Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Occurrence of Some Spoilage Microorganisms in Cheese Highlighting The Impact of Salt and Starter Culture Activity on The Viability of *Pseudomonas aeruginosa* and *Candida albicans*

Lamiaa Ibrahim Ahmed*, Eman M. Taher, Karima Mogahed Fahim and Zeinab I. Ali

Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University, 12211 Giza, Egypt.

Abstract

CHEESE CONTAMINATION with spoilage microorganisms causes significant economic losses in food industry. In this study, 100 cheese verities (Kariesh, Domiatti, Ras, and Processed (25 each)) were gathered from different markets across Cairo governorate, Egypt. Chemical assessment (Salt% and Titratable acidity) and Microbiological evaluation (Total yeast, Mold, Lipolytic, and Proteolytic counts) were done. The results found that both Kariesh and Domiatti cheeses showed high incidence of yeast that reached 2.6×10^{10} and 4.7×10^{10} cfu/g, respectively. Moreover, Kariesh and Ras cheeses exhibited the highest proteolytic and lipolytic counts, nevertheless, Kariesh cheese had the highest mold count with mean value of 4.6×10^{8} cfu/g. The second part of the study studied the effect of salt percentage and starter culture activity represented in its produced acidity on viability of *Pseudomonas aeruginosa* 27853 and *Candida albicans* during the cold storage of lab-manufactured fresh and ripened white soft cheeses. The obtained results demonstrated that *P. aeruginosa* could not be detected in fresh or ripened soft cheese by the end of the sixth and third weeks of storage period, respectively. While *C. albicans* continued to grow in both fresh and ripened soft cheese samples until visible deterioration appeared (holes and yeasty flavor) by the ninth week. In contrast to *C. albicans*, the study's findings revealed that *P. aeruginosa* could be efficiently controlled by a combination of salt and acidity presenting a safe and feasible approach for spoilage control in cheese sector.

Keywords: Acidity, Salt, Spoilage, *P.aeruginosa*, and *C.albicans*.

Introduction

The growing global population has led to greater demand for safe food with high quality since deterioration of food has caused food insecurity in several parts of the world, as well as significant economic losses for both producers and consumers. The rate of dairy food spoiling can be affected by factors such as storage temperature, pH, water availability, the presence of spoilage microorganisms like bacteria and fungi, the initial microbial load, and processing technologies [1]. The post-pasteurization contamination with psychotropic bacteria, yeast and mold can enhance the deterioration of dairy products by producing heat-stable extracellular and/or intracellular hydrolytic enzymes that remain active even after pasteurization [2].

Egypt is one of the main producer and consumer of cheeses in Africa and the Middle East because of their nutritional, economic, and health benefits [3]. On the other hand, cheese is prone to both spoilage and opportunistic microorganisms. The rate of deterioration is influenced by pH, salt percentage, temperature, lactic starter culture characteristics, the kind and viability of contaminating microorganisms, and the properties and quantities of remaining enzymes. Consequently, cheeses spoil differently; soft cheeses with the highest pH values and the lowest salt concentrations decomposed quickly. While ripened cheese, on the other hand, retains its optimal eating qualities for a long time owing to its low pH and high salt content [4,5].

Psychrotrophic pseudomonas and acid-tolerant psychrotrophic fermentative yeast, i.e., some candida species, were recognized as major spoilage

*Corresponding author: Lamiaa I. Ahmed, E-mail: lamiaa_13@cu.edu.eg, lamiaa_13@yahoo.com, Tel.: +201094864330
(Received 06 September 2024, accepted 05 January 2025)
DOI: 10.21608/EJVS.2025.315453.2361

microorganisms of cheese due to their extracellular enzymes and metabolic by-products. In addition, yeast contamination has a significant impact on cheese quality and safety. Since, some species of yeast can metabolize lactate and citrate, resulting in de-acidification of the cheese surface and enhancing mold and microbial growth. Others exhibit proteolytic and lipolytic activity; however, this activity varies by species and strain [6]. Such deterioration is often characterized by off -flavors, and visible changes in color and texture, as well as production of alcohol and CO_2, which causes blowing (Doming - Early gas formation) and sliminess [5,6].

Fermentation is one of the earliest forms of cheese preservation. It suppresses the growth of spoilage and food-borne pathogens by producing organic acid and antibacterial compounds such as bacteriocins, hydrogen peroxide, diacetyl, and CO_2, by the used lactic acid starter culture species [7]. Salting of cheese not only enhances its flavor and serves as a source of dietary sodium, but also is regarded as a primary method of cheese preservation. Salt, together with the appropriate pH, water activity, and redox potential, help to preserve cheese by inhibiting growth of the spoilage and pathogenic microorganisms [8].

Hence, this study was designed in two parts; the survey part aimed to investigate the prevalence of some deteriorating microorganisms (yeast, mold, proteolytic, and lipolytic microorganisms) in various types of cheese, and the second part included viability studies aimed to evaluate the effect of salt and starter culture activity represented in the produced acidity on the viability of *P. aeruginosa* 27853 and *C. albicans* (major deteriorating microorganism) in laboratory-manufactured fresh and ripened soft cheese model over storage period.

Material and Methods

Survey study:

Sample Collection

A total of hundred random samples of Kariesh cheese, ripened soft cheese (Domiatti), hard cheese (Ras), and Processed cheese (25 of each) were collected from local dairy markets in Cairo governorate, Egypt, during the period from September/2022 to June/2023. Samples were transported to the laboratory in an insulating ice - box as soon as possible for further examination.

Microbiological and chemical analysis

Decimal dilutions of the examined samples were prepared followed by determining the total yeast, mold, lipolytic, and proteolytic microorganisms counts on their specific media (Hi media) according to the method described by APHA [9]. The isolated strains of yeast, lipolytic and proteolytic microorganisms were further biochemically identified. Briefly, microscopical examination, observation of yeast morphology, urea hydrolysis, growth in the presence of cycloheximide 0.01%, growth at 37°C, growth without vitamins, glucose fermentation, carbon and nitrate assimilation test, germ tube test were used for yeast identification. Regarding proteolytic and lipolytic microorganisms, microscopical examination, catalase activity test, sugar fermentation, pigment production, blood hemolysis, coagulase test, thermostable nuclease, oxidase test, Voges Proskauer, growth at 10, 45, 50 °C and 65°C, growth in 2%, 6.5 and 7 % NaCl, starch hydrolysis, egg yolk lecithinase, detection of proteolysis, citrate utilization, arginine dehydrolase, and aesculin hydrolysis. All tests were applied according to Bergey's manual of systematic bacteriology [10]**.** Moreover, titratable acidity and salt percentages were determined according to APHA [9]. All tests were done in triplicates.

Viability studies in lab- manufactured fresh and ripened white soft cheeses:

Used Milk

Raw cow's milk obtained from the Dairy Industry Unit, Animal Production Research Institute, Ministry of Agriculture, Giza, Egypt. Milk was free from any inhibitory substances as approved by Lactic Acid Activity Test [11].

Cheese starter culture

Lyophilized culture for direct vat set (DVS) type Mesophilic/Thermophilic culture blend (Safel T3) was used for ripened cheese manufacture. The culture Chr. Hansen Laboratories, Copenhagen, Denmark was used according to the manufacturer's description.

Preparation of the tested strains

The study used the reference strain of *Pseudomonas aeruginosa* ATCC 27853 that obtained from the Dairy department at the National Research Centre, Dokki, Giza, Egypt, and the isolated and molecularly identified *Candida albicans*. The selected strains were inoculated separately into Tryptic soya broth with 0.6% yeast extract and incubated at 25°C for 2 days (*P. aeruginosa)* and 25°C for 3 days (*C. albicans)*. The first inoculum counts were determined by spreading on Pseudomonas agar base and Sabaroud dextrose agar (Himedia) for *P. aeruginosa* and *C. albicans,* respectively from both the incubated tryptic soya broth and milk at the time of inoculation according to the method reported by Ahmed *et al.* [12].

Viability of P. aeruginosa and C. albicans in lab-manufactured fresh and ripened soft cheeses

Lab-pasteurized cow's milk was divided into two portions; the first one was inoculated with the tested *P. aeruginosa* (6×10^7cfu/ml) and the second part was inoculated with *C. albicans* (3×10^6 cfu/ml) followed by gentile mixing. Each group was then subdivided into three treatments as follows; T1: supplemented with 3% salt (fresh cheese; for studying the effect of

salt only); T2: supplemented with 3% salt and cheese starter culture (ripened cheese; for studying the effect of salt and acidity), and T3: left without salt and starter culture addition as control group (fresh unsalted cheese; for observing the viability of the inoculated microorganisms in absence of salt and acidity factors). The prepared treatments were then processed according to the method illustrated by Ahmed *et al.* [12]. Briefly, the exact amount of rennet as described by the manufacturer and calcium chloride ($CaCl_2$) at a rate of 0.02% as maximum were added to the previously prepared milk of the different treatments, followed by stirring/5minutes/and left undisturbed in a thermostatically controlled water bath at 32ºC till curd formation. Curd was cut into 6–8 mm cubes and whey was drained off through a colander with a cheese cloth, followed by light hand pressure. Curd was then distributed into cylindrical mold and cheese was pressed overnight at 4ºC using 0.015 kg/cm^2 pressure to drain the excess whey. The produced cheese groups were stored in brine solution with 7 % salt and stored at 4 ºC until the inoculated organisms disappeared or the cheese deteriorated. Samples were examined from the inoculated milk, cheese after manufacture as zero time, after one day for *P. aeruginosa* and after three days for *C. albicans*, and afterward, examined every week during the storage period for total colony counts of the inoculated microorganisms, titratable acidity, and salt percentages by Volhard method according to APHA [9].

Statistical analysis

All experimental trials were carried out in triplicate, and the mean results were recorded. The mean ± standard error of mean (SEM) was used to express the quantitative variables using IBM SPSS statistics for Windows, version 25.0. The correlation between the total counts of the inoculated microorganisms, acidity and salt percentages was determined using Pearson's test for correlation.

Results

The obtained results of microbiological analysis revealed that both Kariesh and Domiatti cheeses had high yeast count of mean 2.6 $\times10^{10}$ and 4.7$\times10^{10}$ cfu/g, respectively while, Ras and Processed cheeses recorded 3.9$\times10^{7}$ and 2.97$\times10^{5}$ respectively. Regarding the mold count, Kariesh cheese was the highest 4.6X10^{8} while Domiatti, Ras and Processed cheese recorded 1.7$\times10^{4}$, 3.1$\times10^{5}$, and 7.6$\times10^{5}$ respectively. Ras and Kariesh cheese, the highest proteolytic count was observed 6.1$\times10^{10}$ and 1.7$\times10^{10}$ respectively. Processed cheese were the lowest contaminating samples with mean lipolytic count of 1.7$\times10^{10}$, while Kariesh and Ras were the highest mean of 7.2$\times10^{8}$ and 3.1$\times10^{8}$ (Table 1).

Table 2. demonstrated the chemical assessment where salt% in Kariesh, Domiatti, Ras, and Processed cheese was 0.59, 5.79, 3.5 and 1.024% respectively. The titratable acidity was 0.67, 1.0, 0.6 and 0.83% in Kariesh, Domiatti, Ras, and Processed cheese respectively.

Incidence of the proteolytic isolated strains demonstrated in Fig.1 revealed the presence of varying percentage of *Bacillus spp., Enterococcus spp., Micrococcus spp., P. aeruginosa* and *Staphylococcus aureus. Bacillus licheniformis* was the predominating proteolytic bacteria in all cheese samples followed by *B.mycoides* and *Ent. Durans*.

Fig. 2 presented the incidence of yeast isolates in the examined cheese samples. *C. albicans* was the predominating species isolated in Domiatti cheese (18.75%), while *Galactomyces geotrichum* was presented (16.98%) in Ras cheese.

Regarding the lipolytic strains isolated, *P. aeruginosa* represented the most prevalent lipolytic isolate in all cheese types predominantly in Domiatti cheese and Kariesh cheese (Fig. 3).

In Fig. 4, the viability of *P. aeruginosa* was observed in fresh and ripened cheeses over the storage period. Briefly, in fresh cheese supplemented with 3% salt and stored in 7% brine solution (T1), the inoculated *P. aeruginosa* proliferated until reached its maximum count (4x10^{15} cfu/g). After one week of refrigerated storage; *P. aeruginosa* counts dramatically decreased to neglectable level of 2 x 10^{3} cfu/g that did not have any deteriorating effect on fresh cheese throughout the storage period. The inoculated cheese without salt supplementation (T3) exhibited distinct signs of cheese deterioration by the end of the third week as a result of the continuous growth of *P. aeruginosa*, which reached 55 x 10^{19}cfu/g.

Data represented in Fig. (5) demonstrated that *C. albicans* survivability exhibited no alterations between fresh salted (T1), ripened cheese (T2), and the unsalted fresh cheese (T3), the results showed normal growth curve and increasing log numbers of viable yeast counts over nine weeks of storage with clear spoilage signs represented in sliminess, holiness, and offensive yeasty fermented odor by the end of 7th week in T3 and 9th week in T1 and T2 thought the salt and acidity percentages reached 3.5 and 0.4, respectively, confirming the resistance of *C. albicans* to the high acidity and salt together with the cold storage condition.

Discussion

Over the past decade, there has been an increasing concern regarding the issue of food loss and waste. Spoilage microorganisms encompass both gram-negative and gram-positive bacteria, as well as a variety of fungal organisms [13]. Cheese and cultured products demonstrated an increased susceptibility to contamination by spoilage microorganisms from production to the consumer's hands. Spoilage of

cheese can be linked to several factors, primarily the production of extracellular enzymes that break down nutrients, leading to undesirable odors, flavors, and noticeable defects in appearance. Additionally, visible fungal growth resulting from spoilage fungi, along with the production of bacterial and fungal pigments [14].

Ras, Kariesh, and Domiatti are three of the most renowned and authentic types of Egyptian cheese. Domiatti cheese is a soft white cheese that is usually made from cow or buffalo milk [15].

Microbiological examination for assessing the prevalence of some deteriorating microorganisms in the collected cheese samples showed high level of microbial contamination both kariesh and Domiatti cheeses showed high incidence of yeast counts that reached 2.6×10^{10} and 4.7×10^{10} cfu/g, respectively. However, mold, proteolytic, and lipolytic counts were higher in kariesh cheese than the Domiatti cheese. In Ras cheese, the highest proteolytic count was observed (6.1×10^{10}) with high lipolytic, yeast and mold load. Processed cheese were the lowest contaminating samples with mean mold count of 2.97×10^{5}. Nearly similar data was obtained by [16] who reported the bad quality of the examined Ras cheese samples which were totally not agree with the Egyptian specifications for yeast and mold. According to the Egyptian standards (hard cheese; 1007-1/2005; soft cheese; 1008-1/2005 and processed cheese; 999-2/2005); total mold count must not exceed 10 cfu/g in soft and hard cheese while, total yeast count should not exceed 100 cfu/g in hard cheese, whereas in soft cheese should not exceed 400 cfu/g. Accordingly, all kariesh and Ras cheese, in addition to 80 % of Domiatti cheese samples were disagreed with these standards. Moreover, it was found that all examined cheese samples were heavily contaminated with mold and disagreed with the Egyptian standards. These findings were agreed with previously published data [17-19] who reported that yeast and mold contaminated all the tested white soft cheese and Ras cheese samples, while [20, 21] found yeast in 77.1% and 25% and mold in 94.3% and 20%, respectively. Lower yeast and mold contamination in processed cheese spreads was recorded by [22, 23].

The wide variation of microbial counts in different cheese varieties can be attributed to many factors such as the microbiological quality of raw milk, inadequate hygiene practices, and the presence of post-processing contamination [24]. According to the obtained results in Table (1), Ras and Kariesh cheese had higher lipolytic and proteolytic counts compared to the findings of [25]. Regarding Processed cheese samples, lower counts were obtained previously [6, 20-26] and that may be attributed to application of chemical preservatives to keep the quality of manufactured Processed cheese [27]. The presence of microbial contamination in cheese can be linked to inadequate sanitary practices throughout the manufacturing process, as well as the use of raw milk [28].

The chemical assessment revealed that Domiatti cheese showed the highest acidity (1.0 ± 0.075) and salt percentages (5.79 ± 0.12) (Table 2). While kariesh cheese had the lowest salt content (0.59 ± 0.044). In Ras cheese, similar physicochemical results were obtained by previous study [29]. On the other hand, lower salt was also recorded [16]. While the mean titratable acidity was higher (1.5 ± 0.50) than our results. Salt acts as a preservative by reducing the water activity which decreases or prevents the growth of microorganisms and germination of their spores in addition to its essential role in flavor enhancement [30].

Pseudomonas species contribute significantly to off-flavor problems in dairy products. This is because they produce volatile compounds and metabolize amino acids, resulting in production of undesirable odors. Furthermore, they could produce enzymes that can break down proteins and fats, which negatively impacts the quality and shelf-life of protein-rich foods such as cheese [5, 31]. In addition, they can produce pigments in spoiled foods resulting in their discoloration [32- 34].

Regarding yeast contamination of cheese, it was draw backed to their diverse range of species, their ability to grow at low temperatures, the assimilation of lactic and citric acid, organic acids like succinic, resistance against high salt concentration, their proteolytic and lipolytic activities, low water activity (αw) and the relative resistance to cleaning compounds and sanitizers [35,36]. Furthermore, some strains of these yeasts have been observed to act as opportunistic pathogens, particularly in individuals with impaired immune systems [37, 38]. Some species of yeast proved to have the ability to generate biogenic amines, which may also be linked to undesirable flavors in cheese [39]. It has been determined that *C. lipolytica*, a proteolytic yeast, possesses the most threatening potential among cheese yeasts in terms of generating biogenic amines such as tyramine, cadaverine, putrescine, and 2–phenylethylamine [40]. Souring and yeasty off-flavors usually develop when the yeast count exceeds 10^{5}cfu/g [41]. Fruity esters may be formed when ethanol produced by yeasts combines with short-chain fatty acids released by lipolysis [36].

Keeping the microbial quality throughout the shelf-life of food items is a critical issue for the dairy sector. Traditionally, natural weather conditions, including sun drying in summer and freezing in winter, along with natural fermentation, were utilized for cheese preservation [42]. Excessive consumption of chemical preservatives, including sorbates, sulfites, acetic acid, propionates, benzoates, hexamethylenetetramines, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA), has led

to considerable health repercussions for example, Benzoic acid and its salts have been reported to provoke asthma, urticaria, metabolic acidosis, and seizures [43]. The enduring demand for safe, wholesome, and chemical-free food is driving scientists to create novel and sustainable food preservation technologies [44,45]. Consequently, active and intelligent packaging, together with non-thermal technologies, have arisen to avert the spoilage of cheese [46,47]

Therefore, the second part of this study assessed the influence of two major intrinsic factors affecting the microbial cheese quality; salt percentages that reflected on the water activity of cheese and subsequently the survival rate of the contaminated microorganisms, in addition to the starter culture activity represented by the amount of acidity produced. Indeed, experimental models of both fresh and ripened cheese were used to evaluate the influence of salt and acidity percentages on viability of two main deteriorating microorganisms frequently isolated from the different cheese types [5].

In the first cheese model, throughout the storage period, the counts of *P. aeruginosa* experienced a significant decline, resulting in a negligible level of 2 x 10^3 cfu/g. This level did not have any detrimental effects on the fresh cheese. This protective effect was drawn backed to the salt percentage that reached 3.8% inside the cheese core. Accordingly, there was a negative non-significant correlation between *P. aeruginosa* count and salt percentage (r= -0.337, p=0.311), the salt content in cheese core increased with increasing time interval, which confirmed statistically by strong significant positive correlation between salt content and time interval (r= 0.838, p=0.002). In contrast, by the end of the third week, the inoculated cheese without salt supplementation (T3) exhibited spoiled. This is attributed to the continuous growth of *P. aeruginosa.* This growth resulted in the production of pigments, which caused yellowish to brownish color defects, and extracellular hydrolytic enzymes, including proteases and lipases, which decomposed the cheese fat and proteins, resulting in slimness and the production of an offensive odor. This result was in accordance with [37,49] who reported that *P. aeruginosa* exhibited spoilage features (slimness, bitter taste, off flavors and color defects) due to its proteolytic and lipolytic activities which can affect the cheese quality. It has been reportedthat all laboratory manufactured cheese samples inoculated with 1% salt showed spoilage at 14th day (slimness, off flavour and yellow pigmentation), while samples from cheese manufactured with high salt concentration (5%) showed extended shelf life up to 35th day [50].

Furthermore, the combined effect of acidity and salt content in the lab-manufactured ripened cheese (T2) revealed that the added ripening condition and starter culture activity drooped the growth of the *P. aeruginosa* four logs during the first week and completely suppressed its growth by the end of third week of storage where the titratable acidity and salt percentages were 0.8 and 3.8, respectively. There was negative non- significant correlation between *P. aeruginosa* count and acidity percentage in ripened cheese (r= -0.537, P=0.350), and strong negative non-significant correlation with salt content (r= -0.839, p =0.076); thus confirmed the effective combined role of high titratable acidity percentage (0.8) and salt content (3.8) on inhibiting the growth of *P. aeruginosa* (Figure 3). Additionally, there was strong significant positive correlation between the time interval and acidity percentage of ripened cheese (r= 0.984, p =0.00) and strong positive non-significant correlation between the time interval and salt content (r= 0.809, p =0.051).

The yeast found in cheese might not only derive from milk, but also from the processing surrounding and during ripening process [37]. The majority of yeasts are capable of metabolizing lactate produced by lactic acid bacteria (LAB) and generating ammonia from amino acids. Thus, give it the capability to tolerate acid and salt [51]. Yeast plays diverse roles in cheese quality and safety [52,53]. Yeasts are crucial to the production of almost all traditional matured cheeses. Nevertheless, yeasts can also contribute to significant defects in cheese, resulting in premature blowing, undesirable flavors, dark staining, and other noticeable changes [53]. Reduced pH, presence of mold, increased salt content, ripening, and storage conditions promote yeast multiplication in cheese [54]. In the second cheese model, there was a weak positive non-significant correlation between *C. albicans* count and salt percentage (r= 0.247, P = 0.439) in fresh cheese, despite the increased salt level in cheese along the storage period that proved statistically by the strong positive correlation between time interval and salt level (r= 0.71, P = 0.01). Astonishingly, the findings of ripened cheese showed positive non- significant correlation between *C. albicans* count and the percentage of salt and acidity (r= 0.473, P = 0.237) and (r= 0.260, P = 0.533) respectively. In spite of the strong positive correlation between the time interval and acidity percentage (r= 0.607, P = 0.111) and salt content (r= 0.708, P = 0.05) of ripened cheese.

These results were agreed with that reported by Zhang et al. [55] who concluded that the growth characteristics of all examined yeast species with 5% NaCl was not affected.

The presence of yeast in cheese is anticipated due to the cheese's favorable conditions for yeast growth, including high acidity, low temperature storage, low moisture content, and high salt concentration [37]. Among the limitations of our study, resistance of *C. albicans* to salt and acidity, therefore, further studies are required to manage the fungal contamination in dairy products, particularly cheese using novel,

natural, and effective additives. Novel bio-preservatives agents are currently under investigation to be assessed in our future research. In addition, absence of molecular characterization of the isolated species, however given that the primary focus of this study was to collectively investigate the different spoilage microorganisms with their possible control measures independent of their molecular specific species confirmation yet, molecular identification of such isolated organisms will be addressed for the further investigations in our future studies.

Conclusion

The current study highlights the prevalence and impact of various spoilage microorganisms in common Egyptian cheese varieties. Investigation of the effect of salt and starter culture activity on viability of these microorganisms provides a promising approach for mitigating the issue. The findings demonstrated that a combination of salt and acidity can effectively control *P. aeruginosa*, while *C. albicans* continues to proliferate resulting in cheese deterioration and subsequent economic losses. In conclusion, this study highlights the crucial of implementing strong quality control and food safety measures throughout the entire process of cheese manufacturing and distribution. Moreover, our findings presented the potential application of natural preservation (salt and starter cultures activites) to control cheese spoilage particularly the soft one. Finding a novel, safe, and effective control measures for the fungal contamination in dairy products especially cheese is of paramount importance and our future research will focus on this issue.

Acknowledgments

Not applicable.

Funding statement

This study didn't receive any funding support

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

Not applicable.

TABLE 1. Microbiological analysis of the examined cheese samples

	Microbial counts (Mean± SEM)			
Samples	**Kariesh cheese**	**Domiatti cheese**	**Ras cheese**	**Processed cheese**
Yeast	$6 \times 10^{10} \pm 2.2 \times 10^{10}$	$4.7 \times 10^{10} \pm 2.8 \times 10^{10}$	$3.9 \times 10^{7} \pm 6 \times 10^{6}$	$2.97 \times 10^{5} \pm 1.5 \times 10^{5}$
Mold	$4.6 \times 10^{8} \pm 3.2 \times 10^{8}$	$1.7 \times 10^{4} \pm 1.04 \times 10^{4}$	$3.1 \times 10^{5} \pm 2.8 \times 10^{5}$	$7.6 \times 10^{5} \pm 7.1 \times 10^{5}$
Lipolytic	$7.2 \times 10^{8} \pm 3.4 \times 10^{8}$	$1.5 \times 10^{5} \pm 10^{5}$	$3.1 \times 10^{8} \pm 1.9 \times 10^{8}$	$17.9 \times 10^{3} \pm 6.6 \times 10^{3}$
Proteolytic	$1.7 \times 10^{10} \pm 8.6 \times 10^{9}$	$4.5 \times 10^{6} \pm 1.8 \times 10^{6}$	$6.1 \times 10^{10} \pm 3.7 \times 10^{10}$	$6.1 \times 10^{6} \pm 1.8 \times 10^{6}$

TABLE 2. Chemical assessment of the examined cheese samples

	Chemical analysis (Mean± SEM)			
Samples	**Kariesh cheese**	**Domiatti cheese**	**Ras cheese**	**Processed cheese**
Salt %	0.59 ± 0.044	5.79 ± 0.12	3.5 ± 0.098	1.024 ± 0.061
Titratable acidity %	0.67 ± 0.045	1.0 ± 0.075	0.6 ± 0.047	0.83 ± 0.064

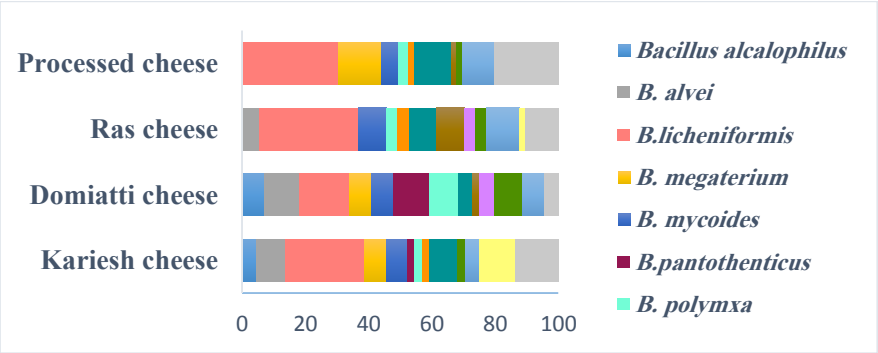

Fig. 1. Incidence of the proteolytic isolates in the collected cheese samples

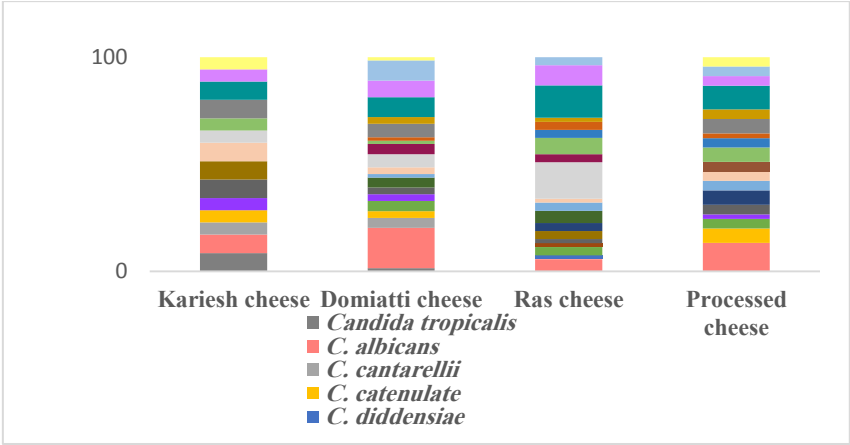

Fig. 2. Incidence of yeast isolates in the collected cheese samples

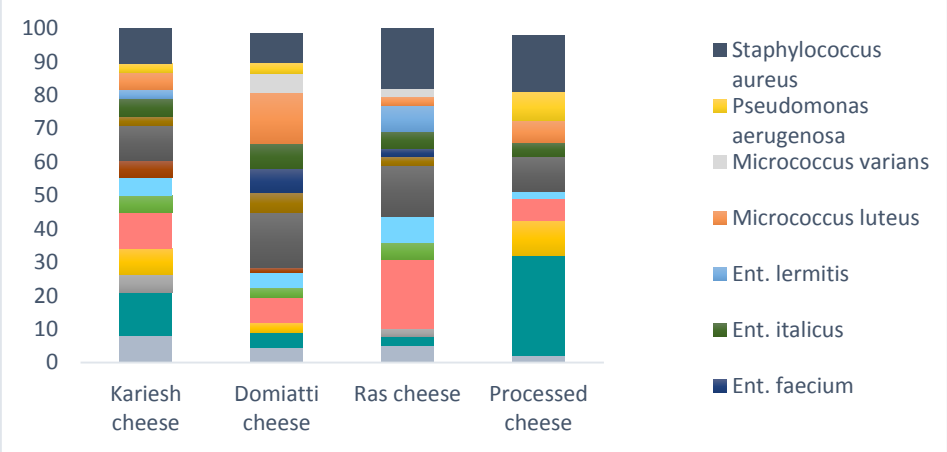

Fig. 3. Incidence of lipolytic isolates in the collected cheese samples

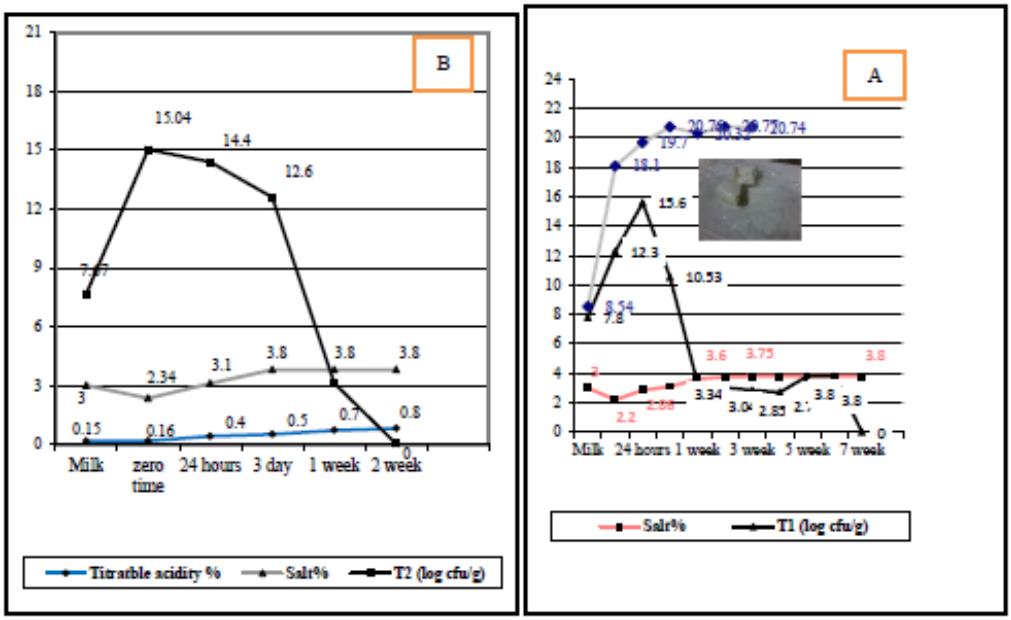

Fig. 4. Viability of *P. aeruginosa* in fresh and ripened cheeses over the storage period

A: Fresh cheese, **B:** Ripened cheese, **T1:** count of *P. aeruginosa* in salted fresh cheese, **T2:** count of *P. aeruginosa* in ripened soft cheese, **T3:** count of *P. aeruginosa* in control group (unsalted fresh cheese)

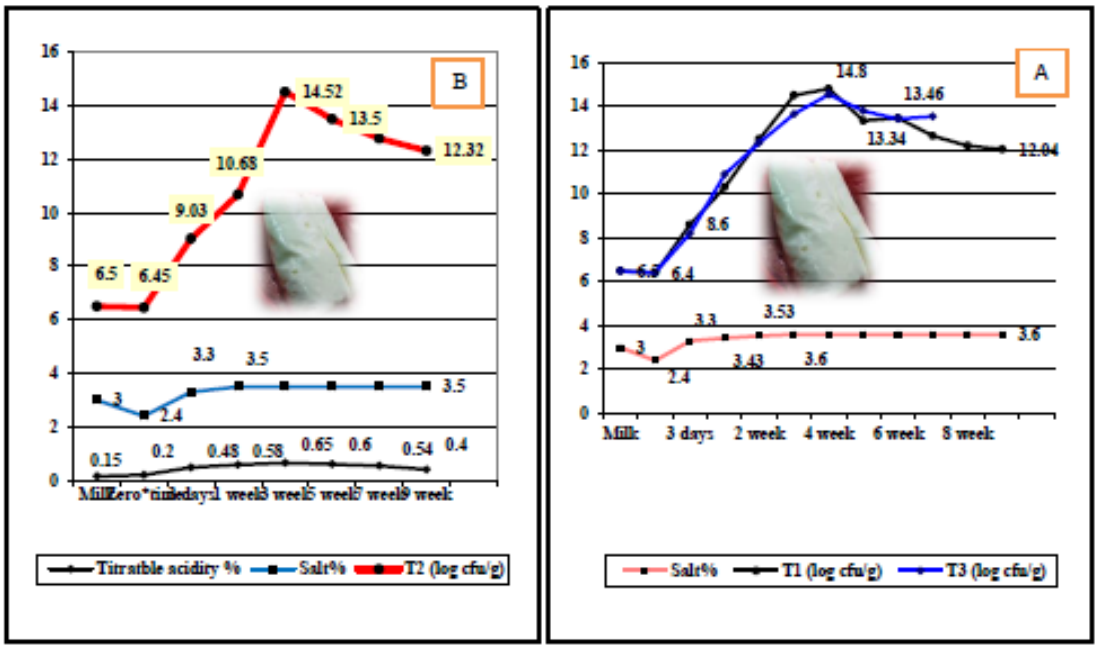

Fig. 5. Viability of *C. albicans* in fresh and ripened cheeses over the storage period

A: Fresh cheese, **B:** Ripened cheese, **T1:** count of *C. albicans* in salted fresh cheese, **T2:** count of *C. albicans* in ripened cheese, **T3:** count of *C. albicans* in control group (unsalted fresh cheese**).**

References

1. Odeyemi, O. A., Alegbeleye, O. O., Strateva, M. and Stratev, D. Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin. *Comprehensive Reviews in Food Science and Food Safety,* **19**(2), 311-331(2020).
2. Machado, S. G., Baglinière, F., Marchand, S., Van Coillie, E., Vanetti, M. C., De Block, J. and Heyndrickx, M. The Biodiversity of the Microbiota Producing Heat-Resistant Enzymes Responsible for Spoilage in Processed Bovine Milk and Dairy Products. *Front. Microbiol.,* **8**, 302(2017). DOI: 10.3389/fmicb.2017.00302.
3. Ali, Z. I., Shawki, M. A., Abdel-Latif, E. F., & Saudi, A. M. (2024). Assessment of fatty acid content and lipid nutritional quality indicators for some cheese kinds using gas chromatography–mass spectrometry. *Journal of Advanced Veterinary Research*, **14**(7), 1258-1263.
4. Nájera, A. I., Nieto, S., Barron, L. J. R. and Albisu, M. A review of the preservation of hard and semi-hard cheeses: Quality and safety. *International Journal of Environmental Research and Public Health,* **18**(18), 9789(2021).
5. Nadi, W., Ahmed, L., Awad, A. and Taher, E. "Occurrence, antimicrobial resistance, and virulence of Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa isolated from dairy products." *International Journal of Veterinary Science*, **90**, 403-409 (2023).
6. Abd El Tawab, A., El-Hofy, F., EL-diasty, E., Abo-Hamdah, E. and Al-Khayat, M. "Diversity of some food born fungi associated with raw milk and some cheese in Egypt." *Benha Veterinary Medical Journal,* **38**(1), 48-51(2020).
7. Admassie, M. "A review on food fermentation and the biotechnology of lactic acid bacteria." *World Journal of Food Science and Technology,* **2**(1), 19-24(2018).
8. Oliveira, R. B., Ramos, G. L., Sá, P. B., Pereira, A. P. M., Conceição, D. A., Cruz, A. G., Alvarenga, V. O. and Sant'Ana, A. S. Controlling Clostridium sporogenes spoilage of "requeijão cremoso" processed cheese: Modeling the growth/no-growth probability as a function of pH, sodium chloride and nisin. *Food Control,* **162**, 110435 (2024).
9. APHA Standard methods for the examination of dairy products, American Public Health Association. (2004). https://doi.org/10.2105/9780875530024.
10. Vos, P., Garrity, G., Jones, D., Krieg, N. R., Ludwig, W., Rainey, F. A., Schleifer, K.-H. and Whitman, W. B. Bergey's manual of systematic bacteriology: 3: *The Firmicutes, Springer Science & Business Media* (2011).
11. Ahmed, L., Morgan, S., Hafez, R. and Abdel-All, A. Influence of yoghurt starter culture on viability of some pathogenic microorganisms in yoghurt. *International Journal of Dairy Science,* **9**(3), 82-88(2014).

12. Ahmed, L. I., Ibrahim, N., Abdel-Salam, A. B. and Fahim, K. M. Potential application of ginger, clove and thyme essential oils to improve soft cheese microbial safety and sensory characteristics. *Food Bioscience,* **42**, 101177(2021). DOI: https://doi.org/10.1016/j.fbio.2021.101177

13. Martin, N. H., Torres-Frenzel, P. and Wiedmann, M. Invited review: Controlling dairy product spoilage to reduce food loss and waste. *Journal of Dairy Science,* **104**(2) 1251-1261(2021).

14. Saha, S., Majumder, R., Rout, P., & Hossain, S. Unveiling the significance of psychrotrophic bacteria in milk and milk product spoilage-A review. *The Microbe*, 100034 (2024).

15. Farag, M. A., Ashaolu, T. J., Guirguis, H., & Khalifa, I. Implementation of HACCP in the production of Egyptian cheeses: A review. *eFood*, **4**(2), e69(2023)..

16. Shawki, M. A., Abdel-Latif, E. F., Darwish, S. F., Saudi, A. M. and Ali, Z. I. Assessment of the Chemical and Microbiological Quality of Some Cheese Assortment in Egypt: Highlighting the incidence of multidrug-resistant Staphylococcus Species. *Journal of Applied Veterinary Sciences,* **9**(1), 85-93(2024). DOI: 10.21608/javs.2023.243014.1285

17. El-Sayed, M., Hosny, I., El-Kholy, W., El-Dairouty, R. and Mohamed, S. H. S. Microbiological evaluation of Egyptian white soft cheeses style. *The Journal of American Science,* **(7),** 5, 517-526 (2011).

18. Ibrahim, J. I., Salama, E., Saad, A. and Helmy, A. A. Microbial quality of some dairy products in Ismailia city. *2nd Conference of Food Safety, Suez Canal University, Faculty of Veterinary Medicine,* (2015).

19. El-Sherif, W. and Shaker, E. M. Detection of Molds in Egyptian Ras Cheese with Special Reference to Aspergillus flavus Aflatoxins. *International Journal of Comprehensive Veterinary Research,* **2**(2),1-9 (2024).

20. ELbagory, A., Eid, A. M., Hammad, A. and Dawood, S. Prevalence of fungi in locally produced cheese and molecular characterization of isolated toxigenic molds. *Benha Veterinary Medical Journal,* **27**(2), 9-20 (2014).

21. Moharram, A., Abd El Haleem, A. and Refaie, R. Mycological and mycotoxins analysis of Kareish and soft cheese in Assiut, Egypt. *Am. J. Microbiol Research,* **6**,165-172(2018).

22. EL-Alfy, M., Shenana, M., Gemiel, D. G. and Abdou, S. M. Evaluation of some processed cheese spreads and sauces available in the Egyptian market. *Annals of Agricultural Science Moshtohor,* **55**(2) 321 –332 (2017).

23. Moawad, A. A. and Ibrahim, A. S. Assessment of Selected Varieties of Cheese Analogues sold in Egyptian Markets. *Egyptian Journal of Chemistry,* **66**(10), 509-520 (2023).

24. Rodrigues, D. E. B., da Paixão, L. L., Santana, V. R., Cardozo, R. M. D. and Machado, S. G. Proteolytic potential of psychrotrophic microorganisms isolated from fresh cheese. *Revista do Instituto de Laticínios Cândido Tostes,* **75**(4), 232-242(2020).

25. Hassan, M G., Meshref, M.S. A., Zeinhom, M.A. M. and Abdel-Halim, S. M. Impact of spoilage microorganisms on some dairy products. *Assiut Veterinary Medical Journal,* **65**(161), 133-141(2019).

26. Hameedm, K. G. A. Fungal diversity in different types of cheese and the effect of natamycin on their survival during Feta cheese manufacture and storage. *Journal of Advanced Veterinary Animal Research*, **3**(3), 214-220(2016).

27. Mohamed, A. G., Ibrahim, O. A. E. H., Gafour, W. A. and Farahat, E. S. Comparative study of processed cheese produced from sheep and cow milk. *Journal of Food Processing and Preservation*, **45**(1), e15003 (2021).

28. Elbassiony, T. A. R., El-Khawas, K. M. and Mostafa, N. M. Microbiological quality of Kareish cheeses. *New Valley Veterinary Journal*, **1**(1), 16-22(2021).

29. Eldenary, A., Hassan, M., Amer, D. and Bakr, A. Assessment of Quality and Safety of Ras Cheese in Egyptian Delta Governorates. *Journal of Sustainable Agricultural and Environmental Sciences,* **2**(2), 77-99 (2023).

30. 30-Bansal, V. and Mishra, S. K. Reduced-sodium cheeses: Implications of reducing sodium chloride on cheese quality and safety. *Comprehensive Reviews in Food Science and Food Safety,* **19**(2), 733-758 (2020).

31. Raposo, A., Pérez, E., de Faria, C. T., Ferrús, M. A. and Carrascosa, C. Food spoilage by Pseudomonas spp.—An overview. *Foodborne Pathogens and Antibiotic Resistance*, 41-71(2016).

32. Tirloni, E., Bernardi, C. and Stella, S. Pseudomonas spp.: are food grade organic acids efficient against these spoilage microorganisms in fresh cheeses? *Foods,* **10**(4), 891(2021).

33. Urgancı, N. N., Yılmaz, N., Alaşalvar, G. K. and Yıldırım, Z. *Pseudomonas aeruginosa* and its pathogenicity. *Turkish Journal of Agriculture-Food Science and Technology,* **10**(4), 726-738 (2022).

34. Bansal, V. and Veena, N. "Understanding the role of pH in cheese manufacturing: General aspects of cheese quality and safety. *Journal of Food Science and Technology* **61**(1), 16-26 (2024).

35. Awasti, N., and Anand, S. The role of yeast and molds in dairy industry: An update. *Dairy processing: Advanced Research to Applications*, 243-262 (2020).

36. Bintsis, T. "Yeasts in different types of cheese." *AIMS Microbiol.,* **7**(4), 447-470(2021). DOI: 10.3934/microbiol.2021027.

37. Rodrigues, R. d. S., Machado, S. G., de Carvalho, A. F. and Nero, L. A. Genome-based analysis of *Pseudomonas paracarnis* RQ057, a strain responsible for blue discoloration spoilage in processed cheese. *International Journal of Dairy Technology,* **76**(4), 974-986 (2023).

38. Geronikou, A., Srimahaeak, T., Rantsiou, K., Triantafillidis, G., Larsen, N. and Jespersen, L. Occurrence of yeasts in white-brined cheeses: Methodologies for identification, spoilage potential and good manufacturing practices. *Front. Microbiol.,* **11**, 582778 (2020).

39. Martin, N. H., Torres-Frenzel, P. and Wiedmann, M. Invited review: Controlling dairy product spoilage to reduce food loss and waste. *Journal of Dairy Science,* **104**(2), 1251-1261(2021). DOI: https://doi.org/10.3168/jds.2020-19130

40. Snyder, A. B., Martin, N. and Wiedmann, M. Microbial food spoilage: impact, causative agents and control strategies. *Nature Reviews Microbiology,* **22**(9), 528-542(2024). DOI: 10.1038/s41579-024-01037-x

41. Banjara, Nabaraj, Mallory J. Suhr and Heather E. Hallen-Adams. Diversity of yeast and mold species from a variety of cheese types. *Current Microbiology,* **70,** 792-800 (2015).

42. Ali, Ali Muhammed Moula, Anderson S. Sant'Ana, and Sri Charan Bindu Bavisetty. "Sustainable preservation of cheese: Advanced technologies, physicochemical properties and sensory attributes." *Trends in Food Science & Technology,* **129**, 306-326 (2022)

43. So, J. S., Lee, S. B., Lee, J. H., Nam, H. S. and Lee, J. K. Simultaneous determination of dehydroacetic acid, benzoic acid, sorbic acid, methylparaben and ethylparaben in foods by high-performance liquid chromatography. *Food Science and Biotechnology*, **32**(9), 1173-1183. (2023).

44. Ali, Z. I., Saudi, A. M., Albrecht, R. and Talaat, A. M. The inhibitory effect of nisin on Mycobacterium avium ssp. paratuberculosis and its effect on mycobacterial cell wall. *Journal of Dairy Science*, **102**(6), 4935-4944 (2019).

45. Sweet, Ryan, Paul A. Kroon and Mark A. Webber. Activity of antibacterial phytochemicals and their potential use as natural food preservatives. *Critical Reviews in Food Science and Nutrition,* **64**(8), 2076-2087(2024).

46. Du, H., Sun, X., Chong, X., Yang, M., Zhu, Z., & Wen, Y. A review on smart active packaging systems for food preservation: Applications and future trends. *Trends in Food Science & Technology*, **141**(10),104200 (2023).

47. Khezerlou, A., Zolfaghari, H., Forghani, S., Abedi-Firoozjah, R., Alizadeh Sani, M., Negahdari, B. and McClements, D. J. Combining non-thermal processing techniques with edible coating materials: an innovative approach to food preservation. *Coatings*, **13**(5), 830 (2023).

48. Garnier, L., Valence, F. and Mounier, J. Diversity and Control of Spoilage Fungi in Dairy Products: An Update. *Microorganisms,* **5**(3), 5030042(2017). DOI: 10.3390/microorganisms5030042.

49. Soliman, S. A. E.-A. Identification of Pseudomonas aeruginosa Isolated from Karish Cheese by PCR Technique. *Al-Azhar Journal of Agricultural Research,* **48**(3), 528-537(2023).

50. El-Leboudy, A. A., Amer, A. A., Nasief, M. E. and Eltony, S. M. Occurrence and behavior of Pseudomonas organisms in white soft cheese. *Alexandria Journal of Veterinary Sciences,* **44**(1),74-79 (2015).

51. Maicas, S. The Role of Yeasts in Fermentation Processes. *Microorganisms* **8**(8),142-1150(2020). DOI: 10.3390/microorganisms8081142

52. Mendes Ferreira, A. and Mendes-Faia, A. "The Role of Yeasts and Lactic Acid Bacteria on the Metabolism of Organic Acids during Winemaking. *Foods,* **9**(9)1131 (2020). DOI: 10.3390/foods9091231.

53. Merchán, A. V., Benito, M. J., Galván, A. I. and de Herrera, S. R.-M. S. Identification and selection of yeast with functional properties for future application in soft paste cheese. *Lwt Food Science and Technology,* **124**, 109173 (2020).

54. Zhang, L., Huang, C., Malskær, A. H., Jespersen, L., Arneborg, N. and Johansen, P. G.The effects of NaCl and temperature on growth and survival of yeast strains isolated from Danish cheese brines. *Current Microbiology,* **77**, 3377-3384 (2020).

55. Zheng, X., Shi, X. and Wang, B. A review on the general cheese processing technology, flavor biochemical pathways and the influence of yeasts in cheese. *Frontiers in Microbiology*, **12**, 703284. (2021).

تواجد بعض الميكروبات المسببة للفساد في الجبن مع تسليط الضوء على تأثير الملح والبادئ على حيوية السودوموناس اريجنوزا و كانديدا البيكانس

لمياء ابراهيم احمد ، ايمان مصطفى طاهر ، كريمة مجاهد فهيم و زينب ابراهيم علي

قسم الرقابة الصحية علي الأغذية - كلية الطب البيطري- جامعة القاهرة - مصر.

الملخص

يسبب تلوث الجبن بالميكروبات المسببة للفساد خسائر اقتصادية كبيرة لصناعة الغذاء. في هذه الدراسة، تم تجميع مائة عينة من الجبن (القريش، الدمياطي، الراس، والجبن المطبوخ) 25 عينة لكلاً منهم من الأسواق المختلفة في محافظة القاهرة، مصر. .تم عمل تحليل كميائي (نسبة الملح و الحموضة) و تحليل ميكروبيولوجي (عدد الخمائر الكلي ، العفن الكلي، عدد بكتريا التحلل الدهني والبروتيني الكلي). وأشارت النتائج أن كلا من جبن القريش والدمياطى أظهرا نسبة عالية من الخميرة بلغت 2.6×10^{10} و4.7×10^{10} /جم، على التوالي. علاوة على ذلك، أظهرت جبن القريش والراس أعلى نسبة تحلل للبروتين والتحلل الدهني، ومع ذلك، سجلت جبن قريش أعلى نسبة فطريات بمتوسط قيمة 4.6×10^{8} /جم. بحث الجزء الثاني من الدراسة في تأثير نسبة الملح ونشاط البادئ المتمثل في الحموضة المنتجة على حيوية بكتيريا سودوموناس اريجنوزا و كانديدا البيكانس أثناء التخزين البارد للأجبان البيضاء الطرية الطازجة ذوالناضجة المصنعة مخبرياً. أظهرت النتائج المتحصل عليها عدم إمكانية الكشف عن سودوموناس اريجنوزا في الجبن الطري الطازج أو الناضج بنهاية الأسبوعين السادس والثالث من فترة التخزين، على التوالي. بينما استمرت كانديدا البيكانس في النمو في عينات الجبن الطري الطازجة والناضجة حتى ظهور علامات الفساد الواضح على الجبنة (الثقوب ونكهة الخميرة) بحلول الأسبوع التاسع. وعلى النقيض من كانديدا البيكانس، كشفت نتائج الدراسة أنه يمكن السيطرة على سودوموناس اريجنوزا بكفاءة عن طريق مزيج من الملح والحموضة مما يمثل طريقة آمنة ومجدية للسيطرة على الفساد في الجبن.

الكلمات الدالة: الحموضة، الملح، التعفن، سودوموناس اريجنوزا و كانديدا البيكانس