or mix 12.5 µg each) in preparing acidophilus Labneh milk. **Results:** The results revealed that 98.83% of *T. vulgaris* was identified as the major compounds of the oil. Meanwhile, *N. sativa* was rich in polyunsaturated fatty acids where un-saturation was predominant rather than saturation. *T. vulgaris* and *N. sativa* showed antimicrobial activity against some pathogenic bacteria, mold and yeast. The incorporation of these oils in acidophilus Labneh milk extended its shelf life to 30 days at 4°C with good chemical, microbiological and sensory qualities. **Conclusion:** Antimicrobial activities, flavoring properties and fatty acids constituents of *Thymus vulgaris* and *Nigella sativa* raised the potentials of the prepared acidophilus Labneh milk as a new functional dairy product, with enhanced flavor and longer shelf- life stability.

Key words: *Thymus vulgaris*, *Nigella sativa*, antimicrobial activity, fatty acids, shelf- life, acidophilus Labneh milk, lactose-intolerant

Citation: Abdelhamid, S.M., B.A. Alnashi, H.Z. Hassouna and R.K. El Dairouty, 2021. Acidophilus labneh milk flavored with *Thymus vulgaris* and *Nigella sativa*: A new functional dairy product. Int. J. Dairy Sci., 16: 48-57.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plant-derived Essential Oils (EOs) are natural antimicrobials found in many plants and could be used as antimicrobials in food systems[1-3]. For the composition of thyme essential oils, previous studies showed that it contained complex mixtures of organic substances that have different functional groups, mainly terpenoids, thymol and p-cymene, phenolic compounds such as p-cymene, thymoquinone and carvacrol, which are known to have antiseptic, antiviral and antimicrobial activities products[4,5] which are also associated with medicinal and functional values and several food products[6-8]. The N. sativa seed contain 26-34% fixed oil of which the major fatty acids are linoleic acid (64.6%) and palmitic acid (20.4%)[9], where, Black cumin (*Nigella sativa* L.) seeds have been used for thousands of years as a spice and condiment to flavor a variety of foods, including milk products such as Mediterranean cheeses[10,11]. Several studies revealed the potential for the application of *N. sativa* and thyme in processing and preservation of food and dairy products[12,13]. Among the fermented dairy products, Labneh "Strained Yogurt or concentrated yogurt", is a well-known food appetizer in different parts of the world as it has a relatively thick consistency between the conventional yogurt and cheese, with total solid content of around 23.0-25.0%[14]. Besides, Labneh has less lactose than fresh yogurt due to the removal of some of this sugar during the straining process and could be appealing for lactose-intolerant consumers[15].

Therefore, in view of the great nutritional and health importance and benefits of both thyme and black cumin, previously mentioned, the study tended to use them in fermented milk product contain lactic acid bacteria as a functional dairy product. The study included the chemical analysis of fatty acids and evaluation of the microbial activity against pathogenic and spoilage microorganisms for each of thyme and black cumin, separately and in combinations, to produce "Acidophilus Labneh" fermented with yogurt culture and *Lactobacillus acidophilus*. Chemical, microbial and sensory evaluations were carried out for Labneh during 30 days at cold storage.

MATERIALS AND METHODS

Study area: The study was conducted in laboratories of Dairy and Food Science, Department of Nutrition, NRC, Egypt and Department of Food Science and Nutrition, Collage of Health Science, Kuwait, from January, 2018-March, 2019.

Materials

Essential oils source: *Nigella sativa* (Black seed) oil and *Thymus vulgaris* oil were obtained from squeezing and extracting natural oils unit, National Research Centre, Giza, Egypt.

Milk: Fresh buffalo's milk (6% fat and 16% TS) was obtained from the Faculty of Agriculture, Cairo University, Giza, Egypt.

Bacterial strains

Starter culture: *Lactobacillus delbrueckii* sub sp. *bulgaricus* and *Streptococcus thermophilus* (as yogurt starter culture), for Labneh preparation, were obtained from stock cultures of the Dairy Microbiology Laboratory of Egyptian National Research Center, Dokki, Egypt. While *Lactobacillus acidophilus* N4495 strain was obtained from Chr. Hansen Denmark.

Pathogenic strains: The tested pathogenic strains: *Bacillus cereus* (ATCC 33018), *Salmonella typhimurium* 9027, *Staphylococcus aureus* (ATCC 20231) were obtained from the stock cultures of the Agricultural Research Centre in Giza, Egypt. *E. coli* O157:H7 (ATCC 6933) and *Listeria monocytogens* V7 and *Yersinia enterocolitica subsp. enterocolitica* ATCC9610TM were obtained from Liofilchem S.r.l., Italy. *Helicobacter pylori* and *Klebsiella pneumonia* were obtained from the stock cultures of Microbiology Lab of Dairy Science, National Research Centre, Dokki, Egypt. *Saccharomyces cerevisiae* Y-2223 (provided by the Northern Regional Research Laboratory Illinois, USA), *Aspergillus niger* and *Penicillium roqueforti J5* (obtained from Department of Microbiology, Swedish University of Agricultural Sciences).

Methods

Chemical analysis of *T. vulgaris* and *N. sativa* oils: Chemical analysis of *T. vulgaris* and *N. sativa* oils was carried out using the GC analysis. Methyl esters of the fatty acids of the oils were prepared according to Luddy *et al.*[16]. The chromatographic analysis was performed by using a Perkin Elmer Auto System XL equipped with Flame Ionization Detector (FID). A fused silica capillary column ZB-5 (60mXo.32 mm i.d.) was used. The oven temperature was maintained initially at 150°C and programmed from 150-230°C at a rate of 3°C min^{-1} then programmed from 230-240°C at rate 1°C min^{-1}, held for 20 min. Helium was used as the carrier gas, at a flow rate of 1.0 mL min^{-1}. The injector and detector temperature were 230 and 250°C, respectively.

Antimicrobial activity of *Thymus vulgaris* and *Nigella sativa* oils: Before the incorporation of essential oil into the plain skimmed yogurt, *T. vulgaris* and *N. sativa* oils antimicrobial activity was estimated against some food pathogenic and spoilage organisms by the agar well diffusion assays according to Sabina *et al*.[17]. The test was conducted against: *Bacillus cereus*, *Salmonella typhimurium*, *staphylococcus aureus*, *E. coli* O157:H7, *Listeria monocytogenes* and *Yersinia enterocolitica* subsp. *Enterocolitica*, *Helicobacter pylori* and *Klebsiella pneumonia*, separately. Each organism was cultured in brain heart infusion broth and then diluted in sterile saline solution. The nutrient agar plates were surface inoculated with 0.1 mL of the diluted cultures. The wells (8 mm in diameter) were cut from the agar and inoculated with essential oil. The plates were kept for 1 h at room temperature to allow the diffusion into the medium a nd then incubated at 37°C for 18 hrs. After incubation, all plates were examined for any zone of growth inhibition around the wells. The experiment was repeated two times and the results (zone of inhibition, mm) were expressed as average values[18,19]. *Helicobacter pylori* were grown under anaerobic conditions, using the Anaerobic Jar (Oxoid, Ltd., Basingstoke, UK). In the case of *each of A. flavus, A. niger* and *Penicillium* spore suspension (10^6 spores mL^{-1}) were prepared and 100 µL of each was spread on potato dextrose agar (PDA) dishes. After absorption, the cork borer was used to bore. The dishes were incubated for 5 days at 25°C. Also *Saccharomyces* was spread on potato dextrose agar and incubated for 5 days at 25°C (PDA) dishes. Visible inhibition zone around bore was followed according to Freire *et al*.[20].

Minimal inhibitory concentrations (MICs): The Minimum Inhibitory Concentrations (MICs) of extracts of *N. sativa* and *Thymus vulgaris* oil were determined against pathogenic bacteria, mold and yeast strains following the micro-dilution and disk diffusion method, using 96-well micro-titer plates[21,22]. Minimal Inhibitory Concentrations (MICs) were calculated based on the degree of the growth (count) of each organism with oils and that was the lowest oil concentration that resulted in an 80% reduction in growth compared the oil-free growth control. A 100 µL (volume) of different concentrations was added to the wells and inhibition was measured, after incubation of the plates. Tween 80 (0.5%) as well as a mixture of Tween 80 (0.5%) and ethanol (1%) were assayed and considered as the negative controls, while also oils show no inhibition for any of the tested bacterial strains were recorded as negative results.

Manufacturing of acidophilus labneh flavored with thyme and black cumin: Acidophilus Labneh was made according to the method described by Ozer[23], Abd El-Sattar *et al*.[24] and Hatice and Cakmakc[25] with some modifications. Standard buffalo's milk 4% fat was used for the manufacture of Thyme and *N. sativa* Acidophilus Labneh. Buffalos milk 4% fat was heated 85°C for 20 min and then cooled to 42°C, mixed with 1.5% salt, Thymes and *N. sativa* (25 µg/100 mL) and inoculated with (3%, v/v) S*. thermophilus and Lactobacillus delbrueckii* spp. *bulgaricus (1:1) and* (1%) *Lactobacillus acidophilus,* that made three separate treatments. The mixtures (control and 3 treatments) were incubated at 40°C until uniform coagulation was obtained. The resulting acidophilus Labneh was divided into 4 batches as follow:

Control : S. *thermophilus* and *Lactobacillus delbrueckii* spp. *Bulgaricus* (1:1) and (1%) *Lactobacillus acidophilus*

T_1 : S. *thermophilus* and *Lactobacillus delbrueckii* spp. *Bulgaricus* (1:1) supplemented *with* (1%) *Lactobacillus acidophilus* and mixed well with thyme 25 µg/100 mL

T_2 : S. *thermophilus and Lactobacillus delbrueckii* spp. *bulgaricus* (1:1) supplemented with (1%) *Lactobacillus acidophilus* and mixed well with *N. sativa* 25 µg/100 mL

T_3 : S. *thermophilus* and *Lactobacillus delbrueckii* spp. *Bulgaricus* (1:1) supplemented with (1%) *Lactobacillus acidophilus* and mixed well with both of thyme 12.5 µg/100 mL and *N. sativa* 12.5 µg/100 mL

Then the 4 batches were transferred to cloth bags and hung at 7°C overnight in the refrigerator to allow whey drainage. The resulting coagulation scooped into plastic cups (250 mlL stored at 7°C and analyzed for microbiological and organoleptic properties when fresh and after 7, 15, 21 and 30 days of storage. The whole experiment was repeated in triplicates.

Microbiological analysis: The samples of Labneh were microbiologically examined for each treatment after 0, 7, 15, 21 and 30 days of cold storage. Twenty-five grams of Labneh samples were added aseptically to 225 mL of sterile solution (2% w/v) of sterile Buffered Peptone Water (BPW) and homogenized. *Lactobacillus acidophilus* was enumerated using MRS agar medium and plates were incubated anaerobically using anaerobic gas pack Jar at 37°C for 48 hrs

according to Collins[26], Gillil and Walker[27]. Total Aerobic Colony Count (TACC), using plate count agar (Oxoid), molds and yeast count, using acidified potato dextrose agar (Mu96, Himedia, Mumbai), were carried out in acidophilus Labneh samples during storage period according to FDA[28]. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus* enumerated using modified M17 and MRS agar and the plates were incubated at 37°C for 48 hrs according to Harrigan and McCance[29], El Kholy *et al.*[30], Shenawy *et al.*[31], Dave and shah[32].

Sensory evaluation of acidophilus labneh with thymes and black cumin: Fresh and cold stored (4-7°C) acidophilus Labneh samples were evaluated for their sensory properties according to the score card recommended by Keating and Randwhite[33]. Sensory evaluation was carried out by 10 panelists, researchers at the Department of Dairy, National Research Center, Dokki, Egypt. The score card includes, flavor (50 points), body and texture (40 points) and appearance (10 points).

RESULTS

Chemical composition of thyme essential oil: The compounds identified in thyme essential oil are presented in Table 1. GC analysis of the oil resulted in the identification of 23 constituents, accounting for 98.83% of the oil. The chemical analysis of the oil showed that P-Cymene (30.87%), Thymol (30.72%), Y-Terpinene (8.98%), Bornyl acetate (6.54%), β-Caryophyllene (3.56%), Linalool (2.23%) and Carvacrol (1.87%).

Fatty acid composition of the fixed oil of *Nigella sativa*: The fatty acid profile of *Nigella sativa* fixed oil is presented in Table 2. It is obvious from the results that linoleic acid, oleic acid and palmitic acid were major fatty acids amounting to 59.94, 19.07 and 12.19%, respectively. Stearic acid and arachidic acid were present in percentages of 3.14 and 2.77% of the fixed oil, respectively. Similarly, linolenic acid and lauric acid were also present with percentages of 1.54 and 1.17%, while myristic acid was in meager proportions of 0.18%. It is evident from the results that sum up of saturated and monounsaturated fatty acids were i.e., 19.45 and 19.07%, respectively, while polyunsaturated fatty acids were 61.48%.

***Thymus vulgaris* and *Nigella sativa* Antimicrobial Activity:** Results of antibacterial activity of the *T. vulgaris* essential oils (0.1%), shown in Table 3, reveal high antibacterial activity against *Yersinia enterocolitica, Klibsiella pneumonia, Salmonella typhimurium, Listeria monocytogenes* and *S. aureus,* in descending order, then *N. sativa* and oils in combination. Furthermore, the results in Table 3 indicated that *Bacillus cereus*, *E. coli* and *H. pylori* were less sensitive against the thyme and N. sativa oils compared with the other tested bacterial species. On the other hand, results of antifungal activity, in Table 4, clear that *T. vulgaris* and *N. sativa* oils showed higher antifungal activity in mixes than in single oils against *Penicillium roqueforti, Aspergillus flavus*, *Aspergillus niger* and *Saccharomyces cerevisiae*, in descending order.

Minimal inhibitory concentrations of *T. vulgaris* and *N. sativa* (MICs): Results of MICs as shown in Table 5, revealed

Table 1: Chemical composition of thyme essential oil

Compounds	(%) RP*	Compounds	(%) RP*	Compounds	(%) RP*
α-Thujene	1.27	1,8-Cineol	0.68	Linalyl acetate	0.67
α-Pinene	1.22	Y-Terpinene	8.98	Bornyl acetate	6.54
Camphene	0.91	Cis-Sabinene	0.31	Thymol	30.72
1-Octen-3-ol	1.39	Linalool	2.23	Carvacrol	1.87
Mycrene	1.67	Camphor	0.48	B-Caryophyllene	3. 56
α-Terpinene	1.41	Terpinen-4-ol	1.24	ð-Cadinene	0.30
p-Cymene	30.87	Cis-Dihydrocarvone	0.95	Elemol	0.29
Limonene	0.32	Thymol methyl ether	0.95		

*RP: Relative percentage

Table 2: Fatty acid composition of the fixed oil of *N. sativa*

Fatty acids	Relative (%)
Lauric acid (C12:0)	1.17
Myristic acid (C14:0)	0.18
Palmitic acid (C16:0)	12.19
Stearic acid (C18:0)	3.14
Oleic acid (C18:1)	19.07
Linoleic acid (C18:2)	59.94
Linolenic acid (C18:3)	1.54
Arachidic acid(C20:0)	2.77

Table : 3 Antimicrobial activity of *Thymus vulgaris* and *N. sativa* (in diameter of inhibition zone, mm)

Essential oil type	*H. pylori*	*E. coli* O157: H7	S. aureus	*Y. enterocolitica*	*B. cereus*	*Sal. typhimurium*	*K. pneumonia*	*L. monocytogene*
Thyme in single	10^{Be}	10.0^{Be}	22.0^{Ad}	55.0^{Aa}	2.3^{Af}	50.0^{Ab}	55.0^{Aa}	40.0^{Ac}
N. sativa in single	1^{Ce}	2.0^{Cd}	2.5^{Cf}	22.0^{Ca}	2.0^{Af}	11.0^{Cb}	8.0^{Cc}	11.0^{Cb}
Thyme + *N. sativa* in combination	11^{Af}	15.0^{Ae}	5.0^{B}	33.0^{Ba}	2.0^{Ag}	26.0^{Bb}	18.0^{Bc}	16.0^{d}

Means with the different capital (A, B, C) superscript letters within the same raw indicate significant ($p \leq 0.05$). While, means with the different small (a, b, c⋯.g) superscript letters within the same column are Significantly ($p \geq 0.05$)

Table 4: Antifungal activity of *Thyme* and *N. sativa:* Diameter of inhibition zone (mm)

Essential oil types	*Aspergillus niger*	*Aspergillus flavus*	*Penicillium roquiforte*	*Saccharomyces cerviciae*
Thyme in single	7.0^{Bc}	18.0^{Ab}	19.0^{Bb}	5.0^{Ad}
N. sativa in single	5.0^{Cb}	2.0^{Cc}	15.0^{Ca}	2.0^{Bc}
Thyme+*N. sativa* in combination	16.0^{Ab}	15.0^{Bb}	26.0^{Aa}	6.0^{Ac}

Means with the different capital (A, B⋯) superscript letters within the same raw indicate significant ($p \leq 0.05$). While, means with the different small (a, b, c) superscript letters within the same column are Significantly ($p \geq 0.05$)

Table 5: Minimal inhibitory concentrations of *T. vulgaris* and *N. sativa* (MICs)* oils against different food borne microorganisms (µg/100 mL)

Organism	*Bacillus cereus*	*S. aureus*	*Listeria monocytogenes*	*Klebsiella pneumonia*	*Yersinia enterocolitica*	*Salmonella typhimurium*	*E. coli* O157:H7	*A. niger*	*A. flavus*	*Penicillium roqueforti*	*Saccharomyces cerevisiae*
MIC of *T. vulgaris* µg/100 mL	25	25	25	10	10	25	10	10	10	20	25
MIC of *N. sativa* µg/100 mL	30	30	20	30	20	20	30	30	30	25	25

MICs: Lowest oil concentration resulted in an 80% reduction in the growth of organism compared with the oil-free growth control

Table 6: Total bacterial count, molds and yeasts counts (Log CFU g^{-1}) in plain and *T. vulgaris* and *N. sativa* flavored acidophilus Labneh during storage at 4°C

Labneh treatments	Storage time (days)	Total bacterial count	Mold and yeast
Control	Fresh	7.55±0.42	Nil
	7	7.75±0.22	Nil
	14	7.81±0.14	Nil
	21	7.62±0.16	1.6±0.29
	30	7.55±0.21	2.9±0.33
T_1 (theme oil)	Fresh	7.61±0.18	Nil
	7	7.50±0.09	Nil
	14	7.10±0.25	Nil
	21	6.95±0.14	Nil
	30	6.89±0.41	Nil
T_2 (black cumin oil)	Fresh	7.96±0.33	Nil
	7	7.32±0.42	Nil
	14	7.11±0.40	Nil
	21	6.71±0.36	Nil
	30	6.91±0.08	Nil
T_3 (mix oils)	Fresh	7.71±0.11	Nil
	7	7.5±0.09	Nil
	14	7.21±0.15	Nil
	21	7.11±0.29	Nil
	30	7.00±0.25	Nil

that MIC of thyme oil for *Yersinia enterocolitica, Klebsiella pneumonia, E. coli* O157:H7, *A. Niger* and *A. Flavus* was 10.0 µg/100 mL. While, MIC for *S. aureus, Salmonella typhimurium, Bacillus cereus, Listeria-monocytogenes* and *Saccharomyces cerevisiae* was 25 µg/100 mL and MIC for *Penicillium roqueforti* was 20 µg/100 mL. On the other hand, MIC of *N. sativa* oil for *Yersinia enterocolitica, Salmonella typhimurium and Listeria monocytogens* was 20 µg/100 mL. MIC for *Klebsiella pneumonia, S. aureus,* Bacillus cereus, E. coli O157:H7, A. Niger and A. Flavus was 30.0 µg/100 mL. Meanwhile, MIC for *Penicillium roqueforti* and *Saccharomyces cerevisiae* was 25 µg/100 mL.

Microbiological analysis of acidophilus labneh flavored with thyme and *N. Sativa*: Results in Table 6 indicated a very slight decrease in TPC in the acidophilus Labneh samples, especially those not treated with oils (control), then samples that treated with an oily mixture and then came the other treatments which showed ~1 log cycle decrease after the thirty days of cold preservation. Meanwhile, results did not show any growth of the fungus and yeast in all oily treatments except in the non-oil treated samples (control) as mold appeared after 21 days. On the other hand, yoghurt starter culture bacteria, *S. thermophilus* and *L. bulgaricus,* were slightly decreased in control (<1 log cycle) than in the oily treatments (>1-1.5 log

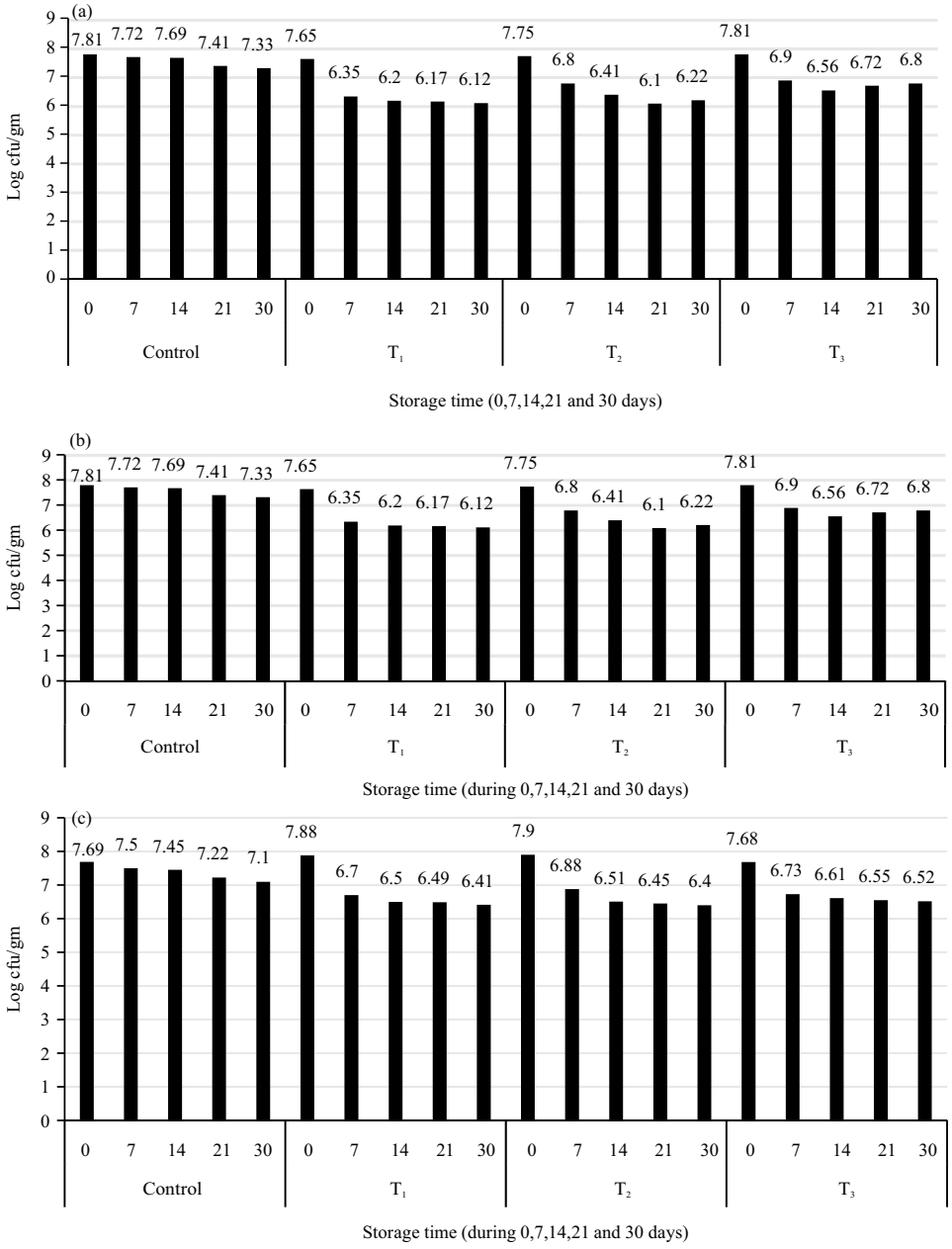

Fig. 1(a-c): Viability count of *L. acidophilus* as probiotic, (b) *S. thermophylus* starter and (c) *L. bulgaricus* the starter culture in plain (control) and treatments of flavored labneh at during storage

cycle), where T_3 (12.5 µL/100 mL of each of the mixed oils) was better than others T_1 and T_2 (25 µL/100 mL of each oil) as shown in Fig. 1a-c. A similar trend was shown by the probiotic bacteria, *L. acidophilus* as the counts decreased very slightly, particularly, in the control (<0.5 log cycle) than in the oily treatments (>1-1.5 log cycle), where T_3 was better than others, T_1 and T_2 as shown in Fig. 1c.

Sensory evaluation of the acidophilus labneh flavored with thyme (TV) and *N. sativa* (NS): Data given in Table 7 showed that TV and NS mix oils (T_3) flavored Labneh was tastier and highly preferred compared with the non-oily Labneh as control, the only theme (T_1) and the only N. sativa (T_2) oily Labneh even after 30 days of storage at 4°C. Also, the concentrations of TV or NS oils (25 µg/100 mL for each oil, separately or 12.5 µg /100 mL of each in the mix) played a big role in determining the sensory quality of the Labneh. Besides, results indicated the longer the shelf life of the oily Labneh as the non-oily samples were unaccepted mainly for flavor after 21 days.

Table 7: Sensory evaluation of plain and flavored acidophilus labneh with different treatments of *Thymus vulgaris* and Black *N. sativa* oils

	Days																					
	1				7				15				21				30					
Sensory	Body texture (40)	Flavor (50)	Appearance (10)	Total	Body texture (40)	Flavor (50)	Appearance (10)	Total	Body texture (40)	Flavor (50)	Appearance (10)	Total	Body texture (40)	Flavor (50)	Appearance (10)	Total	Body texture (40)	Flavor (50)	Appearance (10)	Total		
Control	37^{A}	45	9	91	37^{A}	41	8	86	33^{A}	34	7	74	30	SU*	7	SU	30	SU	7	SU		
T_1	37^{A}	40	9	86	37^{A}	38	9	84	37^{A}	37	9	83	37^{A}	37	9	83	37^{A}	33	9	79		
T_2	37^{A}	40	9	86	37^{A}	40	9	86	37^{A}	40	9	86	37^{A}	40	9	86	37^{A}	40	9	86		
T_3	38^{A}	47^{A}	9^{A}	94	38^{A}	47^{A}	9^{A}	94	38^{A}	47^{A}	9^{A}	94	38^{A}	47^{A}	9^{A}	94	38^{A}	44^{A}	9^{A}	91		

SU* : Off sour flavor unacceptable, Control : Without oils, T_1 : Theme, T_2 : Black cumin and T_3 : Mix oils flavored acidophilus lebneh

DISCUSSION

The compounds identified in thyme essential oil were identified as 23 constituents, accounting 98.83% of the oil. The chemical analysis of the oil showed that P-Cymene , Thymol, Y-Terpinene, Bornyl acetate, β-Caryophyllene, Linalool and Carvacrol and present at the highest fractions as these findings are in agreement with Eqbal and Aminah[34] Sartoratto *et al.*[35]. Also linoleic acid, oleic acid and palmitic acid were major fatty acids than Stearic acid and arachidic acid while polyunsaturated fatty acids were 61.48%. This finding came in accordance with reports published by Mamun and Absarr[9].

The high antibacterial activity of the thyme essential oils against *Yersinia enterocolitica, Klebsiella pneumonia, Salmonella typhimurium, Listeria monocytogenes* and *S. aureus,* this is in accordance with Sartoratto *et al.*[35], Roy *et al.*[36], Teixeira *et al.*[37] and Valizadeh *et al.*[38]. The high antimicrobial activity of thyme essential oil originates from the high content of thymol (64%), gamma-terpinene (9%), para-cymene (6%)] and carvacrol (5%)[39,40]. The antimicrobial action of *N. sativa* was originating from the phenolic, thymoquinone and melanin compounds which are considered as powerful active compounds[41-43]. *Bacillus cereus, E. coli* and *H. pylori* were less sensitive against the thyme and *N. sativa* oils compared with the other tested bacterial species and these verifications may be attributed to oil extraction methods, amount of ingredients plant area and season of collection[44,45].

Thymes and *N. sativa* oils showed higher antifungal activity in mixes than in single oils. This finding is interesting because the growth of these surface organisms on the fermented dairy products leads to a rapid deterioration in appearance, taste and general quality of the product and the use of these oils limits this phenomenon. At the same time results revealed that the antifungal activity of thyme and *N. sativa* essential oil showed weaker activity than their antibacterial activities at the same concentration 0.1%. In this respect several types of research indicated high antifungal activity of thyme essential oil against different fungal species and inhibited Aflatoxins (AFs) production[46,47].

MIC of thyme required to prevents visible growth of *Yersinia enterocolitica, Klebsiella pneumonia, E. coli O157:H7, A. Niger* and *A. Flavus* were less than that required for *S. aureus, Salmonella typhimurium, Bacillus cereus, Listeria monocytogens* and *Saccharomyces cerevisiae.* Studies by Maksimov[48], Bussmann *et al.*[49] and Lambert *et al.*[50] evaluated the Minimum Inhibitory Concentration (MIC) of different plant species for their antibacterial properties against Gram-positive

and Gram-negative bacteria. Whereas, Kokoska *et al.*[22] reported that Thymoquinone *N. sativa* constituent exhibited potent growth-inhibiting activity against gram-positive bacteria, with MICs ranging from 8-64 μg mL^{-1}. On the other hand, results for MIC obtained by Darah, *et al.*[51] revealed that methanol extract of Wedelia chinensis leaves against similar Gram-positive and Gram-negative pathogenic bacteria were 3.12-6.25 and 25 μg mL^{-1}, respectively.

The mold and yeast in all oily treatments showed no growth except in the non-oil treated samples (control) as mold appeared after 21 days. The findings indicated that adding thyme or *N. sativa* oils did not significantly affect the total bacterial count, while they remarkably affected mold and yeast growth, in Labneh during storage. These results were following Abd El-Sattar *et al.*[24], while results were inconsistent with Rahim *et al.*[52] for black pepper. Yogurt starter culture bacteria, *S. Thermophilus*, *L. bulgaricus* and probiotic *L. acidophilus* were slightly decreased in control than in the oily treatments where T_3 (mixed oils) was better than others T_1 and T_2. The behavior of the growth of each of *S. thermophilus*, *L. bulgaricus* and *L. acidophilus* as well, was similar to that found by Abd El-Sattar *et al.*[24] and Moritz *et al.*[53]. However, this advantage may turn into liability if that antimicrobial action extends to inhibit the activity of the starter bacteria (*S. thermophilus* and *L. bulgaricus*) and probiotic bacteria (*L. acidophilus*) that fortunately did not appear. These organisms should remain active even after the Labneh or probiotic dairy product have been manufactured and marketed for as long as possible, until the expiration date, in accordance with Schrezenmeir and Michael[54], Ezema[55], Hatice and Cakmakc[25].

Mix oils (T_3) flavored Labneh was tastier and highly preferred compared with control, the only theme (T_1) and the only *N. sativa* (T_2) oily Labneh even after 30 days of storage at 4°C. Good sensory properties were recorded for many probiotics fermented dairy products as reviewed by Okda *et al.*[56] and Ebojie[57]. Moreover, adding plants or its oils, like thyme and *N. sativa*, may elongate the shelf life of the milk products and produce high-quality products with good sensory properties as recommended by Desalegn[58], Wael *et al.*[59], Asala *et al.*[60], Heba *et al.*[61] and Fouad *et al.*[62].

From this study at lab scale, it was possible to produce a new functional structure dairy product that contains thyme and *N. sativa* oils, in combination with yogurt starter culture and acidophilus bacteria that result in a valuable product with high nutritional, health and sensory properties. Therefore, the study recommends producing and applying this product at the industrial, nutritional and health levels for health and common good.

CONCLUSION

The current investigation indicated that using the flavoring property, phytochemical constituents, the antibacterial and antifungal potential of *Thymus vulgaris* and *N. sativa* oils, besides using probiotic bacteria, was a successful trial for preparing a good flavored functional fermented dairy product. Also, the present findings suggested that adding a mix of *Thymus vulgaris* and *N. sativa* oils into acidophilus probiotic yogurt "Acidophilus Labneh" formulation could improve the sensory quality and potential functionality of this new product.

SIGNIFICANT STATEMENT

This study explores the potential synergistic effect of using *T. vulgaris* and *N. Sativa* oils in combination with yogurt-acidophilus bacteria for preparing a new dairy product that could be beneficial in dairy field and people's health. This study will help the researcher to uncover the critical area of preparing functional dairy products that many researchers were trying to explore. Thus, a new theory on these plant extracts-microbe combinations and possibly other combinations, may be arrived.

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