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Enumeration and Identification of Yeast Species Isolated from Egyptian Soft White Cheeses and *"In Vitro"* Anti-yeasts Activity: A Preliminary Study

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ABSTRACT:

The aims of the current investigation were to enumeration and identification yeast species isolated from karish cheese and pickled Domiati cheese in order to study in vitro anti-yeast activity of some natural agents (natamycin and clove oil) and chemical agents (hydrogen peroxide and potassium sorbate) against yeast isolates to enhance the quality and safety of these products. The results showed that all karish cheese samples and 57% of pickled Domiati cheese samples were contaminated with yeasts above the limits required by the Egyptian standard, also karish cheese contained the highest total yeast count (Mean, 3.04 log cfu/g) in comparison to the examined pickled Domiati cheese (Mean, 2.7 log cfu/g). Twenty yeast isolates were obtained from cheese samples and identified by morphological, physiological and biochemical examinations, the colonies observed white, creamy and binky colors, and circular and irregular shapes. All of yeast isolates had inability to grow at 55°C and casein hydrolysis, however certain isolates exhibited the capability to ferment lactose and grow at a temperature of 7°C. The minimum inhibitory concentration (MICs) of natamycin, hydrogen peroxide and potassium sorbate for all yeast clusters were (0.5, 35, and 100 ppm) respectively, but for clove oil was ranged from 500 to 1000 ppm.

Keywords: karish cheese, Domiati cheese, yeast identification, in vitro anti-yeasts activity.

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INTRODUCTION:

Cheese is acknowledged for its rich nutritional content suitable for human consumption. The cheese protein boasts a high biological value, encompassing all essential amino and fatty acids. Additionally, cheese serves as a valuable source of minerals and vitamins (**Mayo *et al.*, 2021**). Soft white cheese manufacturing in Egypt constitutes 75% of the overall cheese production (**Wedad *et al.*, 2017**).

In the Mediterranean region, large quantities of traditional Egyptian dairy products are both manufactured and consumed. These products include acid-coagulated soft cheese or Kariesh cheese and soft cheese or Domiati cheese, which is salted and coagulated by enzymes. Domiati cheese comes in two varieties: fresh and aged, they differ from one another in terms of the salt percent added to the milk used in cheese making as well as the length of time and conditions under which it is stored. Preserved Domiati cheese is heavily salted and stored for several months in a solution of salted whey or brine, whereas fresh Domiati cheese is lightly salted and kept refrigerated for a short duration of weeks. The possibility of contamination with a variety of microorganisms exists because these products are prepared from unheated milk using less hygienic methods than those utilized in large-scale production facilities. Although the bacterial makeup of these products has been the subject of numerous studies **(El-Baradei *et al.*, 2007; El-Sharoud 2009)**, there is limited knowledge concerning yeast diversity associated with these foods.

In the past few years, consumers have shown a preference for food that is natural, safe, high quality and has a longer shelf life. Through advancements in food preservation technologies, products are now able to maintain their initial nutritional and sensory characteristics, resulting in longer-lasting options (**Zhou *et al.*, 2010; Dave and Ghaly 2011, Rodríguez Sauceda, 2011**). The primary reason for food spoilage stems from diverse microorganism types, including bacteria, molds, and yeasts. This issue has economic repercussions for manufacturers, distributors, and consumers alike. It is estimated that over 20% of the global food production is affected due to spoilage by microorganisms (**Rodríguez Sauceda, 2011**).

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Microbial food spoilage occurs when undesired microorganisms proliferate uncontrollably, leading to food spoilage due to the generation of undesirable flavors and toxins, which can also result in foodborne illness. Across European nations, more than 23 million people become ill each year due to consuming contaminated food (**WHO, 2017**). According to **(Shawaf *et al.*, 2014)** and **(Banjara *et al.*, 2015)** yeasts are frequently found in raw milk, air, cloth, brine, production surfaces, curd cutting knives, cheese vats, and other dairy products. Due to these products' low pH, high salt level, low moisture content and refrigerated storage. It is well known that yeasts may have a significant role in the microflora of numerous cheese types **(Devoyod, 2008).** However, yeasts have a dual function based on the type of cheese. They can contribute positively to the development of flavor and texture during aging in some types of cheeses, while in others, they may be considered as organisms causing spoilage. Spoilage caused by yeast is particularly recognized as an issue in cheese and fermented milk (**Brocklehurst and Lund, 1985; Fleet, 1990**).

The food industry historically utilized artificial preservatives with antimicrobial preservatives being the most standard. However, recent research suggests that consuming chemical additives may increase the risk of intoxications, allergies, degenerative diseases and cancer **(Sauced 2011; Aminzare *et al.*, 2016)**. Numerous naturally derived substances, including nisin, natamycin, and plant essential oils, exhibit potent antimicrobial properties against microorganisms responsible for both spoilage and pathogenic effects **(Juneja *et al.*, 2012)**.

Natamycin is synthesized naturally by *Streptomyces natalensis* bacterium, also known as pimaricin (**Deacon, 1997**). The EU recognizes natamycin as a preservative of natural origin (**EFSA, 2009**), while the FDA describes it as a generally recognized as safe (GRAS) product intended for human consumption (**Koontz and Marcy, 2003**). Within the food sector, natamycin is employed as an ingredient (E235) to preserve fermented meat and cheese from molds and yeasts. It is utilized in over sixty countries to protect various other foods as well (**Delves-Broughton *et al.*, 2005; te Welscher *et al.*, 2008**). Natamycin is favored over other preservatives due to its colorless and odorless nature, with no adverse

effects on the food taste. Its application as additive used for treating surfaces of semi-soft, semi-hard, and hard cheeses is regulated by **Commission Regulation EU 1129/2011**.

Essential oils of various plants have been studied for their biological properties, including antioxidant antimicrobial, and antifungal, activities (**Holley and Patel, 2005; Bakkali *et al.*, 2008; Ghasemnezhad *et al.*, 2011**). These substances are categorized as GRAS for food additives in the U.S. (**Kim *et al.*, 1995**). Initially, their usage in foods was predominantly confined to flavor additives in sweets and soft drinks, and in more recent times as preservatives. Indeed, the incorporation of essential oils in foods, particularly in cheese types, is currently a trending subject in the field of food functionalization. This practice aids in prolonging the shelf life of foods and enhancing their functionality (**Artiga *et al.*, 2017; Bedoya *et al.*, 2018; Can Seydim *et al.*, 2020; Fancello *et al.*, 2020**). Limited research has explored the use of clove oil (CO) as a natural preservative for soft cheese (**Smith *et al.*, 2001, Bakheit and Foda 2012**). These studies demonstrated that CO displayed antimicrobial efficacy against various foodborne pathogens and exhibited high antioxidant activity in cheese.

Potassium sorbate, a naturally derived organic acid, has been widely employed as a fungistatic agent in food industry (**Rajapaksha *et al.*, 2013**). It can be directly added to the food or integrated into the packaging process. In accordance with the Code of Federal Regulations it is considered GRAS when utilized in compliance with feeding practice or good manufacturing.

Hydrogen peroxide (H_2O_2) is approved for utilization as disinfectants in the food industry and it has been discovered to be efficient in eliminating biofilms from equipment (**Salo and Wirtanen, 2004**). It is effective against a broad spectrum of microorganisms including yeasts, bacteria, bacterial endospores and viruses (**Aryal and Muriana, 2019**). Hydrogen peroxide is recognized as a successful preservative in various nations worldwide. However, the FAO/WHO strongly advises against preserving milk with chemicals, except the application of H_2O_2 at moderate concentrations (i.e. 100–300 mg/kg). Hydrogen peroxide was recommended as a

preservative for milk by **FAO in 1957**. Hydrogen is considered a favorable antibacterial agent in milk and has been proposed as a method for improving milk quality in developing countries. Nevertheless, milk and its derivatives are biochemically unstable, leading to rapid deterioration. There has been limited research conducted for the utilization of H_2O_2 as a milk preservative in Egypt, and there has been no exploration into its effect on dairy products comparison to other tropical regions. One notable exception discovered in the experiments conducted is that they do not dilute H_2O_2 before adding it to the milk (**Rokhsana *et al.*, 2007**).

The objective of the present work was aimed to enumeration and identification yeasts isolated from types of traditional Egyptian soft white cheese including Karish cheese and pickled Domiati cheese, and determine anti-yeast activity as minimum inhibition concentration (MIC) of some natural (natamycin, clove oil) and chemical (potassium sorbate and hydrogen peroxide) preservatives against yeast isolates.

MATERIALS AND METHODS:

Collection of samples:

A total fourteen samples of Egyptian soft white cheese, karish cheese and Domiati cheese (seven samples each) various local market in EL-Beheira Governorate, Egypt, during January to April 2023. All samples were transported in an icebox to the laboratory where they were refrigerated and analyzed within 12 h of receipt.

Preservative materials:

Natamycin (Natamax®, Danisco specialities, Denemark), Clove oil (*Syzygium aromaticum*), House of Oil Company, ELbeheira Gavernorate, Egypt, Potassium sorbate (Jiangbei additive Co., LTD., China); Hydrogen peroxide, (Divozon 35 from Diversey™).

Enumeration and isolation of yeast species from cheese samples

As per the procedure outlined by **Van der Walt and Yarrow** (**2009**), 10 grams of each cheese samples (both surface and interior) were

diluted in 90 milliliters of a sterile sodium citrate solution 2% (w/v) (Sigma, St. Louis, MO, USA) and homogenized using a Stomacher (PBI, Milan Italy) for 30 seconds. Subsequently, serial dilutions were prepared in seven-fold for all samples in a sterile sodium citrate solution 2% (w/v), and the yeasts counts were determined through surface plating on potato dextrose agar (PDA) medium (Microbiol, Cagliari, Italy) supplemented with chloramphenicol (0.01%) (Microbiol The plates were then incubated at 25°C for 5 days. Colonies with distinct morphological characteristics were isolated, streaked onto PDA medium (2% peptone, 2% dextrose, 1% yeast extract, and 1.5% agar), and incubated at 25°C for 5 days to verify purity. Finally, pure isolates were stored in 15% (v/v) glycerol and skimmed milk (10%), and then frozen at – 20 ^{o}C. Prior to the experiments, all yeast isolates underwent two rounds of sub-culturing in PDA medium at 25°C for 5 days. Each type of colony was counted individually to determine the relative prevalence of different yeast species in the samples. Yeast species counts were quantified as as log cfu/g(the number of colony forming units per gram of sample).

Morphological features:

The colonies were examined and characterized by morphological agar and yeast-malt agar (YM). Additionally, the isolates were cultured in YM broth to assess their cultural characteristics, including textures (mucoid, fluid or viscous) elevation (flat or raised); color (white, creamy, and pinky); surface (smooth, rough, and sectored) and margin (entire, lobed, and filaments)

Identification of yeast isolates by biochemical and physiological examination:

Yeast isolates are exposed to various types of physico-chemical stresses, including: catalase test, lactose fermentation, casein hydrolysis, ability to growth at different temperature and different salt concentrations**.**

Casein hydrolysis test:

An assay to measure proteolytic activity was conducted using casein as the substrate. Each isolate tested was streaked onto a culture plate containing skim milk agar medium. These plates were incubated at

25°C for 5 days according to the method described by **Difco** (**1984**). Growth was monitored in the medium, and an un-streaked culture plate was also incubated under identical conditions to serve as a control. Results were determined by observing clearing of the agar surrounding bacterial growth, indicating casein breakdown.

Fermentation ability:

Lactose fermentation by isolated yeast strains was investigated on phenol red lactose broth medium. Each tested pure isolate was aseptically transferred as an inoculum into a sterile tube containing phenol red lactose broth medium, then incubated at 25°C for 5 days following the protocol described by **Difco** (**1984**). Growth was monitored in the medium, and a tube without inoculation was also incubated under identical conditions to serve as a control. Results were determined by observing color changes; a positive test was indicated by a transition from red to yellow.

Catalase test:

For the catalase test, a colony under examination was subjected to a 3% (v/v) H_2O_2 solution, and effervescence was observed by **Wong *et al.* (1988**).

Growth of isolated yeasts at different temperatures (temperature tolerance):

The evaluation of isolated yeast strains' growth capabilities at 7°C and 50°C proceeded as follows: each tested isolate was streaked onto PDA medium and subsequently incubated at 7°C for 10-12 days and at 50°C for 2-3 days. Growth in the medium was observed, and an un-streaked plate was also incubated under identical conditions to serve as a control.

Growth of isolated yeasts at different salinities (salt tolerance):

The resistance to salt of every isolated yeast strain was assessed, The PDA medium was modified to include NaCl 5, 10, and 15% (w/v). The enumeration of yeast isolates was performed by spreading 0.1 ml inoculation of diluted samples of culture onto duplicate plates of PDA, the plates subsequently incubated for 72 h at 25°C, and the colonies were subsequently enumerated according to **Philip and Verneal (1988**).

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In vitro anti-yeast activity of some preservative agents:

Anti-yeast activity of four preservatives materials were tested individually in three concentrations as follow: natamycin (0.5, 0.75, and 1 ppm), clove essential oil (500, 750 and1000 ppm), hydrogen peroxide (35, 70 and 105 ppm) and Potassium sorbate (100, 200, and 300 ppm) to determine the minimum inhibitory concentrations (MICs) against yeast isolates. The agar well diffusion technique is commonly employed for assessing the antimicrobial efficacy of microbial or plant extracts. Like the protocol utilized in the disk-diffusion technique, the surface of the agar plate is inoculated by distributing a volume of the microbial inoculum across the entire agar surface using a sterilized cotton swab. Subsequently, an aseptic hole with a 5 mm diameter is carefully punched using a sterile tip, and a quantity of the antimicrobial agent at the desired concentration (20µl) is added to the well. The agar plates were then incubated at 25°C for 5 days. The antimicrobial agent spreads within the agar medium, thereby inhibiting the growth of the tested microbial strain. The whole zone area was calculated and the difference in area was reported as the zone of inhibition (**Seydim and Sarikus, 2006**).

Statistical analysis:

In this study, a completely randomized experimental design (CRD) in a 4 x 4 factorial arrangement and three replicates was employed. The collected data underwent analysis of variance (ANOVA) using the GLM Procedure of **SAS** (**2013**) 9.4 software. Differences among the means were assessed using Tukey's test, with a significance level set at 5%.

RESULTS AND DISCUSSION

Enumeration and characterization of yeast isolates:

The results given in **Table (1)** showed that the yeast was present in all of the total tested karish and pickled domiati cheese samples, the viable count of yeasts were ranged from 2.7 to 3.26 and1.97 to 3.96 cfu g^{-1} with a mean value of 3.04 and 2.7 log cfu g^{-1} for karish and pickled domiati cheese, respectively. The results of all karish cheese samples were above the limits required by the **Egyptian standard No.1008-4/2005** for

karish cheese parameters, which should not exceed 10cfu/g for fungi and 400 cfu/g for yeast. While, only 43% of the examined pickled domiati cheese samples were satisfactory for molds and yeasts according to the **Egyptian standard No.1008-3/2005** for domiati cheese parameters. The elevated occurrence observed in the tested samples could be linked to inadequate hygiene practices throughout the processing and storing of the product. These results are agreement with those obtained by (**Kaldes, 1997; Abd-Alla, 2004**). In accordance with **Sharaf *et al.* (2014),** 10 out of 45 Domiati cheese samples collected from markets in Cairo were found to be infected with yeast, reaching as much as $6x10^3$ cfu/g. A subsequent study reported even higher levels of yeast contamination (the average counts were $2.63x10^5$cfu/g in 66% of samples) for Domiati cheese. The primary yeast species identified from Domiati cheese were *D. hansenii*, *Candida krusei*, and *Candida albicans*. Among these species, *C. albicans* was detected in 24% of Domiati cheese according to **Hameed (2016)**.

Various sources can lead to the contamination of cheese with yeasts and molds throughout production and post-processing handling and packaging. This contamination negatively impacts the shelf life and quality of the cheese (**Geronikou *et al.*, 2020**). Yeast contamination in cheese can stem from various sources. These include utilization of milk contaminated, unsanitary premises and utensils noted throughout the collection of samples, manual handling of products with bare hands, equipment, involvement of individuals in manufacturing and handling, poorly cleaned surfaces, and debris entering uncovered raw karish cheese milk. Inadequate processing has led to elevated total yeast counts (**Aly *et al.*, 2010**). Spoilage caused by yeast is a significant economic concern within the cheese sector, leading to undesirable alterations like sliminess, red discoloration, and a yeasty flavor (**Sarais *et al.*, 2009**).

Also as shown in **Table (1)**, twenty distinct yeast colonies from the traditional Egyptian soft white cheeses including karish cheese (12) isolates and pickled Domiati cheese (8) isolates were performed for isolation and purification based on characteristics of colonies. The colony color of each isolate was a white, creamy and binky. Rough and flattened

surfaces with undulate margins were observed in white and pinky colonies, while creamy colonies exhibited shiny surfaces and smooth, with entire margins and convex or raised elevations. Cell shapes were observed using microscopy (at a magnification of_400); Cells of white and pinky colonies with rough and flattened surfaces appeared ellipsoidal-shaped, while those from creamy colonies with smooth and shiny surfaces appeared ovoid or lemon-shaped. Each colony was provisionally treated as a distinct strain due to its unique growth properties and stress tolerance. Yeast-induced spoilage of dairy products can lead to noticeable changes, primarily stemming from their proliferation on the product surface. Examples include the browning reaction caused by *Yarrowia lipolytica* or "toad skin" defect caused by *G. geotrichum*. The latter defect is due to the extracellular accumulation of homogentisic acid, an intermediate of tyrosine catabolism, capable of polymerization and auto-oxidization, leading to the formation of a brown pigment, pyomelanin **(Carreira *et al.*, 2001 a,b).**

Table (1): Total yeast counts, morphological characteristics of the yeast isolates

cheese type	Sample code.	yeast count log cfu g^{-1}		no. of isolates	colonies and cell description			
					margin	color	surface	consistency
Karish cheese	1	2.99		1	+	++	+	++
	2	2.87		2	+	++, +	+, ++	++, +
	3	3.12		2	+	+, ++	++, +	+, ++
	4	3.23		2	+	+, ++	++, +	+, ++
	5	2.70		1	+	+	++	+
	6	3.13		2	+	++, +	+, ++	++, +
	7	3.26		2	+	+, ++	++, +	+, ++
Pickled Domiati cheese	8	1.97		1	+	+++	+	++
	9	2.45		1	+	++	+	++
	10	2.78		1	++	+	++	+
	11	2.95		1	++	+	++	+
	12	2.18		1	++	+	++	+
	13	3.96		2	+, ++	++, +	+, ++	++, +
	14	2.63		1	++	+	++	+

Margin: +: regular, ++: irregular; Color: +: white, ++: creamy, +++: binky;
Surface: +: smooth, ++: rough; Consistency: +: unviscid, ++: viscid. CFU: colony forming unit

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Identification of yeast isolates:

The proliferation of yeast in dairy products, especially in white brined cheeses, is influenced by various factors, including milk composition, nutrient availability, interactions with other microorganisms, in addition to the conditions of production and storage (**Buehler *et al.*, 2017; Lacanin *et al.*, 2017**).

Table (2): Affected of yeast isolates by biochemical and physiological examination

Cheese Type	Yeast isolates code	Biochemical and physiological tests					
		Casein hydrolysis	Lactose fermentation		Catalase test	Growth at 7˚C	Growth at 55˚C
			color	pH			
Karish cheese	1	-	+	5.78	+	-	-
	2	-	+	5.76	+	-	-
	3	-	-	6.53	+	++	-
	4	-	-	6.51	+	++	-
	5	-	+	5.74	+	-	-
	6	-	-	6.55	+	++	-
	7	-	+	5.78	+	-	-
	8	-	-	6.53	+	++	-
	9	-	+	5.79	+	-	-
	10	-	-	6.54	+	++	-
	11	-	-	6.53	+	++	-
	12	-	+	5.76	+	-	-
Pickled Domiati cheese	13	-	-	6.57	+	-	-
	14	-	+	5.80	+	+	-
	15	-	-	6.61	+	+	-
	16	-	-	6.6	+	+	-
	17	-	-	6.55	+	+	-
	18	-	+	5.78	+	+	-
	19	-	-	6.58	+	+	-
	20	-	-	6.58	+	+	-

(+): positive reaction; (-): negative reaction
(-)**:** no growth, (+): normal growth, (++): strong growth

Moreover, it is widely recognized that yeasts exhibit variations in their metabolic functions and biochemical processes, leading to differences in their spoilage characteristics. (**Haastrup *et al.*, 2018; Bayili**

***et al.,* 2019**). Conventional techniques for identifying yeast in dairy products rely on macro-morphological and micro-morphological observations, as well as physiological traits including growth conditions, carbohydrate and nitrogen assimilation, and fermentation abilities (**Garnier *et al.,* 2017**). Biochemical and physiological characterization of yeast isolates were illustrated in **Table (2)**. All yeast isolates, observed positive effect for catalase production, but exhibited negative effect for casein hydrolysis. Lactose fermentation ability was examined, only eight isolates were able to lactose ferment. While the temperature typically employed for the growth of yeasts is 25-30^{o}C (**Belloch *et al.,* 2008**), the growth of yeast isolates were examined at lower temperature (7^{o}C) and higher temperature (55^{o}C). Among of all, 30% of yeast isolates grew well at 7^{o}C (showed high psychrophiles), but 35% of isolates exhibited normal growth, and 35% of isolates hadn't the ability to growth. While, all the yeast isolates hadn't the ability to growth at high temperature (55^{o}C).

Influence of salt on the growth of investigated yeast isolates.

The production process of Domiati cheese encompasses various technological stages, including natural milk fermentation, salting, adding rennet, and maturation in salted whey or brine solutions. It mainly differs from other brined cheese types like Brinza, Telema or Feta cheese, where the milk is salted during the initial stage of the manufacturing process. The amount of salt used, ranging from 5 to 14%, is influenced by the time of year when the cheese is produced and the temperature during cheese aging (**Abou-Donia, 1986**). All 20 isolated yeasts were tested for their tolerance to salt on PDA petri dishes under the conditions of 5, 10, and 15% (w/v) NaCl. The results outlined in **Table (3)** showed that yeast isolates were affected by presence of salt and exhibited various growth rate, five isolates grew well under the conditions of 5.0% NaCl (0.85 mol L^{-1}), but 80% of isolates exhibited inhibition ratio ranged from 0.13 to 1.22%. Under the conditions of 10 % NaCl (1.7 mol L^{-1}) the growth rate of all isolates was affected with inhibition ratio ranged from 0.45 to 2.99%. All yeast isolates did not show any growth with 15 % NaCl (2.6 mol L^{-1}) on PDA plates. This data aligns with **Logothetis *et al.* (2007)**

findings, which demonstrated that while yeasts maintained their viability at a 10% w/v NaCl concentration, their growth rate was negatively impacted. Additionally, it illustrates that sodium chloride effectively inhibited yeast cells at level of 15% w/v, as evidenced by the 100% inhibition rate of yeasts isolated from karish and pickled Domiati cheese samples.

Table (3): Potentiality of yeast isolates to growth in different salt concentration

Cheese Type	Yeast isolates code	Salt concentration (%)			
		Control	5%	10%	15%
		Yeast isolates count log cfu g^{-1}			
Karish cheese	1	6.76	6.76	6.71	N.D
	2	6.55	6.47	6.46	N.D
	3	7.53	7.48	7.42	N.D
	4	7.50	7.47	7.38	N.D
	5	6.66	6.63	6.61	N.D
	6	7.45	7.45	7.38	N.D
	7	6.82	6.76	6.71	N.D
	8	7.65	7.59	7.53	N.D
	9	6.50	6.44	6.41	N.D
	10	7.55	7.49	7.48	N.D
	11	7.51	7.47	7.41	N.D
	12	6.45	6.44	6.41	N.D
Pickled Domiati cheese	13	7.04	7.04	6.99	N.D
	14	6.67	6.65	6.59	N.D
	15	7.70	7.70	7.49	N.D
	16	7.74	7.73	7.52	N.D
	17	7.64	7.60	7.56	N.D
	18	6.74	6.74	6.71	N.D
	19	7.75	7.72	7.52	N.D
	20	7.64	7.55	7.42	N.D

cfu: colony forming unit; N.D: not detected

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In vitro anti-yeast activity of some natural and chemical preservatives substances:

In the current work, according to the obtained results from morphological, biochemical and physiological examination, twenty yeast isolates from traditional Egyptian soft white cheeses were grouped into 4 clusters. Twelve yeast isolates from karish cheese were grouped into cluster-A and cluster-B, while eight yeast isolates from pickled Domiati cheese were grouped into cluster-C and cluster-D.

Preliminary work was done using the agar well diffusion test revealed that concentrations of preservative agents not specified in the study facilitated the growth of all yeast clusters (data not presented), In contrast, the mentioned concentrations resulted in distinct zones of varying diameters based on the yeast clusters. Data in **Table (4)** and **Pic (1)** showed the inhibitory effects of various concentrations of natamycin, clove oil, hydrogen peroxide and potassium sorbate on survival of examined yeast clusters, The inhibitory effect was assessed by quantifying the diameter of inhibition zone. It's crucial to note that the inhibition zone diameter reflects the equilibrium between the rate at which microorganisms grow and the rate at which antimicrobial agents diffuse into the agar.

Nowadays, natamycin is used to prevent the growth of molds and yeasts in certain dairy products (**Pintado *et al.*, 2010, Kallinteri *et al.*, 2013; Ollé Resa, *et al.*, 2014**). Yeast clusters exhibited notable variances in both growth rate and sensitivity to various concentrations of natamycin. Yeast cluster-C displayed the highest growth rate and consequently exhibited a smaller inhibition diameter in the current assay. Conversely, yeast cluster-B demonstrated slower growth and consequently displayed a larger inhibition diameter. The MIC of natamycin was 25 ppm produced a zone of inhibition (11, 12, 8 and 9 mm) for yeast clusters (A, B, C and D), respectively. **Pintado *et al.* (2010)** investigated the impact of incorporating malic acid, nisin, and natamycin into a whey protein concentrate film against *Y. lipolytica.* They found that films containing malic acid and natamycin resulted in inhibition zones measuring 11.9 mm and 8.0 mm, respectively. According to **Galal and Hameed (2016),** Feta

cheese samples containing natamycin (at concentrations of 0.2% or 0.4%) showed no presence of yeasts and molds when stored at either room temperature or refrigeration temperature. The European Union has authorized natamycin is as an additive in food for treating the surface of semi-soft, semi-hard, and hard cheeses. Natamycin is approved as a food additive in the In the USA, and its application on cheese must not exceed 20 $mgkg^{-1}$ in the final product, as outlined by International Dairy Federation (IDF) Standard 140A: 1992 (21 CFR 172.155). In Canada, natamycin is allowed on 47 specified cheeses at levels a maximum of 20 ppm and in grated / shredded cheese at residual levels a maximum of 10 ppm (**Stark, 2003**).

In the current investigation, clove oil demonstrated efficacy in inhibiting yeast activity at high concentrations (500, 750 and 1000 ppm). Yeast clusters showed varied inhibition zone diameters with significant differences to clove oil concentrations. The MICs of clove oil were (500 ppm) produced inhibition zone diameter (9, 10.5 and 13 mm) for yeast clusters (A, C and D) respectively, and (750 ppm) for yeast cluster-B with inhibition zone (7 mm). In the same line (**Saleh, 2018**) added clove oil to kareish cheese manufacturing as antifungal agent in concentration 0.5%, 0.75% and 1.0%. The reported inhibitory concentrations in previous studies on clove essential oil ranged between 0.04% and 2% (**Smith-palmer *et al.*, 1998; Hammer *et al.*, 1999; Thanissery *et al.*, 2014**). Based on findings obtained by Kavas ***et al.*** (**2015**), the application of whey protein isolate- based edible films includes 1.5% (v/v) essential oils of thyme and clove as coatings for Kashar cheese might contribute to prolonging its shelf life. Despite being listed as a 'GRAS' substance by the United States FDA, clove oil is deemed safe when used in food at concentrations not surpassing 1500 ppm across all food groups (**Gulcin *et al.*, 2012**).

The yeast clusters studied had hydrogen peroxide MIC at concentration 35 ppm, with inhibition zone diameter differ significantly and ranged from 14 mm to 20.5 mm for clusters A and C, respectively. The results obtained align with those of **Saha *et al.* (2003),** who determined that a concentration of 0.04 to 0.05% hydrogen peroxide $_{at}$ (30

w/v) was adequate to extend milk shelf life by up to 24 hours. **Collinson and Dawes (1992**) identified the concentrations of H_2O_2 that impacted yeast strain BGWl-7a, they specified that a concentration of 2 mM was selected as the lethal dose for subsequent experiments.

Table (4): Minimum Inhibitory concentration (ppm) of investigated yeast isolates to different preservative agents.

Preservative agents	Conc. ppm	Yeast isolates*			
		A	B	C	D
		Inhibition zone diameter (mm)			
Natamycin	control	0^{c}	0 ^{c}	0 ^{c}	0 ^{c}
	0.5	11.0 ^{ab}	12.0 ^{ab}	8.00 ^{bc}	9.00 ^{ab}
	0.75	13.0 ^{ab}	14.5 ^{ab}	10.5 ^{ab}	10.5 ^{ab}
	1	13.5 ^{ab}	16.5 ^{a}	13.5 ^{ab}	13.5 ^{ab}
Clove oil	control	0 ^{g}	0 ^{g}	0 ^{g}	0 ^{g}
	500	9.00 ^{ef}	0 ^{g}	10.5 ^{def}	13.0 ^{cde}
	750	16.0 ^{bc}	7.00 ^{f}	14.0 ^{cd}	19.0 ^{ab}
	1000	16.0 ^{bc}	8.50 ^{f}	15.0 ^{bc}	20.5 ^{a}
Hydrogen peroxide	control	0 ^{f}	0 ^{f}	0 ^{f}	0 ^{f}
	35	14.5 ^{e}	14.0 ^{e}	20.5 ^{c}	15.5 ^{de}
	70	17.5 ^{d}	15.0 ^{de}	25.5 ^{b}	20.5 ^{c}
	105	20.5 ^{c}	15.0 ^{de}	31.5 ^{a}	21.5 ^{c}
Potassium sorbate	control	0 ^{e}	0 ^{e}	0 ^{e}	0 ^{e}
	100	9.00 ^{cde}	8.00 ^{de}	12.0 ^{bcd}	17.5 ^{bcd}
	200	13.5 ^{bcd}	12.5 ^{bcd}	21.5 ^{b}	17.5 ^{bcd}
	300	19.0 ^{bcd}	15.0 ^{bcd}	35.0 ^{a}	20.0 ^{bc}

* A, B: yeast isolates from karish cheese samples; C, D: yeast isolates from domiati cheese samples

The means followed by the same letter don't differ statically from each other applied at a 5% probability level.

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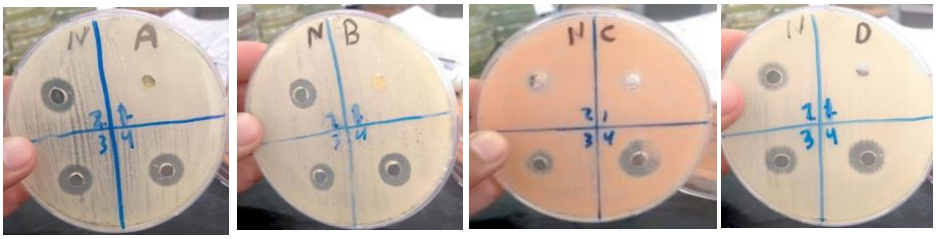
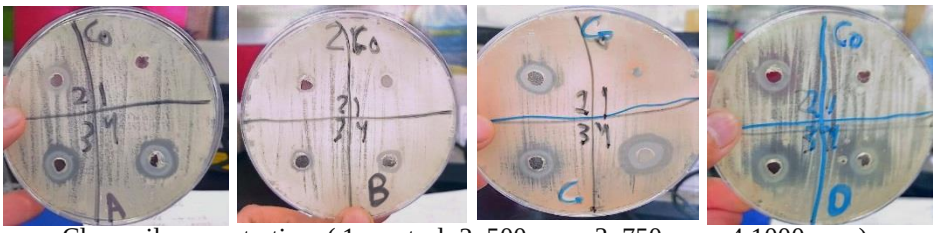
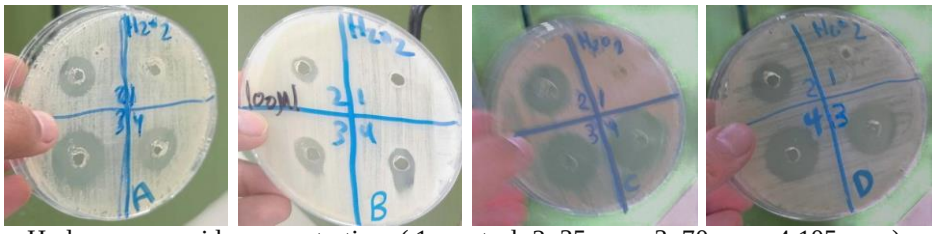


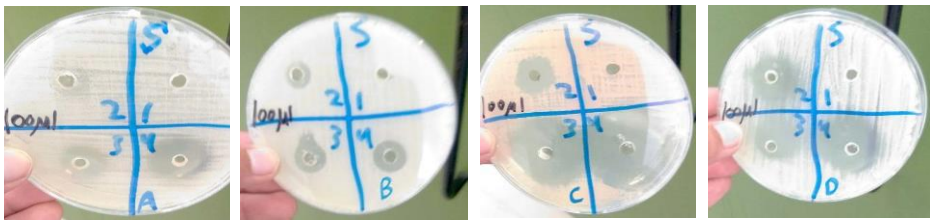
Natamycin concentration :(1: control; 2: 0.5 ppm; 3: 0.75 ppm; 4:1 ppm)



Clove oil concentration :(1: control; 2: 500 ppm; 3: 750 ppm; 4:1000 ppm)



Hydrogen peroxide concentration :(1: control; 2: 35 ppm; 3: 70 ppm; 4:105 ppm)



Pot. sorbate concentration:(1: control; 2: 100 ppm; 3: 200ppm; 4:300 ppm)

Pic. (1): Inhibition zones diameter of yeast clusters (A, B, C and D) exhibited by different concentration of preservatives agents.

Potassium sorbate was used at concentrations (100,200and 300ppm). exhibited significant differences against yeast clusters activity. The MIC of Potassium sorbate was 100 ppm produced a zone of inhibition (9, 8, 12 and 17.5 mm) for yeast clusters (A, B, C and D), respectively. Potassium sorbate showed less inhibitory effect, than natamycin, against the studied mold species. The obtained results agreed with the results of (**Finol *et al.*, 1982)** who stated that cheeses with potassium sorbate as a preservative are sometimes spoiled by molds. In a similar context (**Ombarak and Shelaby., 2017)** investigated the suppressive effect of potassium sorbate at levels 0.5, and 1% on growth of molds in Tallaga cheese during 30 days of refrigerated storage at 4 ±1^{o}C.

CONCLUSION

The current study indicates the poor microbiological quality of the tested products. According to standards limit, the findings showed that the almost Domiati cheese and Karish cheese samples were unsatisfactory. We obtained 20 different isolates, all the isolated yeast exhibited tolerance to 10% (w/v) NaCl, and some of the isolates observed ability to grow under psychrophilic conditions. All of the isolates showed inability to casein hydrolysis, but some isolates showed the ability to lactose fermentation. The results indicate that natamycin is considered an effective anti-yeast natural preservative against the examined yeast isolates, at extremely low levels compared to clove oil, hydrogen peroxide and potassium sorbate.

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العد والتعرف على أنواع الخمائر المعزولة من الأجبان البيضاء الطرية المصرية والنشاط المضاد للخمائر معمليا: دراسة أولية

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الملخص العربي

أجريت الدراسة بهدف العد والعزل والتعرف على أنواع الخمائر الموجودة فى الأجبان المصرية البيضاء الطرية (القريش-الدمياطي الخزين) وكذلك دراسة النشاط المضاد للخمائر لبعض المواد الطبيعية (النتاميسين - زيت القرنفل)، والمواد الكيميائية (فوق أكسيد الهيدروجين- سوربات البوتاسيوم) ضد عزلات الخمائر وذلك لتحسين جودة وسلامة هذه المنتجات. أظهرت النتائج أن جميع عينات الجبن القريش و57% من عينات الجبن الدمياطى الخزين كانت ملوثة بالخمائر أعلى من الحدود المطلوبة بالمواصفة القياسية المصرية، كما وجد أن عينات الجبن القريش احتوت على أعلى خمائر بمتوسط عدد لو$_{10}$ 3.4 مستعمرة /جم مقارنة بعينات الجبن الدمياطي الخزين بمتوسط عدد لو$_{10}$ 2.7 مستعمرة /جم. تم الحصول على عشرين عزلة نقية من الخمائر من عينات الجبن وتم التعرف عليها بالفحص المورفولوجي والاختبارات الفسيولوجي والكيموحيوية و، وأظهرت النتائج ان لون المستعمرات تراوح ما بين الابيض والكريمى والبرتقالى وكذلك الأشكال ما بين الدائرى والغير منتظم ، كذلك أظهرت أن جميع عزلات الخميرة غير قادرة على النمو عند درجة حرارة 55 م ولا تحلل الكازين، لكن بعض عزلات الخميرة كانت لها القدرة على تخمير اللاكتوز وبعضها له القدرة على النمو عند درجة حرارة 7 م. وكان الحد الأدنى للتركيز المثبط (MIC) لكل من النتاميسين ، فوق أكسيد الهيدروجين ، وسوربات البوتاسيوم لكل عزلات الخميرة (0.5، 35، 100جزء في المليون) على التوالي، بينما تراوح الحد الأنى للتركيز المثبط لزيت القرنفل ما بين (500 الى 750 جزء فى المليون).

الكلمات المفتاحية: جبن القريش، الجبن الدمياطي، تحديد الخميرة، النشاط المضاد للخمائر معملياً.