ANTIBACTERIAL AND ANTIFUNGAL EFFECT OF ACHILLEA MILLEFOLIUM ESSENTIAL OIL DURING SHELF LIFE OF MAYONNAISE

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Abstract. The risk of foodborne diseases and consumers' desire to avoid synthetic food preservatives have drawn researchers' attention to natural food preservatives such as essential oils that have antimicrobial qualities. Spoilage of foods by fungi is another major problem, especially in developing countries. The purpose of this study was to evaluate the effectiveness of *Achillea millefolium* essential oil as a natural food preservative in high-fat and low-fat mayonnaise kept at 4°C for 6 months. The mayonnaise samples were divided into four experimental groups treated differently, namely: EO (essential oil added in the concentrations 0.45, 2.41, and 7.2 mg/cm^3), BS (sodium benzoate and potassium sorbate added in the concentration 0.75 mg/cm^3), C_{mo} (control: no preservative, only microorganisms added), and C (control: no preservative and no microorganisms added). The results have shown that essential oil obtained from *Achillea millefolium* acts upon all tested microorganisms in mayonnaise, and prevents the growth of all pathogens and fungi, whereas in the control samples, all the microorganisms grew. The maximum cell counts of bacteria and fungi in low-fat mayonnaise were approximately lower than those in high-fat mayonnaise, and the resistance to inactivation of microorganisms was greater in high-fat mayonnaise than in low-fat mayonnaise (p<0.05). Also, the BS samples exhibited antimicrobial properties against the tested species of microorganisms during storage. As essential oil from *Achillea millefolium* allows controlling the development of foodborne pathogens and food spoilage organisms, it can be used as a natural preservative in the food industry, in particular, in mayonnaise production.

Keywords: *Achillea millefolium*; antimicrobial effect; essential oil; food preservatives; mayonnaise.

Introduction. Formulation of the problem

Foodborne pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella enteritidis* are found in a wide variety of foods. Mayonnaise is extremely susceptible to microbial contamination. It often causes food poisoning, due to its high fat content, the nature of raw materials, and lack of thermal processing. Due to its low pH, aciduric bacteria are the predominant spoilage microorganisms in this product [1-3]. It is an egg-based product vulnerable to the growth of *Salmonella*, particularly *Salmonella enteritidis* [4-6]. *S. enteritidis* can be inactivated by heat treatment, but mayonnaise is not supposed to be heat-treated. This makes it necessary to use other methods of reducing or eliminating this pathogen [6]. Some researchers investigated whether *S. enteritidis* survives being inoculated into mayonnaise with natural antimicrobials added [5-8]. *Escherichia coli* are foodborne pathogens that can damage a variety of acidic foods including unpasteurised apple juice, yoghurt, and mayonnaise [9,10]. In March 1993, there were 40 to 50 cases of *E. coli* infection in the US through consumption of mayonnaise in restaurants [9,11]. There are more than 73.500 cases of this illness each year in the United States [10]. *Staphylococcus aureus* is another health-threatening pathogen a person gets consuming food without heat treatment [12,13].

Analysis of recent research and publications

Protecting different food products against fungal spoilage increases their shelf life. Every year, a lot of food is discarded due to contamination and spoilage by fungi. Fungal spoilage on the food surface alters its appearance, texture, taste, or aroma by formation of fungal metabolites and in some cases, by mycotoxins [7,8]. Yeast contaminate all types of food, but they grow best in foods with high concentrations of organic acids and low pH [14]. Yeast like *Zygosaccharomyces baiili*, *Saccharomyces cerevisiae*, or moulds of the genus *Aspergillus* or *Penicillium* are also responsible for spoilage of mayonnaise [1].

Microbial growth causes spoilage, foodborne diseases, and production of gas and acid that affect the sensory characteristics of mayonnaise [1]. That is why, preventing microbial growth can significantly extend the shelf life of mayonnaise and be beneficial to consumers' health. To achieve these purposes and prevent spoilage in unpasteurisable products, such as mayonnaise, food manufacturers in the past decades have used several synthetic additives with antimicrobial properties – benzoates and sorbates. However, they present some problems – for example, benzene can form from benzoic acid in foods by decarboxylating some spoilage microorganisms [2,3].

Nowadays, there is consumers' growing demand for safe and natural products, such as essential oils (EO) from spices and aromatic plants, with notable antimicrobial activity [4,15-17]. Essential oils can be used as additional ingredients to improve the microbiological quality of mayonnaise without using chemical additives. Moreover, it has been established that essential oils are better antimicrobial agents for mayonnaise than synthetic preservatives, and can be used as additional ingredients to improve its microbiological quality [4,18].

In 2012, it was studied how oregano essential oil acts against *Salmonella enteritidis* in mayonnaise [6]. In 2016, the antimicrobial effect of *Ziziphora clinopodioides* essential oil and its extract on *Salmonella enterica*, *Staphylococcus aureus*, and *Saccharomyces cerevisiae* in low-fat mayonnaise was studied [19]. No study, though, considered the survival of populations of *S. aureus*, *E. coli*, *S. enteritidis*, *P. Glaucum*, and *S. cerevisiae* in high-fat and low-fat mayonnaise acted upon with *Achillea millefolium* essential oil at refrigeration temperatures. While there are some studies showing the efficacy of essential oil of *Achillea millefolium* as an antioxidant, antimicrobial, antitumor, anti-inflammatory, and antidiabetic agent [20-22], very few studies are about the efficacy of the essential oil when incorporated into a food matrix.

The purpose of this study was to investigate the potential role of *Achillea millefolium* essential oil and its antibacterial and antifungal activities in high-fat and low-fat mayonnaise during storage (4°C).

Research objectives:

1. To study the antibacterial activities of essential oil of *Achillea millefolium* in high-fat and low-fat mayonnaise.
2. To study the antifungal activities of essential oil of *Achillea millefolium* in high-fat and low-fat mayonnaise.

Research materials and methods

The microbial strains of the American Type Culture Collection (ATCC) were obtained from the Iranian Research Organisation for Science and Technology (IROST, Iran). All culture media, potassium sorbate, and sodium benzoate were provided by *Merck* (Germany). All chemicals and reagents used were analytical grade. Other ingredients for the mayonnaise formulation were provided by a local supermarket.

Mayonnaise production. In this research, essential oil extracted from *Achillea millefolium* as a natural condiment, like it was in our previous study, was selected to investigate its antibacterial and antifungal properties [20]. An attempt was made to develop a useful formula of mayonnaise free from chemical preservatives. First, high-fat (65%) and low-fat (30%) mayonnaises were produced in batches of 5 kg each with a high speed homogeniser (*Arkan felez*, Iran), in a continuous process in the pilot plant of a local factory (Paknam Sepehr Sepahan Company, Naji Mayonnaise, Isfahan, Iran). The composition of the mayonnaise included soy bean oil, water, vinegar, whole egg, NaCl, sugar, citric acid, mustard powder, starch, xanthan, guar gum, and CMC [23]. Then the mayonnaise was divided into four experimental samples, namely: EO (essential oil in the amount 0.45, 2.41, and 7.5 mg/ml), BS (sodium benzoate and potassium sorbate in the amount 0.75 mg/ml), C_{mo} (control: no essential oil, with microorganisms added in the amount 10^3 CFU/g), and C (control: no essential oil, no microorganisms added).

Introduction of additives into the mayonnaise. According to the calculated amounts of MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) of essential oil [20], it was added to the mayonnaise. Benzoate and sorbate, in the concentration 0.75 mg/ml, were used as reference substances for comparison.

PH determination. The pH values were determined at ambient temperature (20±1°C) with a pH meter (*Metrohm*, Instruments, pH 211) calibrated before using [19].

Inoculation of mayonnaise. Microbial suspensions of *S. aureus*, *E. coli*, *S. enteritidis*, *P. Glaucum*, and *S. cerevisiae* were added to the mayonnaise to give the populations 10^3 CFU/g. The inoculated mayonnaise preparations were thoroughly mixed with a mixer for 3 min to ensure a homogeneous distribution of microbial strains. The prepared mayonnaises were transferred to glass jars and kept at 4°C.

Microbiological analysis. S. aureus was enumerated, the Baird-Parker agar (BPA) surface plate incubated at 37°C for 24 h. Populations of *E. coli* and *S. enteritidis* were determined by surface plating in EC broth and Salmonella-Shigella agar (SSA), respectively, at 37°C for 24 h. The counts of *Penicillium glaucum* and *Saccharomyces cervesiae* were determined by surface plating in dicholoran rose bengal chloranphenicol

(DRBC) incubated at 25°C for 72 h. In all cases, the samples (5 g) serially diluted (10^{-1} to 10^{-5}) in sterile 0.1% peptone were surface plated on appropriate enumeration media. The serial dilutions were prepared in 9 ml volumes of peptone water. Finally, colonies were counted after the plates were incubated. All results were reported as log 10 colony forming units per gram (CFU/g). All of the tests were carried out 72 hours after production of the mayonnaise and during 6 months of storage [19]. All microbiological analyses were performed at 3 replications.

Statistical analysis. The experimental results were statistically analysed using the SPSS statistical package (version 20, SPSS Inc., Woking, Surrey, UK). An ANOVA was performed to determine the statistical significance of the samples ($p< 0.05$). The results were expressed as mean value ± standard deviation of three replications.

Results of the research and their discussion

Microbial contamination of the control samples (C). Tables 1 and 2 show the population of microorganisms in the high-fat and low-fat mayonnaise during storage at 4°C for 6 months. The colonies of *E. coli* and *S. enteritidis* were not detected in the control mayonnaise (C) during storage. Besides, the number of *S. aureus* decreased from 10^3 CFU/g to 4.26×10^1 CFU/g in 72 hours after production. But in the low-fat mayonnaise, the colonies of *S. aureus* were not detected, perhaps due to the lack of eggs. The reduction of *S. aureus* in the control samples (C) probably results from a shock to the bacteria introduced into an acidic environment. Also, cross contamination from raw materials, especially from unpasteurised eggs, dirty equipment, infection-carrying food handlers who gain access to products after commercially processed containers are opened, can be responsible for the fact that the pathogen may survive for one month in high-fat mayonnaise [11,15]. Previous studies have shown that *S. aureus* can grow at low pH values [12,13]. Nevertheless, other factors besides pH should be considered when studying the risk potential of mayonnaise. One of these factors is eggs, which have been reported to increase the growth of *S. aureus* and *Salmonella* [12,24-26]. Although unpasteurised eggs have been out of use since 1970, pathogens including *S. aureus* and *S. enteritidis* can be present in pasteurised eggs in small amounts [37]. The use of pasteurised eggs would minimise the risk of *S. aureus* and *Salmonella.* A pH ≤ 4.1 and the acetic acid concentration ≥ 1.4% are necessary for food products that are manufactured with unpasteurised eggs [25]. In this study, the pH is ≤ 4.1, but the acetic acid concentration is <1.4%. No *S. aureus* were detected in the uninoculated mayonnaises (C) until the end of storage. The first inhibitory factor may be the pH. The lower limit of tolerance for most pathogens is ~ 4, which is close to the pH of the mayonnaise samples in this study [25]. The effect of these conditions in inactivating *S. aureus* and *Salmonella* in mayonnaise was previously reported [12,24]. The effect of mayonnaise pH is only evident from the combination of mayonnaise components and storage temperature [27]. The presence of lysozyme in the whole egg used in the production of mayonnaise has an antimicrobial effect on microorganisms, especially gram-negative bacteria [25]. Similar results indicate that lysozyme effects on gram-positive bacteria such as *S. aureus* [25]. Glass and Doyle reported that acetic acid had a synergistic effect with lysozyme or other antimicrobial substances in egg white on inactivation of *Salmonella* in mayonnaise [28]. The results suggested that several factors affected the inactivation of pathogens independently or synergistically with other compounds of the mayonnaise [25]. Some factors such as difference between the pH of the high-fat (4.1) and low-fat mayonnaise (3.97), nutrients (fat, protein, carbohydrates), water content, and mayonnaise ingredients (eggs) may be partly responsible for the more rapid inactivation of the bacteria in the low-fat mayonnaise than in the high-fat mayonnaise [15,25].

Moreover, the colonies of *P. glaucum* and *S. cervesiae* survived in the uninoculated mayonnaise (C) after 72 hours and grew during storage. The counts of moulds and yeasts exceeded >10^2 CFU/g after the first month. The yeast or mould detected probably resulted from airborne contaminants [19]. The ability to growth in the conditions of high acid and low water activity and at low temperatures results in spoilage of mayonnaise by yeasts and moulds. Proton exporting through the cell membrane allows yeast cells to regulate the pH inside the cell [14]. The order of fungal resistance in the control mayonnaise was *S. cervesiae* < *P. glaucum*. The rate of yeast growth is slower than the mould growth. Because the cell wall of yeast is very strong, it can also be very porous and is composed mostly of mannoprotein, ß1.3 glucan, and chitin. Also it has a more stable plasma membrane. These strains have very few requirements for survival and can grow under anaerobic conditions. Mayonnaise containing protein, fat, and carbohydrates are likely to provide a more favourable growth environment for microorganisms [27].

Microbial contamination of the inoculated samples (C_{mo}). Tables 1 and 2 show that the rate of inactivation of pathogens in the inoculated mayonnaises increased during storage. The reduction of pathogens was clearly seen ($p < 0.05$). In the low-fat mayonnaise containing inoculated *S. aureus*, microbial survival was observed just for one month, but in the high-fat mayonnaises, the colonies of *S. aureus* survived till the second month of storage. In the control mayonnaises containing *P. glaucum* and *S. cervesiae*, microbial growth was also observed, and all microbial groups increased in them in the 0^{th} month to the end of storage. Viable cells were detected after 72 hours of storage in the C_{mo} samples and slightly exceeded

10^{6} CFU/g in the 2nd and 4th months of storage, respectively, to the end of storage. Fungal cells did not grow in the first month of storage, but the cells grew after 1 month of storage at 4°C. When *P. glaucum* was inoculated, it survived for a longer time than when the mayonnaise was inoculated with *S. cervesiae*.

Table 1 – Populations or presence of *S. aureus, E. coli,* and *S. enteritidis* (CFU/g) in high-fat and low-fat mayonnaise during storage at 4 °C for 6 months

Storage time (month)	Treatment	Population (log 10 CFU/g) in:					
		S. aureus		*E.coli*		*S. Enteritidis*	
		H.F	***L.F***	***H.F***	***L.F***	***H.F***	***L.F***
0	$EO_{4.5}$	ND	ND	-	-	-	-
	$EO_{7.2}$	ND	ND	ND	ND	ND	ND
	$BS_{0.75}$	ND	ND	ND	ND	ND	ND
	C_{mo}	$2.13 \times 10^{2} \pm 0.15^{a}$	$9.3 \times 10^{1} \pm 0.30^{b}$	$7.2 \times 10^{2} \pm 0.2^{c}$	$4.36 \times 10^{2} \pm 0.37^{d}$	$6.1 \times 10^{1} \pm 0.15^{e}$	$5.1 \times 10^{1} \pm 0.10^{f}$
	C	$4.26 \times 10^{1} \pm 0.15^{g}$	ND	ND	ND	ND	ND
1	$EO_{4.5}$	ND	ND	-	-	-	-
	$EO_{7.2}$	ND	ND	ND	ND	ND	ND
	$BS_{0.75}$	ND	ND	ND	ND	ND	ND
	C_{mo}	$2.43 \times 10^{2} \pm 0.15^{a}$	$1.20 \times 10^{2} \pm 0.26^{b}$	$5.56 \times 10^{2} \pm 0.25^{h}$	$3.36 \times 10^{2} \pm 0.35^{k}$	ND	ND
	C	$2.53 \times 10^{1} \pm 0.30^{m}$	ND	ND	ND	ND	ND
2	$EO_{4.5}$	ND	ND	-	-	-	-
	$EO_{7.2}$	ND	ND	ND	ND	ND	ND
	$BS_{0.75}$	ND	ND	ND	ND	ND	ND
	C_{mo}	$3.33 \times 10^{1} \pm 0.15^{n}$	ND	ND	ND	ND	ND
	C	ND	ND	ND	ND	ND	ND
3	$EO_{4.5}$	ND	ND	-	-	-	-
	$EO_{7.2}$	ND	ND	ND	ND	ND	ND
	$BS_{0.75}$	ND	ND	ND	ND	ND	ND
	C_{mo}	ND	ND	ND	ND	ND	ND
	C	ND	ND	ND	ND	ND	ND
4	$EO_{4.5}$	ND	ND	-	-	-	-
	$EO_{7.2}$	ND	ND	ND	ND	ND	ND
	$BS_{0.75}$	ND	ND	ND	ND	ND	ND
	C_{mo}	ND	ND	ND	ND	ND	ND
	C	ND	ND	ND	ND	ND	ND
5	$EO_{4.5}$	ND	ND	-	-	-	-
	$EO_{7.2}$	ND	ND	ND	ND	ND	ND
	$BS_{0.75}$	ND	ND	ND	ND	ND	ND
	C_{mo}	ND	ND	ND	ND	ND	ND
	C	ND	ND	ND	ND	ND	ND
6	$EO_{4.5}$	ND	ND	-	-	-	-
	$EO_{7.2}$	ND	ND	ND	ND	ND	ND
	$BS_{0.75}$	ND	ND	ND	ND	ND	ND
	C_{mo}	ND	ND	ND	ND	ND	ND
	C	ND	ND	ND	ND	ND	ND

EO: Essential oil (in the concentration 4.5 and 7.2 mg/ml), BS: sodium benzoate and potassium sorbate (in the concentration 0.75 mg/ml), C_{mo}: control (no preservative, only microorganisms added), C: control: (no preservative and no added microorganisms), HF; high-fat. LF: low-fat, ND: not detected in the samples, -: not used, the means in the columns and rows with a different letter differ significantly ($p < 0.05$).

The colonies of *S. aureus* survived to the second month of storage in the inoculated samples (C_{mo}), because of the fat content protecting the cells [15]. No other significant differences were observed in the first and 0th months of storage. It was because the death rate of the bacteria was equal to their multiplication rate. In other hands, the survival of bacteria was greater than their growth. The reason for this may be the low pH, a_w, temperature, viscosity, etc. It was observed for *E. coli*, that its numbers were slowly reduced compared to *S. Enteritidis*, and it was not detected after 1 month in the mayonnaise samples, whereas *S. enteritidis* was more sensitive than the other pathogens. It strongly reduced in 0 months of storage and was detected in no sample until the end of storage (Table 1). In other words, when *E. coli* was inoculated, its survival was longer than when the mayonnaise was inoculated with *S. enteritidis*.

Table 2 – Populations or presence of *P. glaucum* and *S. cervesiae* (CFU/g) in high-fat and low-fat mayonnaise during storage at 4 °C for 6 months

Storage time (month)	Treatment	Population (log 10 CFU/g) in:			
		P. glaucum		*S .cervesiae*	
		H. F	*L. F*	*H. F*	*L. F*
0	EO $_{0.45}$	ND	ND	ND	ND
	EO $_{2.41}$	ND	ND	ND	ND
	$BS_{0.75}$	ND	ND	ND	ND
	C_{mo}	$8.26 \times 10^{2} \pm 0.15^{a}$	$1.4 \times 10^{2} \pm 0.3^{g}$	$5.7 \times 10^{2} \pm 0.75^{p}$	$6.1 \times 10^{2} \pm 0.18^{y}$
	C	$2.1 \times 10^{2} \pm 0.10^{b}$	$5.1 \times 10^{2} \pm 0.85^{h}$	$4.4 \times 10^{1} \pm 0.15^{q}$	$7.6 \times 10^{1} \pm 0.25^{q}$
1	EO $_{0.45}$	ND	ND	ND	ND
	EO $_{2.41}$	ND	ND	ND	ND
	$BS_{0.75}$	ND	ND	ND	ND
	C_{mo}	$4.3 \times 10^{5} \pm 0.36^{c}$	$3.7 \times 10^{5} \pm 0.55^{c}$	$4.1 \times 10^{4} \pm 0.65^{r}$	$5.2 \times 10^{4} \pm 0.30^{r}$
	C	$3.1 \times 10^{4} \pm 0.30^{d}$	$7.7 \times 10^{4} \pm 0.25^{k}$	$2.9 \times 10^{2} \pm 0.95^{b}$	$7.4 \times 10^{2} \pm 0.15^{y}$
2	EO $_{0.45}$	ND	ND	ND	ND
	EO $_{2.41}$	ND	ND	ND	ND
	$BS_{0.75}$	ND	ND	ND	ND
	C_{mo}	$6.8 \times 10^{6} \pm 0.32^{e}$	$5.7 \times 10^{6} \pm 0.25^{m}$	$2.1 \times 10^{5} \pm 0.65^{t}$	$3.5 \times 10^{5} \pm 0.40^{f}$
	C	$3.5 \times 10^{5} \pm 0.20^{f}$	$9.4 \times 10^{4} \pm 0.50^{n}$	$9.7 \times 10^{3} \pm 0.20^{u}$	$5.6 \times 10^{3} \pm 0.61^{s}$
3	EO $_{0.45}$	ND	ND	ND	ND
	EO $_{2.41}$	ND	ND	ND	ND
	$BS_{0.75}$	ND	ND	ND	ND
	C_{mo}	$10^{6}>$	$10^{6}>$	$8.1 \times 10^{5} \pm 0.10^{v}$	$5.8 \times 10^{5} \pm 0.51^{x}$
	C	$> 10^{6}$	$> 10^{6}$	$4.3 \times 10^{4} \pm 0.12^{r}$	$5.1 \times 10^{4} \pm 0.30^{r}$
4	EO $_{0.45}$	ND	ND	ND	ND
	EO $_{2.41}$	ND	ND	ND	ND
	$BS_{0.75}$	ND	ND	ND	ND
	C_{mo}	$10^{6}>$	$10^{6}>$	$10^{6}>$	$10^{6}>$
	C	$> 10^{6}$	$> 10^{6}$	$5.9 \times 10^{5} \pm 0.10^{x}$	$6.9 \times 10^{5} \pm 0.40^{x}$
5	EO $_{0.45}$	ND	ND	ND	ND
	EO $_{2.41}$	ND	ND	ND	ND
	$BS_{0.75}$	ND	ND	ND	ND
	C_{mo}	$10^{6}>$	$10^{6}>$	$>10^{6}$	$> 10^{6}$
	C	$> 10^{6}$	$> 10^{6}$	$>10^{6}$	$>10^{6}$
6	EO $_{0.45}$	ND	ND	ND	ND
	EO $_{2.41}$	ND	ND	ND	ND
	$BS_{0.75}$	ND	ND	ND	ND
	C_{mo}	$10^{6}>$	$10^{6}>$	$>10^{6}$	$> 10^{6}$
	C	$> 10^{6}$	$> 10^{6}$	$>10^{6}$	$>10^{6}$

EO: Essential oil (in the concentration of 0.45 and 2.41 mg/ml), BS: sodium benzoate and potassium sorbate, C_{mo}: control (no preservative, with microorganisms added), C: control: (no preservative and no added microorganisms), HF; high-fat. LF: low-fat, ND: not detected in the samples, -: not used, the means in the columns and rows with a different letter differ significantly ($p < 0.05$).

The most marked increase in the survival time occurred in the high-fat mayonnaise containing inoculated *S. Aureus*. That is why the order of bacterial resistance in the control mayonnaise was *S. aureus* > *E. coli* > *S. enteritidis*. Thus, *S. aureus* was more resistant than the other tested pathogens in the same environmental conditions. Also, this suggested that *E. coli* is more resistant than *S. enteritidis*. Similar results have been obtained about the survival of *E. coli* and *S. enteritidis* in mayonnaise [15,1,10,28-31]. The longest survival of *E. coli* reported by Hathcox *et al.* was observed in the high-fat mayonnaise [15]. Lock and Board achieved the same results while evaluating the viability of *S. enteritidis* in different mayonnaises. The differences in the researches' data can be explained by the production conditions, the type of ingredients such as pasteurised and unpasteurised eggs, the level of inoculating strains, the type of microorganisms, a_w, pH, etc [32,33]. Pathogens that can grow at low pH include *Salmonella*, *E. coli* and *S. aureus* [13]. The adaptive acid tolerance ability of *Salmonella* and *E. coli* has been documented [9,34-37]. Some researchers reported that strains of *E. coli* might be more tolerant to acidic conditions than other foodborne pathogens and could survive in refrigerated acidic foods including mayonnaise for several weeks [9-11,13,25]. The mechanism of the acid tolerance of *E. coli* is not clearly realised. Miller and Kaspar estimated that the cell is not decomposed being exposed to acidic environments [38]. Junkins and Doyle found that *E. coli* produced visible mucoid colonies with exopolysaccharide mucous layers composed of colonic acid [39]. This mucous layer has chemical or structural properties that provide a physical protection barrier against acidic conditions, thereby blocking or delaying penetration of antimicrobial ingredients into cells. Many foodborne pathogens produce exopolysaccharides, but these cellular protective factors are destroyed in mayonnaises [11].

No *S. aureus*, *E. coli*, and *S. enteritidis* were detected in the inoculated mayonnaise (C_{mo}) at the end of storage. Both the pH and the acidity (acetic acid, citric acid) in the mayonnaise formulation prevented the growth of organisms [2]. The effects of pH and citric acid or acetic acid on *S. enteritidis* in home-made and commercially manufactured mayonnaise were investigated [3,5,32,33]. The authors concluded that *Salmonella*-free mayonnaise prepared with lemon juice and acetic acid (vinegar) prevented the transmission of salmonellosis because low pH could have caused cell death. Some studies confirm that the antibacterial activity of acetic acid (vinegar) is greater than that of citric acid (lemon) and allows it to kill *salmonellae* and *S. aureus* in homemade mayonnaise [15]. In general, acidity should be considered as a factor controlling the growth or survival of pathogenic bacteria, because it is one of the most important properties of mayonnaise. It prevents the growth of microorganisms by damaging DNA [13]. Besides pH and acidity, there are other factors that can influence the viability of cells in mayonnaises [27,40]. Salt and sugar play minor roles, but they prevent the growth of pathogens due to a synergistic effect when they are used in combination with acetic acid [13]. Another antimicrobial agent existing in mayonnaise is mustard. It has a bactericidal effect on foodborne pathogens. Allyl isothiocyanate, a component of mustard, has antimicrobial potential [10,41]. When mustard is combined with acetic acid, its effectiveness increases killing foodborne pathogenic bacteria [15].

As mentioned, it has been shown that mayonnaises inactivate gram-negative bacteria [15,27]. Generally, most foodborne pathogens cannot have pH below 4.5. This means that acid products normally are not the source of major health risks, and limiting the microbial growth depends on the types of microorganisms and the type and amount of acid [10,24,25]. For example, the minimum pH for growth of *E. coli* and *Salmonella* was estimated to be 4.4 and 4.5, respectively, similar to those determined in this study. However, the maximum pH allowing growth of these two species was 9.0 and 7.8, respectively. In conclusion, pH has kills gram-negative bacteria. If *E. coli* cells are sensitive to low pH and high acidity, these cells can be expected to die soon after inoculation into mayonnaise. The death of this population is more rapid in mayonnaise, thus resulting in the apparent elimination of viable *E. coli* cells more quickly [15].

The comparison of the microbial stability of different mayonnaise types has shown that low-fat mayonnaise generally has lower microbial counts than high-fat mayonnaise. It is due to the ability of high-fat mayonnaise to support the growth of microorganisms. The lower pH of low-fat mayonnaise can have an immediate lethal effect on more cells than the higher pH of high-fat mayonnaise [15,40]. Zhao and Doyle found that *E. coli* survived slightly longer in high-fat mayonnaise than in the low-fat formulation made with less acid. They concluded that the low-fat mayonnaise contained an ingredient with anti-*E. coli* properties that was not present in high-fat mayonnaise [26]. The higher fat content in mayonnaise may have protected cells against inactivation [15]. This means that the effect of the mayonnaise pH is only evident when the combination of the food components and storage temperature is less favourable for the growth of microorganisms. Starch in low-fat mayonnaise may not provide sufficient nutrients for growth of microorganisms [15]. Since low-fat mayonnaise contains more water than high-fat mayonnaise, the percentage of acetic acid (wt/wt) in the aqueous phase of low-fat mayonnaise is substantially lower than the percentage in the aqueous phase of high-fat mayonnaise [15]. Any or all of these factors may be partly responsible for the more rapid inactivation of the microorganisms in low-fat mayonnaise than in high-fat mayonnaise.

Antimicrobial effect of sodium benzoate and potassium sorbate (BS) in mayonnaise. Of all the mayonnaise types, the BS samples did not allow microbial growth in these products at 4°C. In the BS samples, no microbial counts were observed. The antimicrobial synergistic interactions between egg white constituents (lysozyme), low pH, acetic acid, potassium sorbate, and sodium benzoate are responsible for rapid inactivation of pathogens. Potassium sorbate has a significant effect on fungi, but sodium benzoate increases the inactivation rate of bacteria [11]. The presence of sodium benzoate in the BS mayonnaise would be expected to affect the viability of microorganisms. Studies revealed that the addition of 0.1% of sodium benzoate increased the rate of inactivation of *E. coli* in high-fat and low-fat mayonnaise [15]. Zhao and Doyle reported that potassium sorbate alone had little effect on *E. coli* in apple cider, but both potassium sorbate and sodium benzoate were effective in controlling the growth of this organism [26]. Sorbates have been applied as antimicrobial food preservatives in a wide range of foods, particularly against yeasts and moulds, though most yeasts show tolerance to benzoates. The activity of sorbates depends on a number of factors including pH, a_w, storage temperature, target microorganisms, and food composition [14,42]. Liewen and Marth studied the inhibition of *Penicillia* and *Aspergilli* by potassium sorbate and found that its effect was significant [43]. The mechanism of antimicrobial action of food preservatives like sorbate against bacteria and fungi includes the cytoplasmic membrane, the genetic material of the cell, and the cellular enzymes [42,44,45]. Sorbate inhibits moulds at all stages of their life cycle, including spore germination, growth initiation, and mycelial growth. Sorbate inhibits cell metabolism by reducing microbial assimilation of carbon from many substrates. It can be argued that disruption of the proton motive force is the main mechanism of inhibiting the microbial growth by sorbate, because it may be inhibited by amino acid uptake which causes a stringent response in bacteria [45]. The inhibitory effects of sorbates and benzoate are increased by sugars, salts, antioxidants, sodium chloride, and high concentrations of metal ions [42].

Antimicrobial effect of essential oil (EO) in mayonnaise. During the storage period, no microbial counts were observed in the samples containing essential oil. 6 months of storage resulted in the death of microorganisms. Thus, the antimicrobial action in the samples with natural and chemical preservatives was similar.

In both formulations containing natural and chemical preservatives, viable cells were not detected. These results are similar to those obtained in studies of the antimicrobial activity of many essential oils against bacteria *in vitro* and in foods [8]. Silva and Franco showed that the essential oil of oregano in mayonnaise resulted in a reduction in the count of *S. enteritidis*, thus becoming an effective barrier to inhibit the growth of the pathogen in the product [6].

The antimicrobial activity of essential oil of *A. millefolium* against *P. glaucum*, *S. cervesiae*, and *S. aureus* was observed *in vitro* with the lowest minimum inhibitory concentration (MIC) [20]. The antimicrobial action of essential oil might be due to its interaction with components of the membranes and the cell wall, resulting in the increased permeability of the membrane bacteria and leakage of cell material from tissues, causing swelling and reduced membrane function. Or, perhaps, its water-binging capacity and inhibition of various enzymes also allow it to absorb nutrients of bacteria and thus prevent their growth [16,40,46].

The antimicrobial potential of essential oil of *A. millefolium* could be due to its high content of camphor, borneol, and cineol [20]. Besides, the volatile terpenes *p*-Cymene, thymol, carvone, terpinene, caryophyllene oxide, *alpha*-Cadinol, pinene, terpinolene, neomenthol, and thujone are probably responsible for its antibacterial activity [24,35,46,47]. All of the substances are confirmed as strong antimicrobials. Kisko and Roller observed that terpinene had an inhibitory activity against *E. coli* in apple juice [48]. In the study [14], scanning electron microscope images taken of *S. cerevisiae* cells exposed to thymol showed severe surface damage and total cell mortality. In another study, carvone exhibited fungicidal activity and protected potato tubers from rotting without altering their taste and quality [16]. Due to the complexity of compounds, it is difficult to establish a correlation between the antimicrobial activities of essential oil and certain compounds [49].

In this study, differential sensitivity was observed when using essential oil *in vitro*, but not in the mayonnaise tests. For example, in the *in vitro* assays, gram-positive bacteria seemed to be more sensitive to the essential oil tested than gram-negative bacteria [20], but in the mayonnaise samples, the growth of the two tested species was inhibited. Antimicrobial effects, particularly against gram-negative bacteria in mayonnaise, are also more pronounced due to the influence of the pH, acidity, NaCl, temperature, storage time, antimicrobial ingredients, physical and chemical properties of mayonnaise, which can disturb the permeability of cell membranes [15,27,1,10,6,26,50]. As a general rule, the sensitivity of microorganisms to the antimicrobial effects of essential oil seems to increase when there is a decrease in the pH, temperature, and oxygen [1,3,9].

Conclusion

The effect of essential oil of *Achillea millefolium* on the microbiologically tested mayonnaise has been studied. It has been established that different essential oil concentrations have similar antibacterial and antifungal power, compared with that of the mixture of sodium benzoate and potassium sorbate, against the tested microorganisms. The growth of all microorganisms in all samples was inhibited. The acidic nature of mayonnaise improved the ability of essential oil to penetrate the bacterial cell membrane, so its use can be an additional protection to increase the safety of commercial mayonnaise. *S. aureus*, *E. Coli*, and *S. enteritidis* died when inoculated into mayonnaise. Therefore, essential oil of *Achillea millefolium* can be considered an alternative natural antimicrobial preservative in food products. We recommend using this essential oil to develop new and safe natural antibacterial and antifungal agents for food preservation. A novel antibacterial compound from natural sources that can be used in the food industry to extend the shelf life and for consumers' health should be searched for.

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