

# Prevalence of Some Deteriorating Microorganisms in Some Varieties of Cheese

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## Abstract

**Aim:** This work throws light on the deteriorating microorganisms in some varieties of cheeses [hard cheese (Ras cheese), and processed cheese]. **Material and Method:** Thirty Ras cheese and processed cheese samples were collected randomly and subjected to chemical & microbiological examination to determine its contamination with deteriorating microorganisms. **Results:** Chemical examination showed that the mean titratable acidity % of Ras and Processed cheese samples were  $0.5 \pm 0.073$  and  $0.93\% \pm 0.062\%$ , respectively, while salt % were  $3.4 \pm 0.078$  and  $1.03\% \pm 0.05\%$ , respectively. Most of samples were contaminated with high numbers of Coliforms, yeast, mold, proteolytic, lipolytic and Enterococci. **Conclusion:** There are poor hygienic measures during production, processing and handling of cheese.

## Keywords

Titratable Acidity, Salt, Proteolytic Microorganisms, Coliforms, Enterococci

## 1. Introduction

Dairy food spoilage varies according to food type, microbial type and numbers that cause changes in color, odor and texture with sliminess, gas and liquid accumulation, microorganisms must multiply and reach “spoilage detection level”. According to the nature of spoilage and types of microbes, the spoilage detection level can range from  $10^6$  -  $10^8$  cells/g or /ml.

Spoilage microorganisms include psychrotrophic bacteria, yeast, mold, hetero fermentative lactobacilli, and spore-forming bacteria. Psychrotrophic bacteria produce extracellular hydrolytic enzymes, and contamination of pasteurized products with these bacteria affect their shelf life. Fungal spoilage of dairy foods is manifested by the presence of a wide variety of metabolic by-products, causing off-flavors, and changes in color or texture. Coliforms, yeast, hetero fermentative lactic acid bacte-

ria and spore-forming bacteria cause gas defects in cheese. There are a wide variety of spoilage microorganisms in dairy foods due to practices used in production, formulation, processing, packaging, storage, distribution, and handling [1].

Fermentation of lactose lower pH acts as a preservative and allows desirable microflora to restrict undesirable microorganisms growth; adding salt to reduce the water activity ( $a_w$ ); packaging with less oxygen and freezing slow spoilage rate of dairy foods [1].

This work was planned to throw light on the deteriorating microorganisms in some varieties of cheeses [hard cheese (Ras cheese), processed cheese].

## 2. Material and Methods

### 2.1. Sample Collection

Thirty each of hard cheese (Ras), and processed cheese was randomly collected as sold to consumers from dairy shops in Cairo and Giza governorates.

### 2.2. Chemical Examination

Titrateable acidity percentage was measured according to [2] and NaCl content according to Volhard method [3].

### 2.3. Microbiological Examination

Preparation of decimal dilutions for the collected samples, Total coliform count with Completed test for *E. coli*, Total proteolytic count, Lipolytic microorganisms count, Enterococci count, Total Yeast and Total Mold count were examined according to [3] with bio chemical identification of the isolated Coliforms & Enterococci species were examined according to [4]. While yeast species were examined according to [5].

## 3. Results and Discussion

### 3.1. Chemical Examination

**Titrateable acidity percentage:** Increase of titrateable acidity is a monitor of freshness and the bacterial activity in milk [1] [6]. While lower pH of milk to less than 4.6 protects the fermented dairy products from contamination with pathogens and considers safe hygienically [7] [8]. Data recorded in **Table 1** showed that the mean titrateable acidity percentage of the examined Ras and Processed cheese samples were  $0.5 \pm 0.073$  and  $0.93\% \pm 0.062\%$ , respectively. Nearly similar

**Table 1.** Statistical analytical results of titrateable acidity % & salt % in the examined samples.

	Total No. of samples	Min.	Max.	Mean $\pm$ S.E.M.
Titrateable acidity percentages	Ras cheese	0.3	0.93	$0.5 \pm 0.073$
	Processed cheese	0.46	1.7	$0.93 \pm 0.062$
Salt percentages	Ras cheese	2.8	4.7	$3.4 \pm 0.078$
	Processed cheese	0.51	1.4	$1.03 \pm 0.05$

findings of Ras cheese were obtained by [9]. Higher results of processed cheese were reported by [10]. Whereas, lower findings of Processed cheese were obtained by [9] [11].

**Salt percentage:** Salt added is an important step in most cheese varieties; it enhances flavor, draws the whey out of the curd and controls the undesirable microorganisms. The salt content of cheese ranges from about 0.5% to 0.7% (w/w) in acid curd varieties, such as cottage cheese to about 4% to 6% (w/w) in pickled cheeses [12] [13]. Regarding the salt percentage in examined cheese samples (Table 1), it ranged from 2.8 - 4.7 and 0.51 - 1.4 with a mean value of  $3.4 \pm 0.078$  and  $1.03\% \pm 0.05\%$  in the examined samples of Ras cheese and Processed cheese, respectively. Nearly similar results in processed cheese were recorded by [9]. While lower obtaining of Ras cheese were recorded by [9].

The variation of salt content in the examined cheese samples is due to the added salt depends on individual dairies, and there is no standard in addition of salt to cheese and the amount of salt relies on discretion of cheese maker [12]. The high salt content in some examined samples can mask the poor bacteriological quality of the raw milk and prevent gas holes formation and off-flavor especially in summer [14].

### 3.2. Microbiological Examination

**Coliforms contents (MPN/g):** Coliform count is an indicator of possible fecal contamination, microbial quality and reflects the hygienic conditions in the food processing [15]. Regarding the results represented in Table 2, coliform could be detected in all of the examined samples of Ras cheese and Processed cheese, with mean values of  $1.2 \times 10^{10} \pm 3.3 \times 10^9$  and  $2.5 \times 10^9 \pm 6.5 \times 10^8$  MPN/g in Ras and Processed cheese, respectively.

Lower results of processed cheese were reported by [9] [16] [17]. Lower results

**Table 2.** Statistical analytical results of the microbiological parameters in the examined cheese samples.

	Total No. of samples	Positive samples		Min.	Max.	Mean $\pm$ S.E.M.
		No.	%			
Coliform count (MPN/g.)	Ras cheese	30.0	100.0	$2.5 \times 10^8$	$1.2 \times 10^{11}$	$1.2 \times 10^{10} \pm 3.3 \times 10^9$
	Processed cheese	30.0	100.0	$9 \times 10^6$	$1.3 \times 10^{10}$	$2.5 \times 10^9 \pm 6.5 \times 10^8$
Total proteolytic count (cfu/g)	Ras cheese	30.0	100.0	$120 \times 10^5$	$7.4 \times 10^{11}$	$5.8 \times 10^{10} \pm 4.5 \times 10^{10}$
	Processed cheese	18.0	60.0	150.0	$3.4 \times 10^7$	$6.3 \times 10^6 \pm 2.8 \times 10^6$
Total lipolytic count (cfu/g)	Ras cheese	30.0	100.0	$10^3$	$4.8 \times 10^9$	$3.0 \times 10^8 \pm 1.2 \times 10^8$
	Processed cheese	12.0	40.0	110.0	$5.7 \times 10^4$	$16.8 \times 10^3 \pm 5.4 \times 10^3$
Enterococci count (cfu/g)	Ras cheese	30.0	100.0	$7 \times 10^3$	$8.8 \times 10^9$	$7.7 \times 10^8 \pm 7.2 \times 10^8$
	Processed cheese	30.0	100.0	$15 \times 10^3$	$67 \times 10^9$	$4.3 \times 10^8 \pm 2.7 \times 10^8$
Total yeast count (cfu/g.)	Ras cheese	30.0	100.0	$6 \times 10^3$	$1.1 \times 10^8$	$4.9 \times 10^7 \pm 5.6 \times 10^6$
	Processed cheese	25.0	83.3	300.0	$3.1 \times 10^4$	$2.57 \times 10^5 \pm 1.8 \times 10^5$
Total mold count (cfu/g.)	Ras cheese	10.0	33.3	$5 \times 10^3$	$4 \times 10^6$	$3.6 \times 10^5 \pm 2.5 \times 10^5$
	Processed cheese	17.0	56.67	200.0	$10^7$	$6.7 \times 10^5 \pm 6.2 \times 10^5$

of Ras cheese were reported by [9] [17]. The results revealed that all of the examined Ras cheese and processed cheese contain high numbers of coliform. Egyptian Standards recommend the count should be less than 10 cells/g [15], the obtained results makes these cheeses of bad quality and cause losses economically [18].

**Isolated coliforms:** Biochemical identification of Coliform organisms in Ras cheese, results revealed that *Enterobacter intermedium* (37.1%) was the most frequent one followed by *Citrobacter diversus* (16.13%), *Citrobacter freundii* (12.9%) and *Klebsiella oxytoca* (11.29%), while *E. coli* could be detected only in one isolate. *Enterobacter intermedium* (21.43), *E. coli* (20%) & *Citrobacter freundii* (20%) had the highest incidence, then *Klebsiella oxytoca* (12.86%), *Citrobacter diversus* (11.4%) and *Erwinia chrysanthemi* (7.14%) in the examined Processed cheese (**Table 3**).

*E. coli* was found in Ras cheese and processed cheese with percentages of 1.61 and 20%, respectively. *E. coli* is due to fecal contamination, indicates that other pathogens of fecal origin may be found [19]. Results presented in **Table 4** illustrated the serological identification of the suspected isolates of *E. coli* by slide agglutination test. In Ras cheese, the suspected isolates were negative, while in processed cheese one strain out of 14 strains was positive and classified as *E. coli* O<sub>25</sub>. *Escherichia coli* is present in the gastrointestinal tract of man and dairy animals. Milk can be contaminated either directly through animal feces or indirectly through contaminated surrounding environment, workers, and equipments. Most strains of *E. coli* are harmless commensals; some causes human gastrointestinal disease with mild to severe symptoms that may be fatal in high-risk individuals. Moreover, *E. coli* was reported for cases of pyelonephritis cystitis, pyelitis, peritonitis and appendicitis [20] [21].

**Total yeast count:** Yeast contamination is economically hazard as it causes

**Table 3.** Incidence of isolated coliforms from the examined samples.

Isolated Coliform	Ras cheese		Processed cheese	
	No.	%	No.	%
<i>Citrobacter diversus</i>	10.0	16.13	8.0	11.4
<i>Citrobacter freundii</i>	8.0	12.9	14.0	20.0
<i>E. coli</i>	1.0	1.61	14.0	20.0
<i>Enterobacter aerogenes</i>	2.0	3.23	0.0	0.0
<i>Enterobacter cloaca</i>	0.0	0.0	0.0	0.0
<i>Enterobacter intermedium</i>	23.0	37.1	15.0	21.43
<i>Erwinia chrysanthemi</i>	4.0	6.45	5.0	7.14
<i>Klebsiella oxytoca</i>	7.0	11.29	9.0	12.86
<i>Klebsiella pneumoniae</i> subsp. <i>ozaenae</i>	0.0	0.0	0.0	0.0
<i>Serratia fonticola</i>	4.0	6.45	3.0	4.3
<i>Serratia rubidaea</i>	3.0	4.84	2.0	2.88
Total	62.0	100.0	70.0	100.0

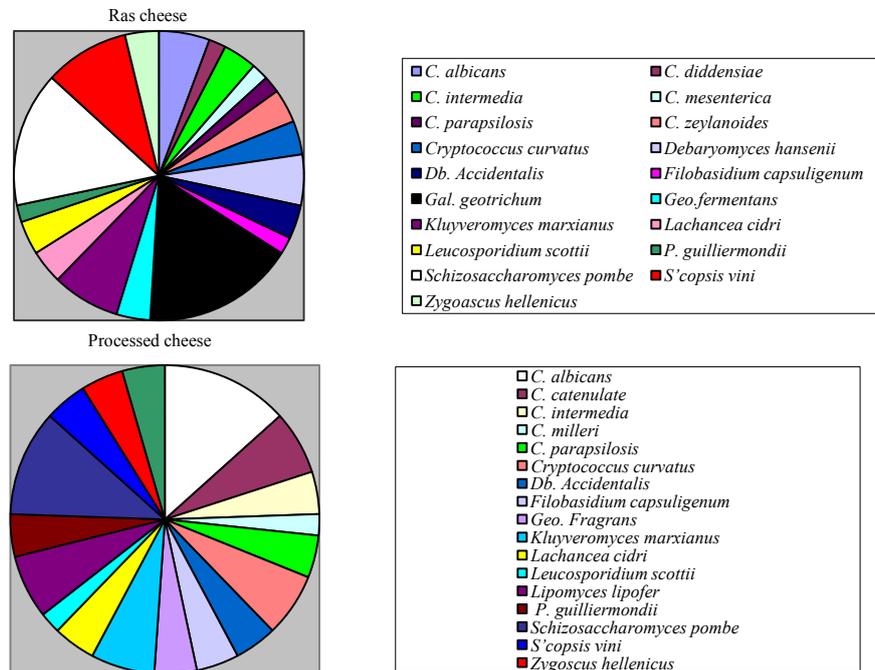
**Table 4.** Serological identification of suspected *E. coli* isolates by slide agglutination test.

Type of sample	No. of suspected <i>E. coli</i>		Slide agglutination test		
	No.	%	ve+	%	Serotype
Ras cheese	1	1.61	0.0	0.0	-
Processed cheese	14	20	1.0	25.0	O <sub>25</sub>

objectionable changes as yeasty flavor and frothy consistency. Some species of yeast cause public hazard as endocarditis, gastrointestinal disturbance, and sometimes, fatal systemic diseases [22]. Data in **Table 2** revealed that contaminated yeast could be detected in 30 (100%) and 25 (83.3%) of Ras cheese and Processed cheese, respectively, with mean values of  $4.9 \times 10^7 \pm 5.6 \times 10^6$  and  $2.57 \times 10^5 \pm 1.8 \times 10^5$  cfu/g, respectively. Nearly similar results of Ras cheese were recorded by [9] [17], while lower findings were recorded by [23]. Lower results of Processed cheese were obtained by [11] [24], while higher results were recorded by [9] [17]. According to the Egyptian standards the count of total viable yeast should not exceed 100 cfu/g in hard cheese [25], 100% of Ras cheese samples was disagreed with this standard. This high level may be due to poor hygienic measures during production or using raw materials of bad quality [26].

**Isolated yeast species:** from **Figure 1**, the obtained results of cheese proved that *C. albicans*, *C. diddensiae*, *C. intermedia*, *C. mesenterica*, *C. parapsilosis*, *C. zeylanoides*, *Cryptococcus curvatus*, *Debaryomyces hansenii*, *Db. Accidental*, *Filobasidiumcapsuligenum*, *Gal. Geotrichum*, *Geo. fermentans*, *Kluyveromyces marxianus*, *Lachanceacidri*, *Leucosporidiumscottii*, *P. guilliermondii*, *Schizosaccharomyces pombe*, *S. copsisvini* and *Zygoascushellenicus* were isolated from the examined samples of Ras cheese with concern that *Gal. geotrichum* (16.98%) and *Schizosaccharomyces pombe* (15.09%) had the highest incidence among the isolated strains. On Processed cheese, *C. albicans* (13.34%) was the most frequent one then *Schizosaccharomyces pombe* (11.13%); *C. catenulate* (6.67%); *Cryptococcus curvatus* (6.67%); *Kluyveromyces marxianus* (6.67%) and *Lipomyces lipofer* (6.67%). High acidity, low moisture content, storage at low temperature, and high salt concentration enhance growth of yeast. These spoilage yeast cause gas production, discoloration, soften texture, and fruity, bitter or yeasty off flavors [27] [28]. *Candida* spp. are the most common fungal infection in immune compromised persons known as candidiasis, which caused by *C. albicans* [29] [30].

**Total mold count:** Presence of mold cause economic losses because it is accompanied by visible spoilage discoloration, off flavor, and rejection of the product, and may be a source of mycotoxins result in outbreaks of human food poisoning and many diseases as cancer, leukemia, and kidney toxicity [31] [32]. Regarding the data recorded in **Table 2** mold was present in 33.3% and 56.67% of the examined Ras cheese and Processed cheese samples, respectively, The counts were ranged from  $5 \times 10^3$  -  $4 \times 10^6$  and  $200$  -  $10^7$  cfu/g, with mean values of  $3.6 \times 10^5 \pm 2.5 \times 10^5$  and  $6.7 \times 10^5 \pm 6.2 \times 10^5$  cfu/g, respectively. Nearly similar

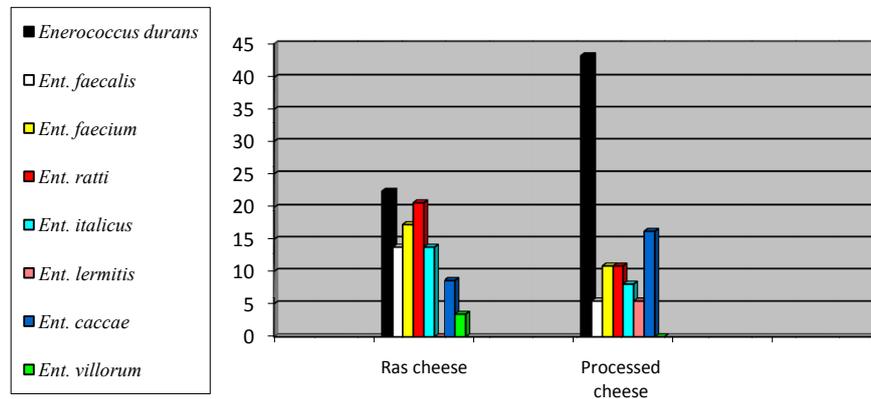


**Figure 1.** Incidence of isolated yeast from the examined sample.

findings of Processed cheese were reported by [9] [17], while Lower results of Processed cheese were recorded by [23] [24]. Nearly similar findings of Ras cheese were recorded by [17]. Higher obtaining of Ras cheese was recorded by [9], while lower outcomes were reported by [23]. It was found that 33.3 and 56.67% of the examined Ras cheese and Processed cheese, respectively were highly contaminated with mold and disagree with the Egyptian standards [15] [33] because of the warm weather and inadequate refrigeration, increased species diversity [34]. The fungi contributed to poor cleaning and unhygienic measures during production and handling and post processing contamination [35] [36].

**Total proteolytic count:** *Bacillus* spp. can survive heat treatment of milk [37] [38]. They can produce thermo stable hydrolytic enzymes are produced by *Bacillus* spp [39] [40]. *B. stearothermophilus*, *B. licheniformis*, *B. coagulans*, *B. cereus*, *B. subtilis* and *B. circulans* are the most commonly isolated species. Moreover, *Bacillus* species such as *B. cereus*, *B. licheniformis* and *B. subtilis* can produce many types of toxins associated with food borne diseases [41] [42]. The proteolytic enzymes may cause undesirable changes in texture [25] [43] and in flavor (especially bitterness). According to the obtained results in (Table 2) it was cleared that proteolytic organisms were present in 30 (100%) and 18 (60%) of the examined Ras cheese and Processed cheese, with a mean count of  $5.8 \times 10^{10} \pm 4.5 \times 10^{10}$  and  $6.3 \times 10^6 \pm 2.8 \times 10^6$  cfu/g, respectively. Lower results of Ras & Processed cheese were obtained by [11] [44].

**Total lipolytic count:** Psychrophilic bacteria like *Pseudomonas*, *Enterobacter*, *Alcaligenes* and some spore-formers produce extracellular lipase enzyme



**Figure 2.** Incidence of isolated Enterococci from the examined cheese sample.

that is adsorbed on milk fat globules and concentrated in the manufactured cheese. These enzymes hydrolyze triglyceride fat constituents of short chain fatty acids, including butyric, caproic, caprylic, and capric acids resulting in unpleasant odors in milk and rancidity in cheese [39] [45]. Results detected in **Table 2** revealed that lipolytic organisms were present in Ras and Processed cheese samples in percentages of 100% and 40%, respectively, with a mean count of  $3 \times 10^8 \pm 1.2 \times 10^8$  and  $16.8 \times 10^3 \pm 5.4 \times 10^3$  cfu/g, respectively. Lower results of Ras & Processed cheese were obtained by [11] [44].

**Total Enterococci count:** Enterococci are useful indicator to the hygienic conditions during food manufacture. The presence of specific virulence factors in some strains of *Enterococcus faecalis* and to a lesser extent in strains of *Enterococcus faecium* makes them pathogenic to human [46] [47]. Using contaminated water supply, equipment and unhygienic conditions during the production, handling, and milk processing are the causes for their presence [48] [49] [50]. The given results recorded in **Table 2** illustrated that Enterococci were isolated from all examined cheese samples with a mean value of  $7.7 \times 10^8 \pm 7.2 \times 10^8$  and  $4.3 \times 10^8 \pm 2.7 \times 10^8$  cfu/g in the examined Ras and Processed cheese samples, respectively. Lower results of Ras cheese were recorded by [17]. Nearly similar findings of processed cheese were recorded by [17], whereas lower results were recorded by [51].

**Isolated Enterococci:** The biochemical identification of Enterococci isolates in the examined cheese samples showed that *Ent. durans* (22.42%), *Ent. ratti* (20.69%) and *Ent. faecium* (17.24%) had the highest incidence, then *Ent. faecalis* (13.79%), *Ent. italicus* (13.79%) and *Ent. caccae* (8.63%) in the examined Ras cheese samples. *Ent. durans* was the most frequent one in processed cheese samples, followed by *Ent. caccae* (16.22%), *Ent. faecium* (10.81%), *Ent. ratti* (10.81%) and *Ent. italicus* (8.1%) (**Figure 2**).

Some enterococci of dairy origin have been recorded to the production of bacteriocins (enterocins) which has inhibitory effect to some pathogenic bacteria (*Listeria monocytogenes* and *Salmonella* spp.) [52]. Some studies have reported that *E. faecium* and *E. faecalis* may be a potential recipient of vancomycin resistance genes, so, [49] has recommended that *E. faecium* should not be considered

as probiotics for human use. Also, Enterococci are pathogens causing endocarditis, bacteraemia, and urinary tract infections [16].

### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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