Utilization of Cinnamon, Clove and Thyme Essential Oils as Antimicrobial and Bioactive Compounds in Kishk Manufacturing

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Abstract: Cinnamon, clove and thyme essential oils (EOS) were used to manufacture of Kishk product for owns preservation effect and biological & nutritive values. EOS-kishk was prepared by adding various concentrations of essential oils (100 and 200 mg/kg) to traditional kishk mixtures. Results showed that cinnamon, clove and thyme essential oils were having 11, 32 and 18 identified compounds by using GC-MS, respectively. EOS-kishk samples contained (20.44-24.74%) protein, (5.13-5.80%) ash and (4.64 to 6.57%) moisture. The titratable acidity (TA % as lactic acid) and pH in the kishk samples ranged from 1.72 to 2.12% and 4.92-5.12, respectively. The water holding capacity (WHC) of the EOS-kishk samples varied from 464.10 to 498.48 g/kg, which was significantly higher than those of the control sample. EOS-Kishk samples had the highest antioxidant activity values (40.69%) at 200 mg/kg thyme oil compared with the control ones (31.54%). Mineral amounts of EOS-kishk had a slight change among all treatments. Microbiologically, the total viable bacterial count in most samples was slightly decreased as well as Lactobacilli, Streptococci and spore forming bacteria counts when compared with control. Whilst, there were notably decrease on coliform bacteria as well as yeast and mold counts in the EOS-kishk samples when compared with control. Overall, EOS-kishk has highly nutritional and biological value.

***Keywords*:** Kishk, essential oils, bioactive components, functional properties, antioxidant activity, microbiological quality

INTRODUCTION

Traditional kishk is one of the common foods in Upper Egypt. It is a healthy, natural and has a great flavor and cultural values that are increasingly attractive to the Egyptian. The main ingredients of Kishk are Zeer-milk with boiled, dried and crushed whole wheat grains (Bulgur). It is rich in nutritive constituents and source for many vitamins, growth factors and other nutrients (Anon, 2007). Fermentation may be increased the nutritional value of dairy-cereal blends by decreasing the content of antinutritional factors, such as phytic acid, thereby increasing inorganic bioavailability (Poutanen *et al.,* 2009); also, by increasing the content of bioactive components (Rahmawati and Suntornsuk, 2016). The sensory quality, composition and nutritional properties of kishk can be varied by changing the starter culture, type of cereal and the ratio of dairy constituent used in the formula (Gadallahand Hassan, 2019; Demirci *et al*., 2019). Essential oils (EOS) are aromatic and important sources of new phytochemical agents that improve physicochemical, organoleptic properties, nutritional values, harmless for human, have shown positive health effects and shelf-life of food products (Sadrizadeh *et al.,* 2018). The formation of EOS varied highly and can be contained a mixture of a small terpenes up to highly complex blend (terpenes, esters, aromatic hydrocarbons, phenols and other natural substances), and their diverse properties are possibly concerning to their role in plant survival (Probst, 2012). EOS included bioactive compounds that allowed antioxidant and antimicrobial activity due to the existence of phytochemicals (Pozzatti *et al*., 2009). Although, in case of EOS are used to change aroma, they are not being regard as food added ingredients. The use of seasoning agents is allowed in agreement with good manufacturing practices (EU, 2008). Also, EOS is categorized as generally recognized as safe (GRAS) (Burt, 2004). Antimicrobial activity of EOS differed with type, composition of plant, concentration of EOS, the tested microorganism, processing and storage conditions (Marino *et al*., 2001; Ozogul *et al*., 2015). The cinnamon essential oil is a normal oily extracted from cinnamon and mostly made up of diverse active components such as cinnamic aldehyde, cinnamic acid and eugenol, where cinnamaldehyde is a main component of cinnamon essential oil (Xing *et al.,* 2014). Cinnamon essential oil and cinnamic aldehyde have been found to have excellent antibacterial activities against a numerous pathogenic microorganisms regarding food borne illness and most used as antioxidant and antimicrobial agents in preservation of foods (Xing *et al*., 2014; Chen *et al*., 2016; Zhang *et al*., 2016; Han *et al*., 2018; Kaliamurthi *et al.,* 2019). Clove essential oil (CLEO *Syzygium aromaticum* L.) is a normal oil and seasoning agent with excellent antioxidant and antimicrobial properties is an efficient, safe, colorant, natural preservative, and spice in various food products, CLEO has bioactive components such as caryophyllene, eugenol, triterpenes, and sesquiterpenes (Ramadan *et al.,* 2013; Aguilar-González *et al.,* 2015; Hasheminejad *et al*., 2019). Also, CLEO has various applications in food preservation, biomedical, pharmaceutical, sanitary, cosmetics industries and active packaging (Chen *et al.,* 2017). Thyme (*Thymus vulgaris* L.) essential oil displayed significant muscle relaxant, antimicrobial and antioxidant activity (Rota *et al*., 2008; Grigore *et al*., 2010). Thyme is usually used in production of beautifying products, perfumes, soaps and cosmetic industries (Schulz *et al*., 2003). When essential oil of thyme is used as a food additive, it

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should be categorized on the food package as a food additive (EU, 2008). The aim of this study was to evaluate the use of some essential oils for improving the quality, antimicrobial, antioxidant, nutritional status and health benefits of traditional kishk.

MATERIALS AND METHODS

Materials:

Dry Wheat grains (*Triticum* spp.) were purchased from local market (Sohag, Egypt). Zeer-milk was obtained from local rural houses (Sohag, Egypt). Commercial sodium chloride was obtained from El-Nasr Saline's Company (Alexandria, Egypt). EOS of cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*) and thyme (*Thymus vulgaris*) were purchase from Natural oil extraction unit, National Research Centre, Cairo, Egypt.

Manufacture of Kishk: Kishk samples were prepared according to Tamime *et al*. (1997) with some modifications as following:

Wheat was sorted from damaged grain, cleaned from extraneous and boiled for 60 min in hot water.

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Air-dried, (for 2-3 days under aseptic conditions), then cracked to small debris (Bulgur)

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Addition of Zeer-milk to wheat Bulgur (3:1 w/w) and blended-well

↓

Addition of salt (1.5%) and mixed well

↓

The mixture was divided into seven equal portions

↓

Addition of essential oils as follow:

Code of treatment	Type and concentration of EOS
T_0	Without (Control)
T_1	Cinnamon (100 mg/kg)
T_2	Cinnamon (200 mg/kg)
T_3	Clove (100 mg/kg)
T_4	Clove (200 mg/kg)
T_5	Thyme (100 mg/kg)
T_6	Thyme (200 mg/kg)

↓

Incubation at 42°C for 24 h

↓

Kishk dough formed into small balls, airy sun-dried for 3 days and stored at 6±2°C for analyses

Fig. (1): Scheme diagram for kishk manufacture

Methods of analyses:

Moisture content was determined according to IDF (1987), Titratable acidity (as lactic acid%) was carried out according to AOAC (2000). The pH values were measured using a pH meter (model 68 ESD 19713), USA. Ash was determined according to AOAC (2007). Total nitrogen (TN) contents was determined as described by IDF (1993). Potassium and sodium content estimated by Flame Photometer (BWB XP Flame Photometer Technologies, UK LTD) according to Page (1982). Phosphorus content was determined according to Tiessen and Moir (1993). Calcium content was estimated by Flame Photometer according to Villanueva *et al*. (2000). Water holding capacity (WHC) was carried out according to Shevade *et al*. (2019). The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay was carried out according to the method described by Lee *et al.* (2003).

Gas chromatography-MS (GC-MS):

The chemical composition of EOS was performed using Trace GC-TSQ Quantum mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 50°C, then increased by 5°C/min to 200°C hold for 2 min and increased to the final temperature 300°C by 30°C/min and hold for 2 min. The injector and MS transfer line temperatures were kept at 260, 260°C, respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 3 min and diluted samples of 1 µl were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source temperature was set at 200°C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database.

Microbiological analyses:

The total viable bacterial count was determined according to Marshal (1992). Lactobacilli count was estimated on the selective medium for lactobacilli (MRS) and streptococci count on M17 agar medium respectively IDF (1997). Coliform bacteria were enumerated according to IDF (1985). Moulds & Yeasts were enumerated according to FDA (2002). Spore forming bacterial count was determined according to APHA (1992).

Sensory evaluation:

Organoleptic evaluation of prepared Kishk samples was carried out according to the scheme of Clark *et al.* (2009), the samples were subjected to organoleptic analysis by sixteen well-trained members of the Food Science and Nutrition Department, Dairy Sciences Department (Fac. of Agric. Sohag Univ., Egypt). The evaluated sensory attributes were: Flavor (1-10 points), body and texture (1-5 points) and appearance and color (1-5 points).

Statistical Analysis:

Analysis of variance was performed on the data using the software program; JMP Student Edition for Windows and Macintosh (The User's Guide to Statistics with JMP Student Edition, SAS Institute, 2009).

RESULTS AND DISCUSSIONS

Chemical characteristics of cinnamon essential oil:

Cinnamon essential oil was fractionated and identified by GC/MS as shown in Table (1). From these data, it is apparent that there were 11 identified volatile compounds in cinnamon oil as a relative concentration varied from 0.97 to 52.90 %. Furthermore, the major constituent of cinnamon essential oil was cinnamaldehyde (52.90%) followed by Trans-13-Octadecenoic acid (23.99%), 9, 12-Octadecadienoic acid (5.91 %), α-copaene (4.45%) and N-Hexadecanoic acid (3.06%). These results are agreed with some previous studies but there were some differences in the content of each component, Jiang *et al*. (2020) found that the major component of cinnamon essential oil was cinnamaldehyde (67.25%). Jayawardena and Smith (2010), Şimşek *et al.* (2013); Jeon *et al.* (2017) they reported that, the cinnamaldehyde as major component of cinnamon oil extracted by different methods was 68.95, 80.0 and 88.2%. The identified components were commonly found in cinnamon essential oil, but with different levels depending on the genetic, differing chemo type, geographic origin, season, environmental factors, drying and extraction methods (Jiang *et al*., 2020).

Table (1): The identified compounds of cinnamon (*Cinnamomum zeylanicum*) essential oil

Peak No.	Compound name	Rt (min)	Rp(%)
1	Cyclohexene	6.06	2.73
2	α-copaene	12.43	4.45
3	Cinnamaldehyde	14.16	52.90
4	À-Muurolene	15.37	1.52
5	Naphthalene	15.88	1.75
6	N-Hexadecanoic acid	22.66	3.06
7	Trans-13-Octadecenoic acid	25.51	23.99
8	9,12- Octadecadienoic acid	25.68	5.91
9	Hexadecanoic acid, 3-[(trimethylsilyl)oxy] propyl ester	28.71	1.02
10	Hydrocinnamic acid	30.89	1.69
11	9-Octadecenoic acid	31.12	0.97

Chemical characteristics of clove essential oil:

Clove essential oil was fractionated and identified using GC/MS and the results are shown in Table (2). From these data it could be noticed that the identified representing compounds were 32. The level of these compounds varied between 0.07 and 78.22%. The major identified compound was Oxabicyclo [4.1.0]heptan-2-one, 3-methy7 (6-1-methylethyl) (78.22%) followed by Caryophyllene (7.25%), 2-Cyclohexen-1-one, 2-hydroxy-3-methyl-6-(1-methylethyl) (3.44%), Tau.-cadinol (1.69%), 1,6-cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl) (1.41%) and Octadien-3-ol, 2,4,4,7-tetramethyl 5,7- (1.35%). These are in agreement with Yu *et al*. (2017) reported that clove essential oil consisted of eugenol (44.88%) and caryophyllene (26.53%). Saricaoglu and Turhan (2019) clove essential oil contained cymol (26.29%), α-pinene (20.65%), eugenol (17.02%) and 3-carene (11.62%) were predominant compounds. These differences could be attributed to such factors as genetic, age and stage of maturity, drying method, weather conditions, soil composition, type of plant parts and extraction conditions (Noori *et al*., 2018).

Chemical characteristics of thyme essential oil:

Thyme essential oil was fractionated and identified using GC/MS and the results as shown in Table (3). It could be noticed that 18 compounds were identified. The identified compounds percentage ranged from 0.49 to 28.88% of total thyme essential oil. The major identified compound was 9-Octadecenoic acid, (E) (28.88%) followed by Phenol (18.46%), Isopropyl-5-methylcyclohexa2-l (14.22%), 9,12-Octadecadienoic acid (10.12%), Ethylidene-2,2-dimethyl3-butyn-2-ol (7.92%), N-Hexadecanoic acid (4.85%), Benzene (3.45%), Thymoquinone (2.04%), Nonacosatriynoic acid (1.86%) and Trimethyl-2-(butylthio) cycloheptatriene (1.34%) of total thyme essential oil. These components are responsible for biological activity of thyme essential oil, these results are in harmony with Alsaraf *et al*. (2020). Saricaoglu and Turhan (2019) found that the main compounds in thyme essential oil were cymol (25.07%), α-pinene (17.0%), acetophenone (12.52%) and γ-terpinene (12.21%). Oliveiraa *et al*. (2020) reported that 28 compounds were identified in thyme essential oil, with thymol (50.5%), p-Cymene (19.4%) and γ-Terpinene (9.1%) as the most abundant components. The differences in chemical composition of essential oil are depended on various factors including time of collection, environment and cultivation methods, drying method and part of plant for distillation (Hudaib and Aburjai, 2007).

Table (2): The identified compounds of clove (*Syzygium aromaticum*) essential oil

Peak No.	Compound name	Rt (min)	Rp(%)
1	Bicyclo [3.1.1] heptane, 6,6-dimethyl-2-methylene	4.06	0.45
2	3-Octanol, acetate	7.30	0.32
3	2-Hexenoic acid, 3,4,4 trimethyl-5-oxo	11.67	1.27
4	5,7Octadien-3-ol, 2,4,4,7-tetramethyl	11.74	1.35
5	Oxabicyclo[4.1.0]heptan-2-7one, 3-methyl-(6-1-methylethyl)	12.69	78.22
6	2-Cyclohexen-1-one, 2-hydroxy-3-methyl-6-(1-methylethyl)	13.15	3.44
7	Isopropyl-7-methyl-nona-3,5-7diene-2,8-dione	13.38	0.11
8	Caryophyllene	13.82	7.25
9	Á-Famesene	14.03	0.31
10	Cycloundecatriene-1,4,8, 2,6,6,9-tetramethyl	14.43	0.84
11	5-allyl-2-methoxyphenol	14.59	0.09
12	1,6-cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)	14.91	1.41
13	Isoledene	15.16	0.10
14	Naphthalene	15.51	0.46
15	Carotol	15.60	0.24
16	5,5-Dimethyl-6-(3-methyl-buta-1,3-dienyl)-7-oxa-bicyclo [4.1.0] hept-1-yl]-methanol	15.84	0.12
17	(2E,4S,7E)-4-Isopropyl-1,7-dimethylcyclodeca-2,7-dienol	16.73	0.20
18	Caryophyllene oxide	17.23	0.30
19	Epicubenol	17.48	0.29
20	Tau.-cadinol	18.05	1.69
21	Caryophylla-4(12),8(13)-dien-5à-ol	18.23	0.14
22	Methoxyimidazo[1,5-5a]quinoline	18.29	0.11
23	À-Cadinol	18.35	0.07
24	Ledene oxide	19.04	0.08
25	2-Pentadecanone, 6,10,14-trimethyl	19.42	0.11
26	Phytol	22.82	0.14
27	1-naphthalenepropanol	23.34	0.12
28	Benzoic acid, 2-[4-(1,1-dimethylethyl) phenoxy]	23.47	0.27
29	1,3- cyclopentadiene butanenitrile	24.64	0.18
30	9-octadecenamide	27.39	0.07
31	Pentatriacontane	27.63	0.10
32	Hexatriacontane	29.45	0.15

Table (3): The identified compounds of thyme (*Thymus vulgaris*) essential oil

Peak No.	Compound Name	Rt (min)	Rp(%)
1	Cyclohexanol	6.07	1.30
2	Benzene	6.58	3.45
3	Isopropyl-5-methylcyclohexa 2-1	9.58	14.22
4	Thymoquinone	12.50	2.04
5	Ethylidene-2,2-dimethyl3-butyn-2-ol	13.03	7.92
6	Phenol	13.29	18.46
7	Trimethyl-2- 2,5,5 (butylthio) cycloheptatriene	14.09	1.34
8	N-Hexadecanoic acid	22.68	4.85
9	10-Octadecenoic acid, methyl ester	24.28	0.49
10	Linoleic acid ethyl ester	24.50	0.56
11	9-Octadecenoic acid, (E)	25.55	28.88
12	9,12-Octadecadienoic acid	25.72	10.12
13	Hexadecanoic acid, 3-[(trimethylsilyl)oxy] propyl ester	28.72	1.17
14	Nonacosatriynoic acid	30.89	1.86
15	2-Oleoylglycerol, 2TMS derivative	31.12	1.31
16	Isochiapin b	31.65	0.42
17	Trimethyl-3-(3,8,12,16-2,2,4 tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	32.34	0.94
18	Alpha-tocopherol (vitamin e)	36.83	0.68

Table (4): Major components in EOS used

Cinnamon		Clove		Thyme	
Commercial name	**Rp (%)**	**Commercial name**	**Rp (%)**	**Commercial name**	**Rp (%)**
Cinnamaldehyde	52.90	Diosphenol (Camphor)	78.22	Petroselic acid	28.88
Petroselic acid	23.99	Isocaryophyllene	7.25	Phenol	18.46
Linoelaidic acid	5.91	Ascaridol	3.44	Menthol	14.22
Isocaryophyllene	4.45	Cedrelanol	1.69	Linoelaidic acid	10.12
Hexadecanoic-d31 acid	3.06	beta-Caryophyllen	1.41	Benzylurea	7.92

Gross chemical composition EOS-kishk:

The chemical composition of kishk samples is present in Table (5). Protein content ranged from 20.44 to 24.74%. Kishk samples contained clove essential oil at 100 and 200 mg/kg showed highest protein content (24.74 and 23.80%, respectively) compared with the control sample T_0 (21.02%), these results are in agreed with Sadrizadeh *et al.* (2018), they found that EOS have phytochemical agents that improve physicochemical, organoleptic properties and nutritional values. The protein content of all kishk samples were higher than in previous studies and exceeds the requirements, outlined by the World Food Program (WFP) for wheat soy blend (WSB) which should contain a minimum of 16% protein (World Food Program, 2015). Ash content ranged from 5.13 to 5.80%, where T_0sample showed the highest value (5.80%). EOS-Kishk samples blend with various essential oils showed approximate levels of ash content. The moisture content of kishk samples decreased by the addition of essential oils compared to the control sample. The sample T_1 contained 100 mg cinnamon essential oil/kg had the lowest moisture content (4.81%) compared to the control sample (6.57%). Also, the moisture content of kishk samples decreased by increasing the concentration of essential oils added. Tamime *et al*. (1999) found that the moisture content in kishk samples ranged from 6.8 to 10.8%, and ash levels from 4.1 to 9.4%, thus, this minor variance in the moisture content is foremost linked to the composition of the ingredients used in kishk formulations and the drying technique (Erkan *et al*., 2006).

Table (5): Gross chemical composition of EOS-Kishk

Treatments of kishk	Protein % (dwb)*	Ash % (dwb)*	Moisture %
T_0 (Control)	21.02±0.646[bc]	5.80 ±0.043 [a]	6.57±0.252[a]
T_1(Cinnamon 100 mg/kg)	21.74± 0.700[b]	5.28 ±0.337 [c]	4.81±0.153[cd]
T_2 (Cinnamon 200 mg/kg)	21.40±0.00b[c]	5.13 ±0.038 [c]	4.64±0.562[d]
T_3 (Clove 100 mg/kg)	24.74±0.336[a]	5.74 ±0.006 [a]	5.84±0.510[b]
T_4 (Clove 200 mg/kg)	23.80±0.782[a]	5.40 ±0.331 [bc]	5.34±0.329[bc]
T_5 (Thyme 100 mg/kg)	21.34±1.176[bc]	5.68 ± 0.136 [ab]	5.01±0.239[cd]
T_6 (Thyme 200 mg/kg)	20.44±0.312[c]	5.69 ±0.056 [ab]	4.93±0.225[cd]
LSD	**1.1679**	**0.3297**	**0.6205**

*dwb: On dry weight basis
Means of triplicates
Means having the same letter within each column are not significantly different at *p*= 0.05

Physicochemical properties of EOS-kishk samples:

The titratable acidity (TA%) and pH:

The titratable acidity (% as lactic acid) and pH values of Kishk samples are presented in Table (6). The TA% in the kishk samples ranged from 1.72 to 2.12%. Kishk samples which contained cinnamon 100,200 mg/kg and clove 100 mg/kg, had significantly (P<0.05) higher than either samples. Furthermore, Kishk blended with thyme 200 mg/kg had the highest pH value (5.12) while, the lowest value (4.92) observed in the sample contained cinnamon 100 mg/kg. Thus, there was variable in the TA and pH values in Kishk samples. These results could be attributed to the differences of the major substance found in the essential oils. From these results, it can be concluded that the essential oils had a specified effect on the kishk fermentation; these results were in same line with other studies (Mahmoudi *et al*., 2014).

Water holding capacity (WHC):

The WHC is indicative of an increase in water absorption and swelling of the reconstituted powder (particles) during stirring and heating, and hence is likely to impact on the viscosity of the resultant soup/porridge (Wani *et al*., 2012). WHC for kishk samples were presented in Table (6). There were significant differences (P < 0.05) in WHC of kishk samples treated with essential oils and the control (T_0). The WHC in the kishk samples ranged from 464.10 to 498.48 pellet, g/kg. Also, WHC increased with essential oils concentration from 100 to 200 mg/Kg. Kishk samples contained clove essential oil at 200 mg/kg showed highest WHC (498.48 pellet, g/kg) compared with the control sample T_0 (464.10 pellet, g/kg). The WHC was also likely to be influenced by some factors, other than starch content, such as starch granule dimensions, relative proportions of amylose-to-amylopectin, and the presence of other components including lipids, proteins, salt, and acids, and their interactive effects (Kett *et al*., 2013; Kumar *et al*., 2018). The variable interactive effects may be occurred between the components of kishk mixture and the kind of additive EOS which affected the WHC values.

Antioxidant activity of EOS- kishk:

DPPH characteristics of kishk samples showed significant differences when using some types of essential oils (Table 6). Kishk samples blended with essential oils had the highest antioxidant activity values compared with control sample (T_0). The addition of thyme essential oil at 200 mg/kg to kishk (T_6) had the highest DPPH radical scavenging value (40.69%). The DPPH radical scavenging values of kishk samples increased by increasing the level of the added essential oils. These results could be attributed to the differences of phenolic compounds in essential oils. These results are in harmony with El-Den (2020), which found that probiotic kishk containing 0.3% olive leaves extract had the highest antioxidant value followed by that containing 0.2 and 0.1% olive extract, respectively. Kishk containing 0.3% moringa extract had DPPH radical scavenging values higher than those containing 0.1 and 0.2% moringa extract, respectively.

Table (6): Physicochemical properties of EOS-kishk samples

Treatments of kishk	Titratable acidity TA %	pH	WHC pellet, g/kg	DPPH %
T_0 (Control)	1.88 ±0.084 [b]	4.94 ± 0.015 [de]	464.10 ±1.408 [b]	31.54±0.343[e]
T_1 (Cinnamon 100 mg/kg)	2.07 ±0.030 [a]	4.92 ±0.020 [e]	492.20 ±3.992 [a]	32.42±0.175d
T_2 (Cinnamon 200 mg/kg)	2.09 ±0.030 [a]	5.00 ±0.010 [c]	495.16 ±3.011 [a]	32.76±0.066[d]
T_3 (Clove 100 mg/kg)	2.12 ±3.011 [a]	4.97 ±0.040 [cd]	484.52 ±14.94 [a]	30.69±0.286[f]
T_4 (Clove 200 mg/kg)	1.78 ±0.040 [c]	5.06 ±0.010 [b]	498.48 ± 8.630 [a]	35.39±0.238[c]
T_5 (Thyme 100 mg/kg)	1.79 ±0.050 [c]	5.04 ±0.015 [b]	486.70 ±17.81 [a]	38.06±0.686[b]
T_6 (Thyme 200 mg/kg)	1.72 ±0.00 [c]	5.12 ±0.010 [a]	494.76 ±5.334 [a]	40.69±0.114[a]
LSD	0.092	0.0347	17.137	0.5823

Means of triplicates
Means having the same letter within each column are not significantly different at $p = 0.05$

Minerals content of EOS-kishk samples:

The concentrations of Na, K, Ca and P of EOS-Kishk samples were shown in Table (7). It was clear that samples contained clove essential oil (100 and 200 mg/kg) had the highest content of Na (1945.17 and 1894.43 mg/100g) and K (542.78 and 544.50 mg/100g), respectively. Whereas, the sample contained clove essential oil at 100 mg/kg given high Ca content (547.42 mg/100g) compared with other Kishk samples. In addition, samples contain clove essential oils (100 mg/kg) had the highest amount of P (371.9 mg/100g) followed by (328.9 mg/100g) in the sample contained clove essential oil 200 mg/kg. While, T_5 sample contained thyme 100 mg/kg had the lowest amount of phosphorus (259.2 mg/kg). The levels of potassium and calcium were approximated in most of the experimental treatments. All Kishk samples in this study surpassed on the specifications of the WFP which recommends that fortified blended foods (Super cereal plus) should contain calcium at concentrations of 362 mg/100g (WFP, 2015).

Table (7): Minerals profile (mg/100g) of EOS-kishk samples

Treatments of kishk	Na	K	Ca	P
T_0 (Control)	1807.90±3.001 [c]	512.77 ± 6.444 [bc]	508.61 ±2.882 [bc]	300.4±1.90 [cd]
T_1 (Cinnamon 100 mg/kg)	1840.29±8.189 [bc]	530.32 ±1.926 [ab]	520.58 ±2.069 [b]	305.0±2.11 [c]
T_2 (Cinnamon 200 mg/kg)	1868.90 ±74.46 [b]	537.10 ±24.41 [a]	506.59 ±6.104 [c]	293.7±6.24 [d]
T_3 (Clove 100 mg/kg)	1945.17±19.56 [a]	542.78 ±4.869 [a]	547.42 ±9.510 [a]	371.9±13.48 [a]
T_4 (Clove 200 mg/kg)	1894.43±22.50 [ab]	544.50 ±9.330 [a]	513.56 ±12.26 [bc]	328.9±2.12 [b]
T_5 (Thyme 100 mg/kg)	1745.04±5.429 [d]	509.06 ±1.546 [c]	493.17 ±0.247 [d]	259.2±5.52 [e]
T_6 (Thyme 200 mg/kg)	1813.25±17.20 [c]	528.22 ±4.570 [ab]	508.49 ±10.10 [bc]	298.1±3.77 [cd]
LSD	**54.721**	**18.43**	**13.113**	**11.03**

Means of triplicates
Means having the same letter within each column are not significantly different at $p = 0.05$

Microbiological counts (log CFU/g) of EOS-kishk samples:

Table (8) showed that the total viable bacterial count (TVBC) of Kishk samples of all treatments were significantly ($P < 0.05$) compared with the control (T_0). The TVBC was decreased by increasing the levels of added essential oils. Kishk sample contained 100 mg thyme/kg (T_5) had the lowest total viable bacterial count. TVBC of different Kishk samples were ranged from 5.64 to 7.64 log CFU/g in the same line with Tamime *et al*. (2000) finding. The data in Table (8) showed that, all treatments of Kishk samples had lactic acid bacterial count lower than T_0, however, there were no significant differences ($p < 0.05$) among all EOS-kishk samples in their lactic acid bacterial count. These results are in agreement with Özcan and Ekinci (2013) and Abd El-Khalek *et al.* (2016), they found that the phenolic compounds positively affected the growth and metabolism of lactic acid bacteria. Lactic acid bacterial counts were ranged from 5.13 to 6.03 log CFU/g agree with Nurliyani *et al*. (2013).

Also, there were significant ($p < 0.05$) differences in *Streptococci* count between EOS-kishk treatments and the control (T_0). While, the *Streptococci* counts were decreased with increasing the addition of the studied essential oils. Thyme essential oil recorded limited ability to stimulate the growth of *Streptococci* depending on the added concentration. Kishk sample containing thyme 200 mg/kg (T_6) had the lowest numbers of *Streptococci*. The coliform bacterial counts of all kishk treatments were significantly ($P < 0.05$) lower than the control (Table 8) and ranged from 0.95to 2.60 log CFU/g. The coliform count was clearly significantly decrease ($p < 0.05$) by increasing the levels of essential oils. Kishk sample containing clove essential oil 200 mg/kg (T_4) had the lowest coliform counts. Data presented in Table (8) also showed that, yeasts and moulds were detected in all samples of tested products and their counts ranged from 1.74 to 2.67 log CFU/g. The results also showed close counts of yeasts and moulds in kishk samples.

The spore forming count of all EOS-kishk samples (Table 8) were significantly ($P < 0.05$) compared with the control (T_0). While, EOS-kishk samples supplemented with essential oils recorded variable decrease in spore forming bacterial counts depending on the concentration of the essential oils added. These results are in harmony with those of Tamime *et al*. (2000), Gadallah and Hassan (2019) and El-Den (2020).

Table (8): Microbiological counts (log CFU/g) of kishk samples

Treatments of kishk	Total bacterial count	Lactobacilli count	Streptococci count	Coliform bacteria	Yeasts and moulds	Spore forming bacteria
T_0 (Control)	7.64±0.459^{a}	6.03±0.133^{a}	4.08±0.103^{a}	2.60±0.48 ^{a}	2.34±0.359^{ab}	5.04±0.083^{a}
T_1 (Cinnamon 100 mg/kg)	6.22±0.200^{d}	5.50±0.320^{ab}	3.71±0.237^{b}	1.51±0.40 ^{bc}	2.42±0.252^{ab}	4.23±0.200^{bc}
T_2 (Cinnamon 200 mg/kg)	6.82±0.337^{bc}	5.21±0.582^{b}	3.15±0.055^{cd}	1.11±0.36 ^{c}	1.74±0.208^{b}	3.88±0.100^{d}
T_3 (Clove 100 mg/kg)	6.19±0.214^{d}	5.47±0.266^{ab}	3.52±0.321^{b}	1.50±0.69 ^{bc}	2.67±0.208^{a}	4.17±0.149^{c}
T_4 (Clove 200 mg/kg)	6.37±0.331^{cd}	5.30±0.575^{b}	3.02±0.081^{d}	0.95±0.47 ^{c}	1.74±0.551^{b}	3.61±0.100^{e}
T_5 (Thyme 100 mg/kg)	5.64±0.200^{e}	5.62±0.321^{ab}	3.50±0.320^{bc}	1.88± 0.07 ^{ab}	2.81±0.153^{a}	4.43±0.200^{b}
T_6 (Thyme 200 mg/kg)	6.94±0.170^{b}	5.13±0.086^{b}	2.85±0.100^{d}	1.06± 0.31 ^{c}	1.81±0.737^{b}	3.82±0.115^{de}
LSD	0.508	0.652	0.358	0.760	0.710	0.25

Means of triplicates
Means having the same letter within each column are not significantly different at $p = 0.05$

Sensory evaluation of kishk samples:

The flavor scores of different Kishk samples were ranged from 7.78 to 9.06 (Table 9). The lowest score of taste was given by Kishk samples (T_3) prepared from clove essential oil (100 mg/kg). The body and texture scores of different Kishk samples were ranged from 3.78 to 4.40. The lowest score of body and texture was given by Kishk samples (T_2) contained cinnamon essential oil (200 mg/kg). The appearance and color scores of different Kishk samples varied from 3.93 to 4.53. The Kishk sample contained cinnamon essential oil 200 mg/kg (T_2) recorded the lowest score of appearance and color. There were significant differences ($p<0.05$) in total scores between kishk from different treatments and the control except T_5 (Table 8). Kishk containing thyme oil (100 mg/kg) (T_5) gained the highest overall score followed by T_6 sample. Besides, Kishk sample containing cinnamon or clove essential oil (100 mg/kg) had more overall acceptability scores than that contained 200 mg/kg of the same EOS. Also, the highest score of all sensory evaluation given by control sample.

However, each essential oil has its own effect on organoleptic properties of final product, but scientific studies mainly reported that these EOS in specific concentration not only has positive effect on shelf-life of foods, but they can also improve organoleptic properties.

Table (9): Sensory evaluation of kishk samples

Treatments of kishk	Flavor	Body and Texture	Appearance and colour	Overall scores
T_0 (Control)	9.06±0.77[a]	4.40±0.61[a]	4.53± 0.62[a]	18.00±1.317[a]
T_1 (Cinnamon 100mg/kg)	8.28±1.09 [bcd]	4.00±0.41[bcd]	4.06±0.57[bc]	16.34±1.221[cd]
T_2 (Cinnamon 200mg/kg)	7.84±0.63[cd]	3.78±0.41 [d]	3.93±0.68[c]	15.56±1.352[d]
T_3 (Clove 100 mg/kg)	7.78±0.91 [d]	3.96±0.46[bcd]	4.25±0.58[abc]	16.00 ±1.252[cd]
T_4 (Clove 200 mg/kg)	7.81±0.75[cd]	3.90±0.27[cd]	4.09±0.46[bc]	15.81± 0.929[d]
T_5 (Thyme 100 mg/kg)	8.75±0.93[ab]	4.25±0.45[ab]	4.37±0.59[ab]	17.38±1.638[ab]
T_6 (Thyme 200 mg/kg)	8.37±0.72[bc]	4.18±0.66[abc]	4.31±0.68[abc]	16.88±1.489[bc]
LSD	**0.590**	**0.338**	**0.421**	**0.932**

Means of triplicates
Means having the same letter within each column are not significantly different at $p= 0.05$

CONCLUSION

The addition of essential oils clearly had lowering effect on coliform bacteria, spore forming bacteria, moulds and yeasts counts in the prepared Kishk product. Addition of essential oils to Kishk may be improved its health benefits, whereas, this product had high antioxidant activity, indicating that the product had potential health effect.

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إستخدام زيوت القرفة والقرنفل والزعتر كمواد مضادة للميكروبات ومركبات نشطة بيولوجياً في تصنيع الكشك

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تم استخدام الزيت الطيار لكل من القرفة والقرنفل والزعتر لتصنيع منتج الكشك نظراُ لتأثيرهم الحفظي وزيادة القيمة الغذائية للكشك. تم تحضير كشك الزيوت بإضافة تراكيز مختلفة من الزيوت الطيارة (١٠٠ و ٢٠٠ مجم/كجم) من كل زيت إلى خلطات الكشك التقليدية. ثم التعرف علي ١١ و ٣٢ و ١٨ مركبًا في زيوت القرفة، القرنفل والزعتر على التوالي باستخدام كروماتوجرافى الغاز المتصل بمطياف الكتلة. أظهرت نتائج تحليل عينات كشك الزيوت العطرية احتوائها على البروتين بين ٢٠.٤٤-٢٤.٧٤٪ ، ومحتوى الرماد من ٥.١٣ -٥.٨٠٪ بينما محتوى الرطوبة من ٤.٦٤ إلى ٦.٥٧٪. كما تراوحت الحموضة والأس الهيدروجيني في عينات الكشك من ١.٧٢ إلى ٢.١٢٪ و ٤.٩٢ - ٥.١٢ على الترتيب. كما تراوحت قيم القدرة على الاحتفاظ بالماء (WHC) لعينات الكشك عند طبخها في وسط مائي من ٤٦٤.١٠ إلى ٤٩٨.٤٨ جم/كجم وكان متوسط هذه القيم في عينات كشك الزيوت أعلى من قيم معاملة الكنترول. احتوت عينات الكشك الممزوجة بالزيوت العطرية على قيم أعلى في مضادة الأكسدة مقارنة بعينة الكنترول حيث زادت قيم مضادات الأكسدة من ٣١.٥٤٪ في العينة الكنترول إلى ٤٠.٦٩٪ في العينة المحتوية على ٢٠٠ مجم/كجم زيت الزعتر. يختلف المحتوى المعدني للكشك المحضر بالزيوت العطرية باختلاف تركيز الزيوت المضافة. من الناحية الميكروبيولوجية، في معظم العينات، انخفض العد الكلى البكتيري عند مقارنته بعينة الكنترول. كما انخفض عدد الـ *Lactobacilli* و الـ *Streptococci* والبكتريا المكونة للجراثيم بإضافة الزيوت إلى معاملات الكشك. وكان واضحاً أن هناك انخفاض ملحوظ في أعداد بكتريا القولون وكذلك الخمائر والفطريات في العينات المعاملة بالزيوت العطرية عند مقارنتها بعينة الكنترول.