Gas chromatography-mass spectrometry analysis of Olive Leaf (*Olea europaea L.)* Extracts and their effects as Natural Preservative for local cheese

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ABSTRACT

This study aimed to assessment of some microbiological and physicochemical properties of local cheese as the addition of olive leaf extract influenced them. The most abundant components in olive leaf based on gas chromatography-mass spectrometry analysis were methyl alpha-D-mannoside, tetradecanoic acid, oleic acid, 3 alpha-acetoxy-25-ydroxy-olean-12-en-28-oic acid, and carbamic acid. Powdered olive leaf extract was added to the cheese at concentrations of 10%, 20%, . The chemical and microbiological properties of the cheese were then determined at storage times of 0, 8, and 19 days at 4°C. The results showed that raising the percentage of olive leaf extract lowered the titratable acidity of the cheese. However, when the pH level rose, so did the few dried olive leaves added. Total plate counts in the cheese samples of the treated groups were lower ($p<0.05$) than in the untreated groups throughout the refrigerated storage. Olive leaves at the level of 20 % were more effective in inhibiting bacterial growth than 10 %. According to the findings of this study, extracts of olive leaves have the potential to be used to extend the shelf life of food products and can be used as a natural preservative.

Keywords: Gas -mass spectrometry analysis, Olive Leaf, Preservative, Cheese, Titratable acidity.

Introduction

Dairy products are susceptible to bacterial contamination: many sources of bacterial contamination have been documented in the dairy products, particularly in small farm production, including the usage of raw milk (Cardinali et al., 2017). The great danger of contamination by pathogenic and spoilage microbes is a significant issue that affects practically the whole food chain. Pathogenic and rotting bacteria may contaminate cheese. Using these preservatives is possible in cheese making. Consumers desire healthy food that is free of synthetic preservatives. An extensive study has been conducted on the use of aromatic herbs in food preservation, with their antibacterial properties well acknowledged (Hyldgaard etal., 2012). Plant polyphenols and their antioxidant capabilities are primarily responsible for the protective abilities of bioactive compounds (Dzotam et al., 2018), Furthermore, in the pharmaceutical, food, and cosmetics sectors, plant polyphenols are chosen over bioactive chemicals as food additives, preservatives, and nutritional supplements (Kim & Kim, (2007).

Antimicrobial activity of natural components has been shown, which may delay or prevent the development of bacterial pathogenic in the food (Ritota & Manzi, 2020). Olive leaves (Oleaeuropaea L.) are a low-cost raw resource that may be employed to

manufacture high-quality goods and are one of the most effective plants and leaves. Historically, ancient cultures employed olive leaves to treat a variety of illnesses. The natural components in this material may combat free radicals, inflammation, and heart disease. It possesses anti-cancer and antimicrobial effects.(Borjan et al., 2020). It is financially feasible as a result of these characteristics. It is widely available as a dietary supplement in Mediterranean nations. Phytochemicals and their antioxidant capabilities are primarily responsible for the protective properties of bioactive compounds (Cho, et al., 2005); furthermore, in the pharmaceutical, food, and cosmetics sectors, plant phytochemicals are chosen over synthetic compounds as food additives, preservatives, and nutritional supplements (Daljit, & Jasleen 1999). Olive leaf extract was shown to have several compounds that may increase the olive tree's resistance to insects and microbial damage (Pooley et al., 1997). Olive leaf extract also possesses antibacterial effects. As with many natural products, the extract's content may vary depending on geographical region, cultivar, and plant nutrition (Sudjana et, al.,2009). This research aimed to investigate the determination of the bioactive compounds of olive leaf and its extract on the chemical composition and antimicrobial properties of local cheese.

Methods and methods:

Preparing olive leave extraction OLE :

Olive leaves were collected from Nabali Baladi tree in Diwanihia city. At room temperature, the fresh leaves were dried. The powder was kept at room temperature and in the dark until extraction. Methanol extraction was performed according to (Cör Andrejč etal 2022) 400 gm; powdered olive leaves were macerated for 48 hours in 500 ml of (80% solvent). (At 40°C, adjust with 0.1 N hydrochloric acid) for separating and filtering the particles and evaporating in the tank at 40°C in an open atmosphere (classical technique) but under a vacuum (modern technique). Until used, the extracted OLE was kept at 4–6°C.

Preparing local white cheese sample:

The white cheese was made from raw milk provided from dairies in diwanyhia city, as described by Vergara (2018). Cow's milk was filtered and pasteurized for 15 seconds at 72°C before being cooled to 32°C. A quantity of the pasteurized milk was used to create control cheese groups without any additions or treatments. The same procedures were followed for the remaining portion. After finishing the coagulation process for all groups, including the control, olive leaf extract in two concentrations of 10 and 20 µl per 100 gm of cheese curd was added, and table salt NACL was added at a concentration of 1.5 %. Packaged and stored at 4 c^{o}. To assess the olive extract's microbiological activity during the study period, 10 g were used for total plate count number (TPC) and another for chemical analysis. The analysis was carried out in triplicate during Zero, nine, and eighteen days of storage

1- Methods used for analysis:

1.1- Chemical analyses

The properties (pH and titratable acidity) of the cheese (f C) were analyzed according to the standard procedures (AOAC, 1990). Cheese pH was determined by a special meter, while the titratable acidity was determined by mixing sample (9) grams with distilled water (9) grams titrated against 0.1 N NaOH (UnionChemical Works Ltd., Hsinchu, Taiwan), in the zero, 9 then storage for (18) days.

1.2- Microbiological analyzing:

Ten grams of the treated cheese sample were homogenized with distil water aseptically for 2.5 minutes, in peptone water (90) mL (0.1 %) at 4000 rpm. There were (10) fold dilutions made. The total plate count was tested by plate count agar after two days of incubation at 37 C.

1.3 - GC-MS analysis for OLE:

The active extract was analyzed to identify olive leaves' bioactive compounds using Shimadzu QP2010/Japan version 2010 gas chromatography/mass spectroscopy at the Iraqi Ministry of Science and Technology. A Gas Chromatograph was connected to a Mass

Spectrometer. Capillary column (Gl Sciences, Japan, InertCap 1MS, 0.25mm, 30m, 0.25m). Helium (99.999 %) carrier gas; constant flow rate 1 ml/min; Shimadzu AOC-20i auto-injector. 5 l injection volume Oven in the column. The temperature is 100°C. Oven temp software: 100 °C for (3) min; 240 °C for (9) minutes; 280 °C for (5) min; and 300 °C for (2) min. Various analyses were used to get percentages for all samples. Each chemical's relative peak area is expressed as a percentage (Table 1).

2- Result and discussion:

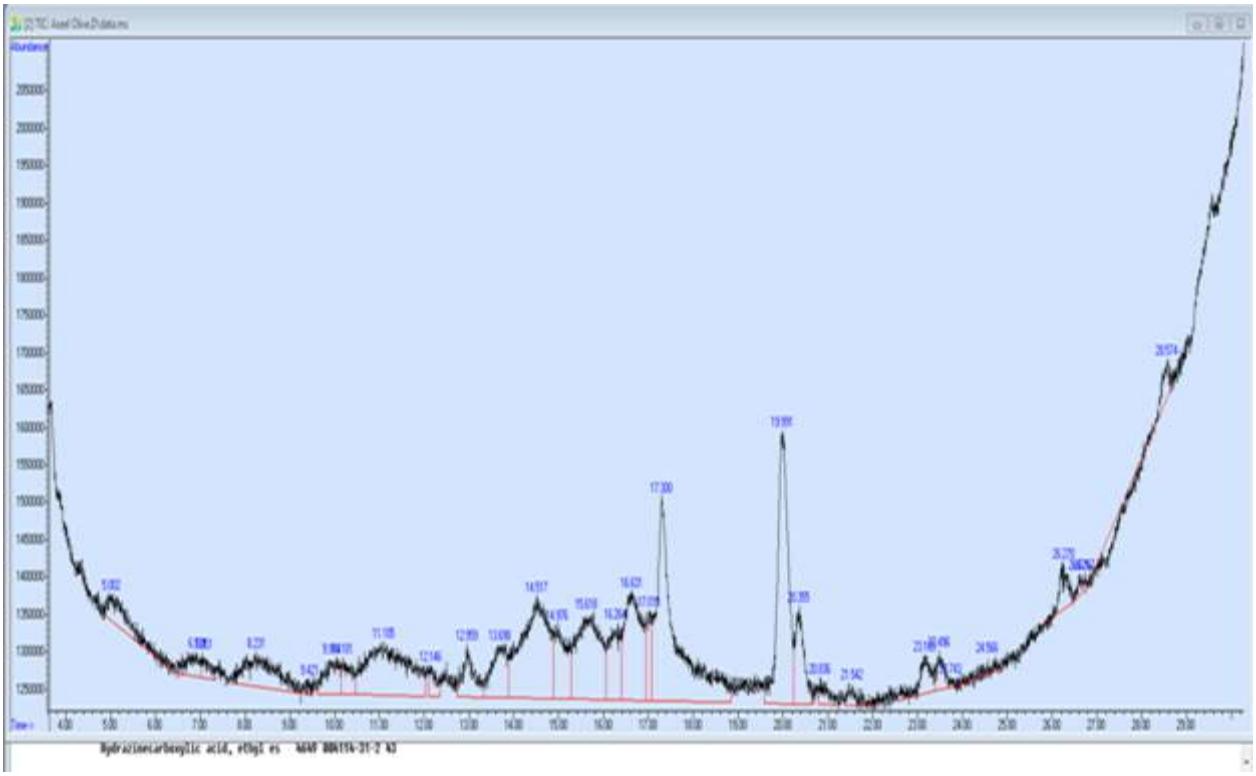

Figure (1): GC/MS spectra of the olive leaves metabolic extract

Table (1): GC-MS volatile organic compounds of Olive leaves

NO.	Compound	Rt	Area %	M.W.	Activity
1	Acetic acid	5.000	2.35	88.0621	Has antibacterial activity against Pseudomonas aeruginosa.
4	Ethylene glycol diglycidyl ether	8.229	4.01	174.1944	derived polysaccharides antimicrobial applications
6	pentanoic acid	9.915	1.98	116.16	Food additives a straight, saturated chain, alkyl carboxylic acid.
7	Ethanedioic acid, diethyl ester	10.182	1.27	146.14	
8	3alpha-acetoxy-25-ydroxy- olean-12-en-28-oic acid	11.104	8.64	456.7	Triterpenoid potent compound has many biological effects

10	Propanoic acid, 2-oxo-, methyl ester	12.959	2.23	161.16	
11	1,2-Hydrazinedicarboxylic acid,	13.695	3.27	120.06	
12	n-Amyl nitrite	14.519	10.97	117.15	A vasodilator medically to treat heart diseases
14	methyl alpha-D-mannoside	15.616	8.43	194.18	exhibited antibacterial and antifungal activities against some microorganisms
15	N-Acetylpropionamide	16.265	3.37%	115.13	
16	Carbamic acid, (cyanoacetyl)-,	16.630	7.62	156.1393	Antimicrobial activity.
17	Formylic acid	17.018	1.70	46.025	
18	Tetradecanoic acid	17.301	15.91	228.37	fatty acid occurring in most animal and vegetable fats
19	Oleic Acid	19.991	11.88	282.47	omega-9 fatty acid.
20	ETHYL THIOCYANATE	20.356	3.52	87.15	as antiproliferative agents against Trypanosoma cruzi,
21	ACRYLIC ACID	20.836	0.79	72.06	
23	butyl 2-oxoacetate	23.166	1.81	130.14	
26	oxalic acid ethyl ester chloride	24.568	0.47	153.54	
27	Octadecenyl aldehyde	26.270	1.40	266.5	9-Octadecenal is a fatty aldehyde.
30	cis-vaccenic acid	28.572	0.44	282.5	antibacterial activity

Table (2): Titratable acidity of Cheese (Mean± SE)

Treatment	**Storage day**		
	zero	**9**	**18**
Control group (without treat)	**0.106±0.05Aa**	**0.113±0.04Ab**	**0.137±0.05Aa**
Cheese treated with 10 %of extract	**0.94 ±0.02Ba**	**0.88±0.003Bb**	**0.76±0.04Bb**
Cheese treated with 20 %of extract	**0.84±0.02Ca**	**0.75±0.01 Bb**	**0.69±0.09Cc**
LSD(P<0.05)	**0.291**		

The capital letters are used for vertical compassion; the small letters are used for horizontal compassion. The different letters mean that there are significant differences at P<0.05

Table (3): PH active acidity of the cheese

Treatment	Storage day		
	zero	9	18
Control group (without treat)	5.79±0.02Aba	5.88±0.005Aa	6.48±0.144Aa
Cheese treated with 10 %of extract	5.85±0.01Aa	5.76±0.03Aa	5.83±0.02Aa
Cheese treated with 20 %of extract	5.66±0.06Ba	5.59±0.04Ba	5.51±0.02Aa
LSD(P<0.05)	0.169		

The capital letters are used for vertical compassion; the small letters are used for horizontal compassion. The different letters mean that there are significant differences at P<0.05

Table (4): the Total Plate Count

Treatment	Storage day		
	Zero	9	18
Control group (without treat)	$7.1\times10^3\pm11.5\times10^2$ Aa	$8.8\times10^5\pm3.78\times10^5$ Ab	$12.23\times10^5\pm5.48\times10^5$Ac
Cheese treated with 10 %of extract	$6.7\times10^3{}_{\pm}15.2\times10^2$ Aa	$7.43\times10^5\pm2.7\times10^5$ Bb	$6.2\times10^5\pm27.09\times10^5$ Bb
Cheese treated with 20 %of extract	$6.9\times10^3\pm52\times10^2$Aa	$6.86\times10^5\pm1.8\times10^5$ Bb	$5.3\times10^5\pm22.8\times10^5$Bb
LSD (P<0.05)	35.69×10^5		

The capital letters is used for vertical compassion; the small letters are used for horizontal compassion. The different letters mean that there are significant differences at P<0.05

Plant antibacterial qualities; have risen due to contemporary issues associated with the usage of chemical preservatives (Nelson et al., 2021). The olive leaves have active biological compounds which have several effects, such as anti-inflammatory, antihypertensive, antioxidant, hypocholesterolemic, and hypoglycemic (El & Karakaya, 2009). The olive leaf extracts are a source of phytochemical materials (Ayala-Zavala et, al 2011). The results of this experiment were collected using GC-MS analysis to determine the components of olive leaf extract. After a 30-minute retention period, pure triterpene, a monounsaturated fatty acid, was determined to contain twenty distinct components. It has been researched. The leaf contains substantial levels of Tetradecanoic acid, which accounts for up to 15.91% of the fatty acid fraction, followed by significant concentrations of oleic acid, which accounts for 11.88 % of the omega-9 fatty acid. n-Amyl nitrite 10.97%, methyl alpha-D-mannoside 8.43%, 3 alpha-acetoxy-25-hydroxy-olean-12-en-28-oic acid 8.62%, and minor amounts of other compounds such as Ethylene glycol diglycidyl ether 4.01%, Acetic acid 2.35%, N-Acetyl Acetylpropionamide 3.37%, and others. The variations in components levels due to many factors such as the polarity of the solvents, The types of

organic solvents that were utilized, the extracts (raw vs purified), and the portions of the plant that were examined were all different. carboxylic acids with carbon chains are known as fatty acids. In many instances, it has been shown that fatty acids are responsible, at the very least in part, for the antibacterial activity that is attributed to the crude extract. This was noticed by (Cerdeiras et al. 2000).

Liu et al. (2017) investigated sufficient concentration of ethanolic have an antibacterial effect of ethanolic extract of the olive leaf against *E. coli, L. monocytogenes*, and *S. enteritidis*, *S. aureus* and *M. kristinae* have been shown to be sensitive to oleic acid (Dilika et, al, 2000) (Liu et al 2017) pH values and Titratable acidity was determined in all cheese treatments when fresh, and In control samples, the mean pH value of local cheese was lower than in control samples. They recorded a significant increase ($p<0.05$). Table 3 showed 5.79±0.02, 5.88±0.005, 6.48±0.144 during zero, 9, and 18 days of storage, respectively, while in treated cheese sample with olive extract recorded a significant decrease in value during storage period from 5.85±0.01 to 5.83±0.02 in 10% and 5.66±0.06 to 5.49±0.02 in 20%. The titratable acidity in all cheese samples was decreased from 0.137±0.05 in control to 0.76±0.04, 0.69±0.09 at 18 days in 10% and 20%, respectively. The acidity changes are significant factors that affect the cheese's quality.

The results in Table (2) show that the cheese acidity decreased as the level of olive leaves increased. Also, the acidity was further increased by cold storage for up to 18 days. The trend of the changes in pH values of all treatments was the opposite of that of acidity, which may have led to lactic acid production due to microorganisms' metabolism (Abd-Allah *et al.,* 1993). These results are similar to those obtained by Hassanein *et al*. (2008). The Total plate count as shown in table 4 of control and treated cheese sample was significantly lower ($p < 0.05$ were, respectively, from 12.23×10^5, $6.2\times10^5$and 5. 3×10^5 log cfu/g in 18 days of storage. It is possible that the action of antibacterial phytochemicals included in the extract was the cause of such a significant drop in the total plate count (Alberle et al. 2020). OLE is a by-product of the olive Industry that is often employed in phytotherapy. However, research conducted in recent years suggests that it may also have the ability to extend the shelf life of food, mostly as a result of its antibacterial and antioxidant properties. It has been established via a series of microbiological tests that OLE is efficient in lowering the viable cell count in cheese when it is being stored at low temperatures for a day or more. The table presents the variations in plate count that occurred during the course of the storage period (4). According to the data, the counts continued to be greater in the control group even after 18 days of cold storage, but then they began to decrease in the treatment groups. The findings presented here are consistent with those found by Hareedy and colleagues (2008).

Conclusion:

Olive leaf extracts inhibited bacterial growth. Further study is required to determine if Olea europaea L. extracts can inhibit the development of a broad spectrum of bacteria under precise application circumstances, ensuring food quality and safety. These findings suggest that Olea europaea L. extracts (methanol) may be used as antibacterial agents in food microbiology.

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