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Effect of Some Improving Processing Techniques on the Microbiological and Sensory Quality of Domiati Cheese

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Abstract

Different trials were made to improve the microbiological quality of the traditionally produced Domiati cheese without effect its sensorial quality. The performed study was depending on using raw, pasteurized and cultured pasteurized milk for cheese manufacture accompanied by: sanitizing of equipment with 0.25% hydrogen peroxide or adding 0.1% potassium sorbate to milk or sanitizing of equipment with hydrogen peroxide accompanied with adding 0.1% potassium sorbate to milk. Samples from fresh and ripened produced cheese from different treatments were examined microbiologically and organoleptically. SPC (Standard Plate Count), coliform, yeast and mould counts proved to be significantly affected by pasteurization or pasteurization and culturing of the used cheese milk. Meanwhile, pasteurization adversely affects the sensory parameters of ripened cheese, whereas, the use of cultured pasteurized milk enhances such parameters. It was seen that sanitizing of equipment with hydrogen peroxide 0.25% had a significant effect on SPC and coliforms count, with an adverse effect on the overall organoleptic acceptability of ripened cheese. While, adding 0.1% potassium sorbate seems to have a significant effect on SPC, yeast and mould counts. Whereas, it has no adverse effect on the sensorial quality of cheese. Therefore, using cultured pasteurized milk with the addition of 0.1% potassium sorbate as a preservative is the best choice for improving the microbial quality, without adverse effect on the sensorial quality of cheese.

Key words: Domiati cheese, Hydrogen peroxide, Potassium sorbate, Coliforms, Yeasts and Moulds.

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1. Introduction

Domiati cheese is the most popular soft white-brined cheese variety made from raw buffalo's or a mixture of buffalo's and cow's milk. It is believed that Domiati cheese originated in Egypt since 332 BC [1]. Domiati cheese is manufactured by the addition of (10 -15%) salt directly to cheese milk and ripened for 2-4 months at room temperature [2, 3]. The resultant Domiati cheese has a unique flavor, its acidity increases during ripening acquiring the cheese a pungent flavor. It has a close texture with no gas holes. Although it is pure white at the beginning of ripening, the color becomes a light brown as ripening proceeds [1, 3].

Small-scale farmhouse Domiati cheese traditionally produced by raw milk and manual manipulation of the curd during manufacture, molding and ripening is still practiced. Cheese made from raw milk has a much better taste, popular to consumer, than that made from pasteurized milk due to the presence of natural microflora of the milk; this is considered to be an important marketing advantage for raw milk cheese. From the other side, this traditional processing technique of cheese made from raw milk enhances the growth of pathogens such as *S. aureus*, *E. coli*, *L. monocytogenes*, *salmonella spp.* Domiati cheese made from raw milk not only has public health effect, but also, it may produce economic losses due to spoilage of cheese including early or late gas blowing, mouldiness, softening, ropiness, discoloration and off-flavors [4].

Early gas blowing is the formation of holes in cheese mass during curdling, drainage, salting or within the first few days of ripening. It is generally considered as the most common defect for white-brined cheese made from raw milk. Early gas blowing is much more frequent in cheese made from raw milk of poor bacteriological quality. This defect is caused by the multiplication of coliforms, yeasts and sometimes heterofermentative lactic acid bacteria during cheese making [5, 6]. Raw milk generally contains between 3 to 5 log CFU/ ml fungi. Their presence considered an indication of secondary contamination during production [7]. The presence of moulds and yeasts in dairy products is undesirable from several

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perspectives, among which alterations of the sensory parameters due to their proteolytic and lipolytic activities. Surface discoloration may due to the outgrowth of a thallus on the product surface [6].

Therefore, the Egyptian food safety and quality authorities enforced dairy product manufacturers to pasteurize the milk or heating it to equivalent temperature during dairy product manufacture [8]. But, pasteurization of cheese milk kills most lactose fermenting organisms which has an adverse effect on the ripened cheese prepared from it which necessitates adding of starter culture for proper fermentation and development of the desired sensorial characteristic of cheese. A benefit of using cultured pasteurized milk was that essentially all of the acid development was from the added starter culture. Thus, the rate and extent of acidification could be controlled and resulted in a more consistent cheese quality [5, 9]. Hydrogen peroxide is a clear, colorless, and odorless with bitter taste liquid and it considered as an environmentally friendly sanitizer, widely used in food industries. Hydrogen peroxide is approved to be used as disinfectants in the food industry and it has been found to be effective in removing biofilms from equipment [10]. It is effective against a broad spectrum of microorganisms including viruses, bacteria, bacterial endospores and yeasts [11].

Potassium sorbate is the most commonly used food preservative worldwide because it is considered as "generally recognized as safe" (GRAS) food additive and is much more soluble in water than the sorbic acid. It is effective up to pH 6.5, but its effectiveness increases as the pH decreases. Potassium sorbate is effective against yeasts, moulds and some selected bacteria and is recommended to be added at 0.025 to 0.1% levels in cheese. Maximum level allowable by law is 0.1% [12, 13].

The sensory attributes of cheese such as appearance, flavor and texture, are properties that are perceived by the human senses, mainly during consumption. Thus, sensory evaluation of cheese is unquestionably essential to determine the relative merits of cheese making processes, and the eating quality of cheese and its consumer acceptability [14]. Therefore, this work was planned to improve some of processing techniques to protect the Domiati cheese producers from economic losses as well as to safeguard consumer's health**.**

Materials and Methods:

A. Raw materials:

- **Raw milk:**

Raw buffalo's milk was obtained from the herd of Faculty of Agriculture, Cairo University, Egypt.

- **Starter Cultures:**

Commercial lyophilized lactic culture (WhiteDaily 40, Chr. Hansen Laboratory, Hørsholm, Denmark), contained *Lactobacillus delbrueckii subsp. bulgaricus, Lactococcus lactis subsp. Lactis*, *Lactococcus lactis ssp. cremoris* and *Streptococcus thermophiles* was used as starter culture.

- **Locally manufactured liquid calf rennet:** was obtained from local market**.**
- **Powder rennet:**

CHY-Max ® powder Extra NB (Chr. Hansen Laboratory, Hørsholm, Denmark) was kindly obtained from Mifad- Misr food additives.

- **Salt:** Iodized salt, produced by EL-Nasr Saline's Co., Alex, Egypt was used.
- **Potassium sorbate:** Potassium sorbate, produced by Z.K.W. China was obtained from Gersy Commercial Co."Alex.", Egypt.
- **Hydrogen peroxide**: 50 vol. H_2O_2 was obtained from Arabian company for chemicals- Clean-Way.

B. Some processing techniques:

Pasteurization of cheese milk: milk was laboratory pasteurized at 63°C/30 minutes in water bath **(Schützart DIN 40050-IP 20)** using the technique described by **[15].**

Culturing of pasteurized cheese milk: starter culture at the rate of 1% (w/w) was added to the pasteurized milk at 40°C, and the milk was left for 30 minutes to develop acidity based on **Abdalla et al., 2010 [16]**.

C. Laboratory production of Domiati cheese:

Domiati cheese was laboratory produced according to **Abd El-Salam et al., 1993 & Abou-Donia, 1996 [17, 18]**.

D. Trials for improving the microbiological quality (please remove these words by red color):

For each trial, 24 liters of raw buffalo's milk were used to produce Domiati cheese. The received milk was divided into 3 portions as follows: portion 1 was left as raw milk; portion 2 was pasteurized milk and portion 3 was cultured pasteurized milk.

Each milk portion was treated by the following methods:

T_1: was used to produce Domiati cheese without any treatment (as control negative).

T_2: was used to produce Domiati cheese after sanitizing the equipment with 0.25% H_2O_2.

T_3: was used to produce Domiati cheese after adding 0.1% potassium sorbate to milk

T_4: was used to produce Domiati cheese after adding 0.1% potassium sorbate to cheese milk accompanied by using sanitized equipment with 0.25% H_2O_2.

Samples from the produced fresh Domiati cheese "representing each treatment" were obtained for determining the microbiological quality. Produced fresh Domiati cheese of each treatment was packed in sterile PPE bags in previously boiled and cooled whey and left to ripen for 2 months at room temperature. Then samples from ripened Domiati cheese

"representing each treatment" were obtained for determining the microbiological and organoleptic quality.

I. Microbiological analysis:

Samples from fresh and ripened Domiati cheese of different treatments were subjected to the following microbiological parameters: SPC, coliform count, yeast, mould and *S. aureus* count. In addition to presence of *E. coli*, *salmonellae*, and *L. monocytogenes* according to **[19]**.

II. Organoleptic evaluation of ripened cheese of different batches:

Sensory evaluation of different cheese batches was conducted by a panel consisting of 7 persons using the technique described by **[20]** for brick cheese. The experimental work was conducted in triplicates and collected data were subjected to statistical analysis.

III. Statistical Analysis:

All obtained data were presented as the mean ± standard error of mean (SEM). The data were analyzed by two way analysis of variance (ANOVA) for main (fixed) effects (heat treatment "raw, pasteurized and cultured pasteurized" & treatments "T_1, T_2, T_3, T_4") and two way interactions between heat treatments and treatments were done by using SPSS version 25. Statistical significant level was considered accepted as 5% (P<0.05).

Results and Discussion

In fresh cheese, the overall statistical analysis revealed that SPC was significantly (p<0.0001) affected by either heat treatment or both heat treatment and culturing of used cheese milk. Where, the overall mean SPC in raw milk cheese was $21\times10^6 \pm 2.1\times10^6$ CFU/g, while, it was $23\times10^4 \pm 9\times10^4$ in pasteurized milk cheese due to the effect of pasteurization on the microbial flora **[21]**. On the other hand, the overall mean SPC of cultured pasteurized milk cheese ($64\times10^4 \pm 18\times10^4$ CFU/g) was higher than pasteurized milk cheese due to the effect of added starter culture **(Table 1)**.

In ripened cheese, the overall mean SPC was significantly (p<0.0001) affected by either heat treatment or both heat treatment and culturing of used cheese milk. The overall mean SPC in pasteurized milk cheese was significantly lower ($45\times10^3 \pm 5\times10^3$ CFU/g) than raw milk cheese ($14\times10^5 \pm 2.5\times10^5$ CFU/g). Indicating the adverse effect of ripening process on the count of thermoduric microorganisms, those resist the pasteurization condition. On the other side, the overall mean SPC of cultured pasteurized milk cheese was $10\times10^5 \pm 0.8\times10^5$ CFU/g this increase in count than pasteurized one could be attributed to the controlled growth of added starter culture bacteria during ripening period **[22]**. These results were similar to those recorded by **[22]**.

Coliforms were significantly (p<0.0001) affected by heat treatment of cheese milk. As the overall mean of coliforms count for fresh raw milk cheese was $67\times10^4 \pm 10\times10^4$ MPN/g. While, it fail to be detected in fresh cheese prepared either from pasteurized or cultured pasteurized milk **(Table 1)**. Therefore, coliforms are used as indicators of processing flaws or post processing contamination of pasteurized foods, because they are easily destroyed by heat and do not survive heat treatment. The obtained results can explain the blowing defects which may appear in cheese made from raw milk due to gas production by coliform **[6]**. So that, this defect in Domiati cheese can easily be controlled by coliforms destruction via pasteurization of raw milk before used in Domiati cheese manufacture. Whereas, in ripened cheese, the overall mean coliforms count was $2\times10^2 \pm 0.8\times10^2$ MPN/g **(Table 1)**.

On the same way, yeasts and moulds were significantly affected by heat treatment (p<0.0001). As, yeast and mould overall mean counts of raw milk fresh cheese were $29\times10^3 \pm 1\times10^3$ Log CFU/g and $15\times10^3 \pm 1\times10^3$ Log CFU/g, respectively. Whereas, in ripened raw milk cheese were $5\times10^2 \pm 2\times10^2$ Log CFU/g and $0.6\times10^2 \pm 0.3\times10^2$ Log CFU/g, respectively **(Table 1)**. Presence of yeasts and moulds in raw milk cheese may be correlated to the higher acidity of the raw milk cheese which may improve their growth. As, several molds grow at pH values below 2.0 and several yeasts even below 1.5 **[19]**. Both yeasts and moulds failed to be detected after pasteurization in pasteurized and cultured pasteurized milk fresh and ripened cheese. **Da silva et al., 2019 [19]** confirmed that fungi have low resistance to heat and are relatively easily destroyed by mild heat treatments **(Table 1)**.

The effect of heat treatment on SPC, coliforms, yeast and mould count in Domiati cheese was in agreement with **[21, 23]**.

Food Grade hydrogen peroxide is available in two concentration w/w 35% or 50%. Hydrogen peroxide used in food processing must meet the specifications of the Food Chemicals Codex **[24]**. Data presented in **(Table 1)** showed the effect of using 0.25% of 50 vol. hydrogen peroxide as sanitizer on the microbiological parameters of Domiati cheese. In fresh cheese, results revealed that the overall mean SPC was significantly affected (p<0.0001) by hydrogen peroxide as in T_1 it was $11\times10^6\pm5\times10^6$ CFU/g and in T_2 it was $62\times10^5\pm29\times10^5$ CFU/g. Indicating the effect of using H_2O_2 as sanitizing agent in improving the microbial quality of cheese. While in ripened cheese, the overall mean SPC indicates a significance difference (p<0.0001) between T_1 ($13\times10^5\pm3.5\times10^5$ CFU/g) and T_2 ($71\times10^4\pm17\times10^4$ CFU/g). Seems that the reduction was due to the effect of ripening on the microbial count rather than the use of H_2O_2, as a sanitizer actually disappeared in fresh and ripened cheese. Therefore, ripening period seemed to have significant effect on improving the quality of the final product through the prevailed adverse conditions as water activity, pH, salt content, temperature and anaerobic condition within the cheese **[25]**.\

Table (1): Effect of different improving processing techniques on the microbiological quality of fresh and ripened Domiati cheese (CFU/g or MPN/g) made from raw, pasteurized and cultured pasteurized milk:

Parameters	Treatment	Fresh cheeses				Ripened cheeses			
		Raw milk cheeses	Pasteurized milk cheeses	Cultured pasteurized milk cheeses	Overall	Raw milk cheeses	Pasteurized milk cheeses	Cultured pasteurized milk cheeses	Overall
SPC	**T_1**	$32\times10^{6\,a} \pm 3.5\times10^{6}$	$85\times10^{4\,a} \pm 0.8\times10^{4}$	$15\times10^{5\,a} \pm 0.5\times10^{5}$	$11\times10^{6\,a} \pm 5\times10^{6}$	$25\times10^{5\,a} \pm 0.6\times10^{5}$	$74\times10^{3\,a} \pm 0.9\times10^{3}$	$14\times10^{5\,a} \pm 0.5\times10^{5}$	$13\times10^{5\,a} \pm 3.5\times10^{5}$
	T_2	$18\times10^{6\,b} \pm 1.4\times10^{6}$	$91\times10^{3\,b} \pm 0.6\times10^{3}$	$75\times10^{4\,b} \pm 2.8\times10^{4}$	$62\times10^{5\,b} \pm 29\times10^{5}$	$90\times10^{4\,c} \pm 6\times10^{4}$	$36\times10^{3\,b} \pm 0.6\times10^{3}$	$12\times10^{5\,b} \pm 0.6\times10^{5}$	$71\times10^{4\,b} \pm 17\times10^{4}$
	T_3	$17\times10^{6\,b} \pm 0.6\times10^{6}$	$71\times10^{3\,b} \pm 0.6\times10^{3}$	$11\times10^{4\,c} \pm 0.5\times10^{4}$	$57\times10^{5\,b} \pm 28\times10^{5}$	$17\times10^{5\,b} \pm 0.6\times10^{5}$	$37\times10^{3\,b} \pm 0.6\times10^{3}$	$81\times10^{4\,c} \pm 0.6\times10^{4}$	$85\times10^{4\,b} \pm 24\times10^{4}$
	T_4	$16\times10^{6\,b} \pm 0.6\times10^{6}$	$21\times10^{3\,c} \pm 0.9\times10^{3}$	$10\times10^{4\,c} \pm 0.5\times10^{4}$	$53\times10^{5\,b} \pm 26\times10^{5}$	$31\times10^{4\,d} \pm 0.5\times10^{4}$	$35\times10^{3\,b} \pm 0.6\times10^{3}$	$74\times10^{4\,c} \pm 6\times10^{4}$	$36\times10^{4\,c} \pm 10\times10^{4}$
	Overall	$21\times10^{6\,A} \pm 2.1\times10^{6}$	$23\times10^{4\,B} \pm 9\times10^{4}$	$64\times10^{4\,B} \pm 18\times10^{4}$		$14\times10^{5\,A} \pm 2.5\times10^{5}$	$45\times10^{3C} \pm 5\times10^{3}$	$10\times10^{5\,B} \pm 0.8\times10^{5}$	
Coliforms	**T_1**	$10\times10^{5\,a} \pm 0.6\times10^{5}$	N.D	N.D	$33\times10^{4\,a} \pm 16\times10^{4}$	$6\times10^{2\,a} \pm 1\times10^{2}$	N.D	N.D	$2\times10^{2\,a} \pm 1\times10^{2}$
	T_2	$23\times10^{4\,c} \pm 0.5\times10^{4}$	N.D	N.D	$77\times10^{3\,c} \pm 38\times10^{3}$	N.D ^{b}	N.D	N.D	N.D ^{b}
	T_3	$10\times10^{5\,a} \pm 0.5\times10^{5}$	N.D	N.D	$33\times10^{4\,a} \pm 16\times10^{4}$	$3\times10^{2\,a} \pm 0.3\times10^{2}$	N.D	N.D	$1.1\times10^{2\,a} \pm 0.6\times10^{2}$
	T_4	$45\times10^{4\,b} \pm 0.6\times10^{4}$	N.D	N.D	$15\times10^{4\,b} \pm 7\times10^{4}$	N.D ^{b}	N.D	N.D	N.D ^{b}
	Overall	$67\times10^{4\,A} \pm 10\times10^{4}$	N.D ^{B}	N.D ^{B}		$2\times10^{2\,A} \pm 0.8\times10^{2}$	N.D	N.D	
Yeast count	**T_1**	$34\times10^{3\,a} \pm 0.5\times10^{3}$	N.D	N.D	$11\times10^{3\,a} \pm 5\times10^{3}$	$2\times10^{3\,a} \pm 0.6\times10^{3}$	N.D	N.D	$7\times10^{2\,a} \pm 3\times10^{2}$
	T_2	$32\times10^{3\,a} \pm 0.9\times10^{3}$	N.D	N.D	$10\times10^{3\,a} \pm 5\times10^{3}$	N.D ^{b}	N.D	N.D	N.D ^{b}
	T_3	$26\times10^{3\,b} \pm 0.6\times10^{3}$	N.D	N.D	$9\times10^{3\,b} \pm 4\times10^{3}$	N.D ^{b}	N.D	N.D	N.D ^{b}
	T_4	$24\times10^{3\,b} \pm 0.6\times10^{3}$	N.D	N.D	$8\times10^{3\,b} \pm 4\times10^{3}$	N.D ^{b}	N.D	N.D	N.D ^{b}
	Overall	$29\times10^{3\,A} \pm 1\times10^{3}$	N.D ^{B}	N.D ^{B}		$5\times10^{2\,A} \pm 2\times10^{2}$	N.D ^{B}	N.D ^{B}	
Mould count	**T_1**	$19\times10^{3\,a} \pm 0.6\times10^{3}$	N.D	N.D	$63\times10^{2\,a} \pm 31\times10^{2}$	$3\times10^{2\,a} \pm 0.8\times10^{2}$	N.D	N.D	$1\times10^{2\,a} \pm 0.6\times10^{2}$
	T_2	$17\times10^{3\,a} \pm 0.6\times10^{3}$	N.D	N.D	$57\times10^{2\,ab} \pm 28\times10^{2}$	$2\times10^{2\,a} \pm 0.9\times10^{2}$	N.D	N.D	$1\times10^{2\,a} \pm 0.4\times10^{2}$
	T_3	$10\times10^{3\,b} \pm 0.5\times10^{3}$	N.D	N.D	$33\times10^{2\,c} \pm 17\times10^{2}$	N.D ^{b}	N.D	N.D	N.D ^{b}
	T_4	$15\times10^{3\,b} \pm 0.4\times10^{3}$	N.D	N.D	$50\times10^{2\,b} \pm 25\times10^{2}$	N.D ^{b}	N.D	N.D	N.D ^{b}
	Overall	$15\times10^{3\,A} \pm 1\times10^{3}$	N.D ^{B}	N.D ^{B}		60 ^{A} ± 30	N.D ^{B}	N.D ^{B}	

*** Data presented as mean ± *SEM*.**

***Mean values with different lowercase letters superscripts within same column are significantly different at (p<0.05) among treatments.**
***Mean values with different uppercase letters superscripts within same raw are significantly different at (p<0.05) among heat treatment.**
***T_1: raw or pasteurized or cultured pasteurized milk cheeses without any additives or sanitization as a control treatment.**
***T_2: raw or pasteurized or cultured pasteurized milk cheeses with cheese molds and utensils were traditionally cleaned and followed by dipping in 0.25% of 50 vol. hydrogen peroxide for sanitization.**
***T_3: raw or pasteurized or cultured pasteurized milk cheeses with addition of potassium sorbate 0.1% as preservative.**
***T_4: raw or pasteurized or cultured pasteurized milk cheeses were made using both sanitization with 0.25% of 50 vol. hydrogen peroxide and addition of potassium sorbate 0.1% as preservative.**

It was obvious from the obtained results in **(Table 1)** that the application of hydrogen peroxide as a sanitizer had a great effect on reducing the total coliforms level in the produced raw milk Domiati cheese. Where, in fresh raw milk cheese, the mean coliforms count was significantly affected (p<0.0001) by using hydrogen peroxide as a sanitizer as in T_1 it was $10\times10^5 \pm 0.6\times10^5$ MPN/g and reduced to $23\times10^4 \pm 0.5\times10^4$ MPN/g in T_2. While in ripened raw milk cheese, the mean coliforms count was significantly affected (p<0.0001) by using hydrogen peroxide as a sanitizer as in T_1 it was $6\times10^2 \pm 1\times10^2$ MPN/g while it fail to be detected in T_2. It was observed that the ripening period has a germicidal effect on coliforms. Sanitizing of equipment with H_2O_2, seems to has insignificantly affected (p>0.05) the yeast and mould counts; where, the mean yeast and mould counts of fresh raw milk cheese in T_1 were $34\times10^3 \pm 0.5\times10^3$ and $19\times10^3 \pm 0.6\times10^3$ CFU/g which reduced to $32\times10^3 \pm 0.9\times10^3$ and $17\times10^3 \pm 0.6\times10^3$ CFU/g in T_2. This may be attributed that fungi are more resistant to H_2O_2 as compared to other microbes **[26].**

After ripening period, the mean yeasts count of raw milk ripened cheese was significantly affected (p<0.0001) as in T_1 it was $2\times10^3 \pm 0.6\times10^3$ CFU/g while it failed to be detected in T_2. It was noticed that the ripening period has a fungicidal effect on yeasts. The mean moulds count of raw milk ripened cheese was insignificantly affected (p>0.05) as it was $3\times10^2 \pm 0.8\times10^2$ CFU/g in T_1 while it was $2\times10^2 \pm 0.9\times10^2$ CFU/g in T_2. Potassium sorbate is used in dairy industry to prolong the shelf life and quality of dairy products through stopping or delaying the nutritional losses due to microbiological, enzymatic or chemical changes of foods **[12]**.

The effect of adding potassium sorbate 0.1% as preservative on microbial quality of Domiati cheese samples was found in **(Table 1)**, a significant difference (p<0.0001) was recorded between the overall mean SPC T_1 ($11\times10^6 \pm 5\times10^6$ CFU/g) and T_3 ($57\times10^5 \pm 28\times10^5$ CFU/g) in fresh cheese. Similarly, a significant difference (p=0.001) was observed between T_1 ($13\times10^5 \pm 3.5\times10^5$ CFU/g) and T_3 ($85\times10^4 \pm 24\times10^4$ CFU/g) in ripened cheese.

The significant effect of potassium sorbate on SPC has been supported by the results of previous studies carried by **[27]**. However, it should be emphasized that there are other studies reporting that potassium sorbate had insignificant effect on SPC as **[28]**. This discrepancy may be due to different quality of raw materials used during cheese manufacture and the composition of raw milk flora **[28]**.

Coliforms count was insignificantly (p>0.05) affected by the addition of potassium sorbate 0.1% as preservative where in fresh and ripened raw milk cheese the mean count in T_1 was $10\times10^5 \pm 0.6\times10^5$ and $6\times10^2 \pm 1\times10^2$ MPN/g, respectively. While, in T_3 it was $10\times10^5 \pm 0.5\times10^5$ and $3\times10^2 \pm 0.3\times10^2$ MPN/g, respectively; this reduction is attributed to ripening environment rather than the effect of potassium sorbate. There have not been sufficient literatures about the antimicrobial effect of sorbic acid and potassium sorbate on growth of these organisms. Although, the absence of significant effect of potassium sorbate on coliforms were similar to those reported by **[27, 28]**

Addition of potassium sorbate 0.1% as preservative to cheese milk directly improved the fungal count; where, in fresh raw milk cheese, the mean yeasts count was significantly (p<0.0001) reduced from $34\times10^3 \pm 0.5\times10^3$ CFU/g in T_1 to $26\times10^3 \pm 0.6\times10^3$ CFU/g in T_3. Similarly, the mean moulds count of fresh raw milk cheese was significantly (p<0.0001) reduced from T_1 ($19\times10^3 \pm 0.6\times10^3$ CFU/g) to T_3 ($10\times10^3 \pm 0.5\times10^3$ CFU/g); indicating the immediate fungicidal effect of added potassium sorbate.

While, in ripened raw milk cheese, the mean yeast and mould counts were significantly (p<0.0001) affected by potassium sorbate to a higher degree; as the mean yeast and mould counts were T_1 $2\times10^3 \pm 0.6\times10^3$ and $3\times10^2 \pm 0.8\times10^2$ CFU/g, respectively. Where, yeasts and moulds failed to be detected in T_3; indicating the increase of fungicidal effect of potassium sorbate during extended storage period as observed **[28]**. The significant effect of potassium sorbate on yeasts and moulds has been supported by the results stated by **[27, 28, 29]**. Data presented in **(Table 1)** showed the effect of using both 0.25% of 50 vol. hydrogen peroxide as sanitizer and potassium sorbate 0.1% as preservative on the microbiological parameters of Domiati cheese.

SPC in fresh and ripened cheese were significantly affected (p<0.0001) by using both 0.25% of 50 vol. hydrogen peroxide as sanitizer and potassium sorbate 0.1% as preservative as the overall mean of fresh cheese in T_1 was ($11\times10^6 \pm 5\times10^6$ CFU/g) and in T_4 was ($53\times10^5 \pm 26\times10^5$ CFU/g). Whereas, in ripened cheese, the overall mean SPC in T_1 was $13\times10^5 \pm 3.5\times10^5$ CFU/g and in T_4 was $36\times10^4 \pm 10\times10^4$ CFU/g. The effect of using combination of sanitizing with H_2O_2 and adding of potassium sorbate on SPC of cheese were significant than using each treatment separately.

Coliforms count in fresh and ripened cheese were significantly (p<0.0001) reduced by using both

hydrogen peroxide as sanitizer and potassium sorbate as preservative, As in fresh raw milk cheese, the mean coliforms count in T_1 was $10\times10^5 \pm 0.6\times10^5$ MPN/g and in T_4 was $45\times10^4 \pm 0.6\times10^4$ MPN/g. It was noticed that using H_2O_2 as sanitizer alone significantly (p<0.0001) reduced coliforms count; whereas, adding potassium sorbate as preservative alone insignificantly (p>0.05) reduced coliforms. Therefore, the effect of using combination of H_2O_2 and potassium sorbate on coliforms may be attributed to the action of H_2O_2 as sanitizer.

While, in ripened raw milk cheese, the mean coliforms count in T_1 was $6\times10^2\pm 1\times10^2$ MPN/g, where, the organisms failed to be detected in T_4. Failure to detect coliforms in ripened raw milk cheese may be due to the effect of ripening period rather than sanitizing with H_2O_2 and/or adding of potassium sorbate. Sanitizing with H_2O_2 in combination with addition of potassium sorbate as preservative were significantly (p<0.0001) affect yeast and mould counts in fresh raw milk cheese. As, the mean yeast and mould counts were $34\times10^3 \pm 0.5\times10^3$ and $19\times10^3 \pm 0.6\times10^3$ CFU/g in T_1, respectively, while, they were $24\times10^3\pm 0.6\times10^3$ and $15\times10^3 \pm 0.4\times10^3$ CFU/g in T_4, respectively. It was obvious that sanitizing with H_2O_2 alone has insignificant effect while adding potassium sorbate alone has a significant effect on the fungal count in fresh raw milk cheese. Therefore, the significant effect of combination of both two treatments was mainly attributed to immediate fungicidal effect of added potassium sorbate.

While, in ripened raw milk cheese, sanitizing with H_2O_2 in combination with addition of potassium sorbate significantly (p<0.0001) reduced yeast and mould counts. As, the mean yeast and mould counts were $2\times10^3 \pm 0.6\times10^3$ and $3\times10^2 \pm 0.8\times10^2$ CFU/g in T_1, respectively, while, yeasts and moulds failed to be detected in T_4. It was observed that disappearance of fungi in T_4 may be attributed to unfavorable environmental conditions during ripening and the increase in fungicidal effect of potassium sorbate during extended storage period. *S. aureus*, *E.coli*, Salmonellae and *L. monocytogenes* failed to be detected in all fresh or ripened cheese samples regardless of the treatment which may be attributed to the high quality of the used raw milk and ingredients accompanied with hygienic processing techniques.

Sensory evaluation of Domiati cheese is a valuable criterion for determining cheese quality and acceptability. Therefore, organoleptic examination was carried out in the present study. Cheese flavor development is one of the consequences of several chemical changes occurring during ripening period. Flavor compounds are produced through the principal biochemical degradation pathways: glycolysis, lipolysis, and proteolysis. Depending on the variety of cheese, technology, microflora, and ripening conditions, flavor compounds are produced to give unique sensory characteristics to each cheese variety **[4]**.

Data illustrated in **(Table 2)** revealed that the flavor score was significantly (p=0.01) higher in raw and cultured pasteurized milk cheese than in pasteurized milk one. Pasteurized milk cheese had low flavor scores which could be attributed to slow down proteolysis which occur during ripening by natural mircroflora that presence in raw milk cheese **[30]**. Whereas, there was insignificant difference (p>0.05) between flavor score of raw and cultured pasteurized milk cheese as they were nearly similar. This may be attributed to the effect of natural flora initially present in raw milk which participates in flavor production in case of raw milk cheese while in cultured pasteurized milk cheese the addition of starter culture increased the total volatile fatty acids and soluble nitrogen contents via hydrolyzing the cheese protein and fats; therefore, it may enhance the cheese flavor in such treatment. Added starter culture has the ability to produce cheese characteristic flavor through controlled fermentation process resembling that naturally produced from natural flora in raw milk **[9]**. The obtained results were in line with those recorded by **[14, 21, 23]**.

In different treatments used, flavor was significantly affected (p<0.0001) by using hydrogen peroxide either alone or in combination with potassium sorbate, while the flavor was insignificantly affected (p>0.05) by the addition of potassium sorbate alone. These data clearly showed the adverse effect of using H_2O_2 as sanitizer even using food grade type in the recommended concentration and volume on the flavor of finally ripened Domiati cheese after the end of ripening period. This may be attributed to the residual effect of using H_2O_2 as sanitizer on the milk flora, as it slow down the proteolysis which occur during ripening giving the ripened cheese its characteristic flavor **[15]**, while potassium sorbate has mild effect on catalase negative bacteria (lactic acid bacteria) **[29]**.

For body and texture score, cultured pasteurized milk cheese was significantly higher (p<0.05) than both raw and pasteurized milk cheese. The preference in body and texture of cultured pasteurized milk cheese was commented by panelist to smooth texture and compact body with fewer holes and cracks. Smoothness may be related to high moisture probably due to the presence of whey proteins in pasteurized milk, since their hydrophilic property allowed higher moisture retention **[31]**. These results were in agreement with those obtained by **[14]** and disagreed with those recorded by **[21, 23]**.

Table (2): Effect of different improving processing techniques on the sensory quality of ripened Domiati cheese made from raw, pasteurized and cultured pasteurized milk:

Criticisms	Treatment	Ripened cheese			
		Raw milk cheese	Pasteurized milk cheese	Cultured pasteurized milk cheese	Overall
Flavor (40)	T_1	39.6 ^{a} ± 0.3	38 ^{a}± 0.6	38.3 ^{a}± 0.7	38.6 ^{a}±0.4
	T_2	35.3 ^{ab} ± 0.9	30.6 ^{c}± 0.7	38.3 ^{a}± 0.3	34.7 ^{b} ±1.2
	T_3	37.3 ^{ab} ±1.2	37.3 ^{a}± 0.3	36.6 ^{ab}± 0.3	37.1 ^{a} ± 0.3
	T_4	34.6 ^{b}± 0.6	34.6 ^{b}± 0.3	36 ^{b}± 1.2	35.1 ^{b} ± 0.6
	Overall	36.7 ^{A} ± 0.7	35.1 ^{B} ± 0.9	37.3 ^{A} ± 0.3	
Body & texture (40)	T_1	39.6 ^{a} ± 0.3	39 ^{a} ± 0.1	38.3 ^{b}± 0.7	39 ^{a} ± 0.3
	T_2	37 ^{b} ± 1	37 ^{ab} ± 0.6	39.6 ^{a}± 0.3	37.8 ^{b} ± 0.6
	T_3	39 ^{ab} ± 0.1	38.3 ^{b} ± 0.3	38.6 ^{b} ± 0.7	38.6 ^{ab} ± 0.2
	T_4	33.6 ^{c} ± 0.7	37 ^{b} ± 0.6	39 ^{a}± 0.6	36.5 ^{c}± 0.8
	Overall	37.3 ^{B} ± 0.8	37.8 ^{B} ± 0.3	38.9 ^{A} ± 0.2	
Color (10)	T_1	9.3 ^{a} ± 0.3	8.6 ^{a} ± 0.8	8.6 ^{b} ± 0.9	8.8 ^{a} ± 0.4
	T_2	8.6 ^{a} ± 0.9	6.3 ^{b} ± 0.3	9.3 ^{a} ± 0.3	8.1 ^{ab} ± 0.5
	T_3	8.6 ^{a} ± 0.9	8.6 ^{a} ± 0.8	8.6 ^{b} ± 0.9	8.6 ^{ab} ± 0.4
	T_4	8 ^{a} ± 1	6.6 ^{b} ± 0.3	8 ^{c} ± 0.6	7.5 ^{b} ± 0.4
	Overall	8.6 ^{A} ± 0.3	7.5 ^{B} ± 0.4	8.6 ^{A} ± 0.3	
Saltiness (5)	T_1	4.3 ^{a} ± 0.7	4.6 ^{a} ± 0.3	3.8 ^{a} ± 0.3	4.2 ^{a} ± 0.3
	T_2	4 ^{a} ± 0.1	3 ^{b} ± 0.5	4 ^{a} ± 0.6	3.6 ^{a} ± 0.3
	T_3	4.3 ^{a} ± 0.3	4.3 ^{ab} ± 0.3	3.8 ^{a} ± 0.7	4.1 ^{a} ± 0.2
	T_4	3.3 ^{a} ± 0.3	4 ^{ab} ± 0.6	3.8 ^{a} ± 0.3	3.6 ^{a} ± 0.2
	Overall	4 ^{A} ± 0.2	4 ^{A} ± 0.3	4 ^{A} ± 0.2	
Style (5)	T_1	5 ^{a}	5 ^{a}	5 ^{a}	5 ^{a}
	T_2	5 ^{a}	5 ^{a}	5 ^{a}	5 ^{a}
	T_3	5 ^{a}	5 ^{a}	5 ^{a}	5 ^{a}
	T_4	5 ^{a}	5 ^{a}	5 ^{a}	5 ^{a}
	Overall	5 ^{A}	5 ^{A}	5 ^{A}	
Overall acceptability (100)	T_1	98 ^{a} ± 1	95.3 ^{a} ± 1.6	94.3 ^{a} ± 0.8	95.8 ^{a} ± 0.8
	T_2	90 ^{b} ± 2.5	81.6 ^{b} ± 0.9	96.3 ^{a} ± 0.3	89.3 ^{b} ± 2.2
	T_3	94.3 ^{ab} ±1.3	94 ^{a} ± 1.5	92.6 ^{a} ±2	93.6 ^{a} ± 0.9
	T_4	84.6 ^{b} ± 1.8	87 ^{b} ± 0.5	94 ^{a} ± 0.6	88.5 ^{b} ± 1.5
	Overall	91.7 ^{B} ± 1.6	89.5 ^{C} ± 1.7	94.3 ^{A} ± 0.6	

*** Data presented as mean ± *SEM*.**
***Mean values with different lowercase letters superscripts within same column are significantly different at (p<0.05) among treatments.**
***Mean values with different uppercase letters superscripts within same raw are significantly different at (p<0.05) among heat treatment.**
***T_1: raw or pasteurized or cultured pasteurized milk cheeses without any additives or sanitization as a control treatment.**
***T_2: raw or pasteurized or cultured pasteurized milk cheeses with cheese molds and utensils were traditionally cleaned and followed by dipping in 0.25% of 50 vol. hydrogen peroxide for sanitization.**
***T_3: raw or pasteurized or cultured pasteurized milk cheeses with addition of potassium sorbate 0.1% as preservative.**
***T_4: raw or pasteurized or cultured pasteurized milk cheeses were made using both sanitization with 0.25% of 50 vol. hydrogen peroxide and addition of potassium sorbate 0.1% as preservative.**

At the same way of flavor, the use of either H_2O_2 alone or in combination with potassium sorbate adversely (p<0.05) affects final ripened cheese body and texture. While, using potassium sorbate alone had insignificant (p>0.05) effect on body and texture due to its mild effect on the lactic acid bacteria **[29]**. Therefore, the negative effect on body and texture in the combination treatment could be attributed to the residual effect of H_2O_2 on the microflora of milk. Also, data presented in **Table 2** revealed that the color score of ripened Domiati cheese was significantly (p=0.04) higher in raw and cultured pasteurized milk cheese than in pasteurized milk cheese. This change in color may be attributed to the initial cheese milk composition, manufacturing procedures, and maturation conditions **[32]**. The obtained results of color were similar to those observed by **[21]**. Whereas, the results were disagree with those recorded by **[14, 23, 31]**.

The color score was insignificantly (p>0.05) affected by using H_2O_2 as sanitizer alone or using potassium sorbate as preservative alone, but it was significantly (p<0.05) affected by using both treatments together. There were insignificant differences (p>0.05) between raw, pasteurized and cultured pasteurized milk cheese either (T_1) or (T_2), (T_3) and (T_4) on saltiness and style scores for ripened Domiati cheese as they were nearly similar.

The overall acceptability of ripened Domiati cheese was significantly (p<0.05) affected by the used cheese milk, as the highest overall acceptability was recorded for cultured pasteurized followed by raw milk then finally pasteurized milk cheese; which explore the benefits of using cultured heat treated milk. As, the Egyptian consumer, used to consume Domiati cheese brands made from raw milk due to its beloved organoleptic characteristics. By using heat treated milk as per the regulatory and statutory requirements, the produced Domiati cheese lost its organoleptic characteristics. So, in trail to improve the

organoleptic characteristics of Domiati cheese, this work was carried out by using starter culture in combination with heat treated milk to substitute the effect of heat treatment on the natural microbiota of milk. For different treatments, using potassium sorbate as a preservative the overall acceptability was insignificantly (p>0.05) affected, while using hydrogen peroxide as sanitizer alone or in combination with potassium sorbate as preservative had adverse effect (p<0.0001) on the overall acceptability of ripened cheese. Therefore, the negative effect on the overall acceptability was attributed to the residual effect of H_2O_2 as sanitizer.

Conclusion:

Findings in this study showed that pasteurization of raw milk used in manufacturing of Domiati cheese positively affect the microbiological quality, but negatively affect the organoleptic parameters of cheese. To overcome this negative effect, starter culture is recommended to be added to pasteurized milk. The use of cultured pasteurized milk in the manufacture of Domiati cheese improved both the microbiological and sensory parameters of final product after the end of ripening period. On the other side, this study emphasized the importance of cleaning and sanitization of utensils and equipment during cheese manufacture. Sanitizing of food contact surfaces with food grade 0.25% hydrogen peroxide as a sanitizer had a significant effect on the microbiological quality of the produced cheese. However, it negatively affects the overall acceptability of organoleptic parameters in addition to leaving foreign flavor aftertaste. It was found that addition of potassium sorbate 0.1% as preservative was effective in controlling the microbial growth with no effect on final product sensory parameters. Therefore, Domiati cheese makers are recommended to use cultured pasteurized milk with addition of potassium sorbate, as they not only had a nearly similar sensory parameters to that arise from raw milk cheese but also more safer to the consumer.

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