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QUALITY ASSURANCE OF KAREISH CHEESE

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ABSTRACT

Recently, aware is become more and more of the problems related to pollution and both government and industries have begun to take steps to avoid further deterioration being arised from of environment. Thereby, quality assurance of Kareish cheese was estimated through the occurrence of both of yeasts and heavy metals in forty samples of Kareish cheese collected from Fayoum and Giza Governorates. Enumeration, isolation and identification of yeasts with API 20 Aux test, revealed that, all of the examined samples contained yeasts, with a mean value of $7.8x10^6 \pm 0.95x10^5$cfu g^{-1}. The most predominant species identified as *Candida* 47.5% whereas, *C. albican* 35%; *C. lipoliticum* 7.5% *; C. curvata* 5%; *C. tenius* 0%, followed by *Saccharomyces* 42.5%; whereas*, S. cervisiae* 37.5% and *S. farinosum* 10%, *Torulopsis* 25% whereas, *T.versatilis* 15%; *T. ernobii* 10%*, Trichosporon cutaneum* 25% and *Yarrowia lipolytica* 13%.

Heavy metals estimation revealed that the values of means ± the standard errors of, cadmium, lead, nickel, cupper, magnesium and manganese are 0.3351± 0.0314, 0.5766± 0.1000, 0.7958± 0.0752, 0.3722± 0.0694, 11.6750± 0.7133 and 1.5090± 0.1997 mg/100g wet weight Kareish cheese, respectively. The concentrations of lead and cadmium in the tested kareish cheese samples were above the Egyptian Standard (2005) and WHO (1993) permissible limits. Possible health risk of these metals was discussed.

INTRODUCTION

Karish cheese is one of the most popular local types of fresh soft cheese in Egypt. The increasing demand by Egyptian consumers is mainly attributed to its high protein content and low price (Osman, *et al*., 2010).

Karish cheese is traditionally made from skim cow's or buffalo's milk which is milked directly into special earthenware pots known as (shalia), and kept undisturbed in a suitable place to allow the fat to rise to the surface, forming a cream layer. Then the cream layer is removed and the curd is poured onto a mat, which is tied and hung with its contents to allow the drainage of the whey. This process of squeezing takes two or three days until the desired texture of the cheese is obtained. Finally, the cheese is cut into suitable pieces, and salted cheese is left for a few hours in the mat till no more whey drains out, then it is ready to be consumed as fresh soft cheese (Ojokoh, 1998). This traditional method affords many opportunities for microbial contamination (Yousef, 2007 and Dawood, *et al.,* 2009).

It is widely recognized that yeasts can be an important component of the microflora of many cheese varieties, because of the low pH, low moisture content, high salt concentration and refrigerated storage of these products (Devoyod, 2008).

The main mechanisms by which yeast growth influences the final quality of cheese are: fermentation of lactose, utilization of lactic acid and lipolytic and proteolytic activities (Tudor and Board, 2010 and Rohm *et al.,* 2010).

In some cheese types, yeasts make a positive contribution to the development of flavor and texture during the stage of maturation; in other varieties, yeasts can be regarded as spoilage organisms (Brocklehurst and Lund, 1985; Fleet, 1990; Ebrahim, 2008 and Mahmoud, 2009). They can produce a characteristic yeasty or fruity flavor and obvious gas formation to cause economic and public health (Dennis and Buhagiar, 2007; Dillon and Board, 2008 and Daryaei *et al*., 2010).

Another risk may be presented in Kareish cheese, is the heavy metals that represent the chemical residues and have a major role in the human health. They are cumulative poisons, causing irreversible accumulation in the body (Alberti and Fidanz, 2002). Heavy metals of toxicological concern are arsenic, lead, cadmium, chromium, mercury, copper and zinc (Kabzinski, 1998). The spill of Lead, Cadmium and Mercury in water may be entered the food chain, because of their extreme persistence, high toxicity, and its tendency to accumulate (Hernandez, *et al*., 1999). However, lactating cows may be exposed to high quantities of toxic metals in the environment from air, water and feeds (El-Shinawy, 2009).

Lead poisoning in cattle, milk and milk product is of public health significance because of the potential for human exposure to lead through ingestion of contaminated meat and milk products derived from lead- poised animals (Senavci, *et al.,* 1997).

Cadmium is considered to be one of the most toxic heavy metals known. It is a non- essential trace element which progressively accumulates inside the body, particularly, kidney and it is a major contributor to thyroid disease (Watanabe, *et al.,* 2000). Chronic inhalation exposure leads to anemia, renal dysfunction, disorder in calcium metabolism, and prostate and lung cancer (Brzoska and Moniuszko, 1998). However, uptake of cadmium from soil by feed crops may result in high levels of cadmium in body. The accumulation of cadmium in the food chain has important implications for human exposure, whether or not significant bio-magnification occurred (USPHS, 1997, WHO, 1993)

The maximum acceptable daily intakes of heavy metals in food in mg /kg body weight are zero for cadmium, lead and mercury, however the maximum permissible limits of arsenic, copper, iron, tin and zinc as daily intakes in mg/ kg body weight are 0.002; 0.05-0.5; 0.8; 20 and0.3-1 respectively, (Egyptian Organization for Standardization and Quality Control, 2005). However, El- Shinawy, (2009) reported that the mean of lead, cadmium and mercury were 0.002-0.003; 0.00004-0.00005 and <0.00007mg/ kg mil,respectively..

In Egypt, the information about the involvement of Karish cheese in human illness and economic losses are unknown. Therefore, this study was designed to cover the following items: (1) enumeration of the yeast populations in kareish cheese samples (2) isolation and identification of the

yeast species using both conventional method and commercial identification system API 20 eux test. (3) Incidence of heavy metals in Kareish cheese.

MATERIALS AND METHODS

Forty samples of kareish cheese were randomly collected from supermarkets and farmers at Fayoum Governorate. Each cheese sample was represented by one whole cheese (500 g). All samples were transported to the laboratory under refrigeration at 5^{o}C and analyzed on arrival for chemical examination as well as for the isolation and identification of yeasts

Ten grams were taken from the interior of the cheese samples, diluted in 90 ml of sterile solution of 2% (w/v) sodium citrate (Sigma, St. Louis, MO, USA) and homogenized in a Stomacher (PBI, Milan Italy) for 30 seconds. For all samples, ten fold serial dilutions were prepared in a sterile solution of 2% (w/v) sodium citrate, and numbers of yeasts were determined by surface plating on yeast potato dextrose agar (PDA) (Microbiol, Cagliari, Italy) with chloramphenicol (0.01%) after incubation at 25 °C for 5 days. All samples were prepared and analyzed in duplicate. Yeast colonies were sorted on the basis of their morphology (smoothness of surface, regularity of border, consistency, color, etc.), streaked to single colonies on yeast potato dextrose agar media (1% yeast extract, 2% dextrose, 2% peptone, and 1.5% agar), incubated for 5 days at 25 °C, and checked for purity. Counts for each individual type of colony were made in order to estimate the relative occurrence of the various yeasts present in the samples. Yeasts species counts were calculated as number of colony forming units per gram of sample. The colonies isolates were characterized according to Van der Walt and Yarrow, (2009).

The isolates were identified using the conventional tests, and were checked using the API 20 eux kits (bio Merieux, Rome, Italy) according to Dolan and Woodward (2007)

As recommended by the manufacturer, each isolate was sub cultured prior to testing to ensure viability and purity. Yeast inoculum suspensions were prepared from 48-h cultures grown on sabouraud dextrose agar plates at 30°C. Yeast cells were suspended in 2 ml of RapID Yeast Plus Inoculation Fluid to achieve a turbidity which completely obliterated the black lines of the Inoculation Card supplied with the kits. Each yeast suspension was dispensed into a Rap ID Yeast Plus panel, and the panels were then incubated for 4 h at 30°C. Immediately after the incubation time, Rap ID Yeast Plus Reagents A and B were added to the designated cavities and color reactions were evaluated by following the manufacturer's directions. A six-digit microcode was derived and compared to the codes in the RapID Yeast Plus Code Compendium for the identification of the isolate. All micro-codes were also sent to the manufacturer for confirmation. Molten (50°C) API basal medium ampoules were inoculated with yeast colonies, and the suspension was standardized to a density below 1+ (lines can be clearly distinguished) on a Wickerham card. Each cupule was inoculated, and the trays were incubated for 72 h at 30°C. Cupules showing turbidity significantly

heavier than that of the negative control cupule (0 cupule) were considered positive. Identification was made by generating a microcode and using the API 20C Analytical Profile Index or the Voice Response System (for profiles not found in the index). Tubes were read after 24 and 48 h, and again after 10 days for evidence of gas production, which indicated fermentation of the carbohydrate substrate.

Ash was determined as described in A.O.A. C (2003), and metals (Pb, Cd, Ni, Cu, Mg and Mn) were estimated using atomic absorption spectrophotometer (ZEISS, AAS 5. Germany). The metal was extracted from a solution of the sample ash. Standards were treated in the same way, and both sample and standard extracts were aspirated in the flame of an atomic absorption spectrophotometer.

Analysis of the data was carried according to Ott (2009)

The results are presented as Mean and Standard errors. The analysis of variance (ANOVA) test was conducted to test the possible significance ($P \geq 0.05$) among mean values of yeast count using Fishers Least Significance Difference (LSD) were calculated.

RESULTS AND DISCUSSION

Occurrence and characterization of yeasts isolated from kareish cheese

Data depicted in Table 1. revealed that yeasts were found in all of the examined cheese samples, with a minimum value of 100 cfu g^{-1} , maximum value of $0.16x10^8$ cfu g^{-1} and a mean value of $7.8x10^6 \pm 0.95x10^5$ cfu g^{-1}

Nearly similar findings were reported by Ahmed, *et al*., (2008); Devoyod, (2008); Aly, *et al.*, (2007) and Qing, *et al*., (2010). Higher results were obtained by Kaldes, *et al*., (2006) while lower results were obtained by Brocklehurst and Lund (1985); El-Kholy (2001) and Said, *et al.,* (2009).

The Egyptian Standards (2005) specify that the total yeast count not exceed 10 cfu g^{-1} detected in the cheese. The International Commission on Microbiological Specifications for Foods (2005) has classified cheese as a high risk potential hazard. A high yeast count often indicates neglected hygienic measures during production and handling, contamination of raw material, unsatisfactory sanitation, or unsuitable time and temperature during storage and/or production. It might also be referred to the suitable pH of cheese for yeast growth as well their wide distribution in the environment (Aponte, *et al.,* 2010).

From the obtained results, it is obvious that most of the examined Kareish cheese samples failed to confirm the Egyptian Standard (2005), as they exceeded the accepted level. The Egyptian standards for Kareish cheese have proposed a limit for the total yeast count to be less than 10 cfu g^{-1}. The high incidence in the examined samples might be attributed to poor sanitation during preparation and or storage of the product. There are numerous sources of yeast contamination. These include the use of contaminated milk, the observed dirty premises and utensils used as observed during sample collection, the use of bare hands in preparing the products (personal communication with the handlers), equipment, through

persons taking part in manufacturing and handling of the product, improperly cleaned servers and debris falling into uncovered raw milk kareish cheese. The high total yeast count has resulted from inadequate processing. Yeast spoilage constitutes major economic losses in the cheese industry through developing undesirable changes such as slimness, red color and yeasty flavor (Sarais, *et al*., 2009). This might be due to their capacity to produce lipolytic and proteolytic (Fleet and Main, 2009 and Tornadijo, *et al*., 2010).

Table 1: Statistical analytical results of total yeast count/g in examined Kareish cheese

Samples	Total No. of samples	Positive samples		Min.	Max.	Mean±
		No.	%			
Kareish	40	40	100	100	$0.16x10^8$	$7.8x10^6 \pm 0.95x10^5$

Table 2. revealed the presence of *Candida* was of 47.5% whereas *C. albican* 35%; *C. lipoliticum* 7.5% *; C. curvata* 5%; *C. tenius* 0%, *Saccharomyces* 42.5%; whereas*, S. cervisiae* was of 37.5% and *S. farinosum* 10%. *Torulopsis* 25% whereas, *T.versatilis* 15%; *T. ernobii* 10%*, Trichosporon cutaneum* 25%, and *Yarrowia lipolytica* 13%.

El-Shafei, *et al.,(*2008) found that the most predominant species isolated from Kareish cheese collected from Giza and Cairo, were *Saccharomyces cerevisia* followed by *Candida* spp.

It was reported that *Trichosporon* spp. caused formation of a surface film on the cottage cheese leading to spoilage (Nichol and Harden, 2006 and Welthagen and Viljoen, 2009). Presence of this species in high concentrations could indicate poor hygiene and ineffective cleaning procedures and show the need for improved sanitization procedures (Seiler and Busse, 2009 and Viljoen, *et al.,* 2010). *Yarrowia lipolytica,* resulted in a browning spoilage of cheese (Vorbeck and Cone, 2009 and Westall and Filtenborg, 2010), while *Candida zeylanoides* has been isolated from Feta cheese but it was not possible to determine whether spoilage was associated with this species (Eklund, *et al*., 2005; Diriye, *et al*., 2007 and Rohm, *et al.,* 2010).

The source of the isolation of *Trichosporon cutaneum* varies considerably, although many of them are of human and animal origin (Kreger-van Rij, 2009). The presence of this species in high concentration could indicate poor hygiene and ineffective cleaning procedures and show the need for improved sanitization procedures. *Yarrowia lipolytica, and Candida zeylanoides* have also been isolated from spoiled Cottage cheese. *Yarrowia lipolytica* in high concentration resulted in an unwanted texture of Feta-cheese due to the degradation of fat through production of lipolytic and proteolytic enzymes (Westall and Filtenborg, 2010).

Candida spp. are the most common cause of fungal infection in immune compromised persons known as candidiasis. Candidiasis is caused by infection of species within the genus *Candida,* predominantly with *Candida albicans. Candida* species are ubiquitous fungi that represent the most common fungal pathogens that affect humans. Oropharyngeal colonization is found in 30-55% of healthy young adults, and *Candida* species may be

detected in 40-65% of normal fecal florae. *Candida albicans* can infect all areas of the skin, as well as the mucous membranes. Infections by *Candida albicans*, especially which are found in the mucous membranes are contagious (Hidalgo, 2011).

Table 2: Incidence of yeast species in examined Kareish cheese samples

Species	Plain	
	N0.	%
Candida	19	47.5
C. albican	14	35
C. lipoliticum	3	7.5
C. curvata	2	5.0
C. tenius	0	0
Saccharomyces	17	42.5
S. cervisae	15	37.5
S. farinosum	2	5.0
Torulopsis	10	25
T.versatilis	6	15
T. ernobii	4	10
Trichosporon cutaneum	10	25
Yarrowia lipolytica	5	13

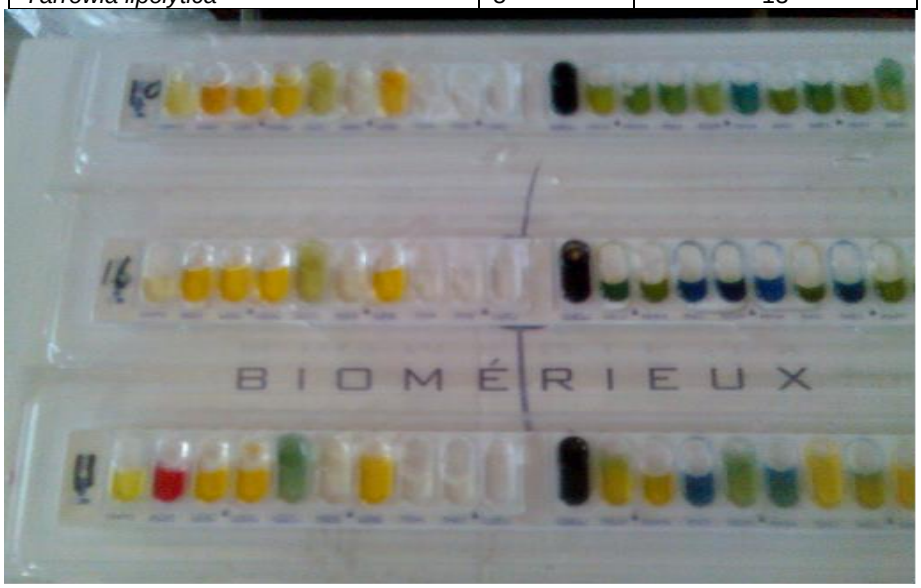

Fig. 1: Identification of yeasts in Kareish cheese using Api 20 eux kits test.

Occurrence of heavy metals in Kariesh cheese

Heavy metals such as lead, cadmium ….etc. have received increasing attention. This attention has been focused due to their adverse toxic effects. The metals that can not be metabolized persist in the body and exert their

toxic effect by combining with one reactive group essential for normal physiological functions.

Table 3. revealed that the mean ± the standard error of cadmium (Cd), lead (Pb), nickel (Ni), copper (Cu), magnesium (Mg) and manganese (Mn) in Kareish cheese are 0.3351± 0.0314, 0.5766± 0.1000, 0.7958± 0.0752, 0.3722± 0.0694, 11.6750± 0.7133 and 1.5090± 0.1997 mg/100g wet weight, respectively. They are 1.3732± 0.1289, 2.3630± 0.4100, 3.2612± 0.3082, 1.5251± 0.2843, 47.8440± 2.9230 and 6.1839± 0.8182 mg/100g dry weight, respectively.

The maximum permissible limits of arsenic, lead, copper, zinc, mercury, cadmium and tin, in processed cheese with plant oils are 0.25, 0.3, 0.3, 20, 0.02, 0.05 and 50 mg/kg, respectively, whereas the maximum permissible limits of Lead in milk caseins is 20 mg/kg, according to Egyptian Organization for Standardization and Quality Control, (2005). Based on chronic toxicity studies, the Provisional Tolerate Weekly Intake (PTWI) of heavy metals in food for adults (60 kg b. w) established by the joint FAO/WHO expert committee on food additives, was 0.05 mg/kg b. w. for lead, 0.007 mg/kg b. w. for cadmium, 0. 005 mg/ kg b.w. for adults.

The mean concentrations of Lead in Kareish and pickled Kareish cheese (in Upper Egypt) were 3.66± 0.94 and 1.44± 0.41 mg/kg dry weight, respectively and 1.2± 0.37 and 0.67± 0.18 mg/kg wet weight, respectively. For cadmium, the mean values were 0±0 and 0.11 ± 0.06 mg/kg dry weight respectively and 0±0, 0.037 ± 0.02 mg/kg wet weight respectively. While, the mean values for manganese were 3.59± 1.69 and 3.4± 3.16 mg/kg dry weight and1.3± 0.8 and 1.22± 0.92 mg/ kg wet weight respectively (Abdou and Korashy, 2009). They added that the high lead content was found in Kareish cheese. Abdou, (2009) added that nitrites, lead, and cadmium in Wadi El Rayan protected area lakes, in Fayoum, Egypt were detected to be above the WHO (1993) permissible limits (3, 0.01 and 0.01mg/l) respectively, while copper, mercury were below the recommended levels, that due to various industrial enterprises, urban infrastructure, agriculture, horticulture.

Results indicated that Kariesh cheese samples have very high content of cadmium, high content of lead and nearly similar findings were found with copper, magnesium and manganese as reported by Abdou, (2009), Abdou and Korashy, (2009), Abdou, *et al*, (2009) and El- Shinawy, (2009), whereas, they were above the Egyptian Standards (2005). The high incidence in the examined samples might be attributed to pollution.

Lead affects both central and peripheral nervous system of human. Lead toxicity inhibits hemoglobin synthesis leading to anemia. However, inhalation of lead dust or fumes results in relatively high concentrations in the blood and tissues with in a few hours or days. The largest amount is found in the bone and the smaller amounts are found in the liver, kidney, heart. Lead poisoning has been a cause of death (Abdou, *et al*, 2009)

Cadmium acts on the sulfhydryl groups of essential enzymes and also binds to phospholipids and nucleic acids. It has been shown to interfere with oxidative phosphorylation. Cadmium can replace zinc in metal enzymes with resulting changes in activity (Brzoska and Moniuszko, 1998). The absorption

of Cadmium is primary directly from lungs with a reabsorption rate of 10-50%, whereas only 1-7% is reabsorbed from the gastrointestinal tract after oral ingestion. Internal reabsorption can be increased by dietary factors for example loss of Ca, Fe, Vitamin D and protein. Cadmium plays an important role in the cause of hypertension and osteomalacia (El- Shinawy, 2009). Cadmium accumulates in plants fertilized with cadmium sewages sludge or phosphate fertilizer, and then accumulates in cattle bodies (Mason, 1991, Gary, 1996). It also used industrially in plastic and fungicides (Peter, 1998)
Copper is trace metal in human beings and is essential to several human enzymes. Human copper deficiency results in anemia. Copper sulfate causes a metallic taste in the mouth, epigastric pain, vomiting, diarrhea, hepatic and renal failure.

Nickel causes little human toxicity other than dermatitis but nickel carbonyl is extremely toxic. Acute inhalation of nickel carbonyl causes severe pulmonary edema and liver necrosis (El-Shinawy, 2009).

Table 3: The mean ± Std. Err of heavy metals in Kareish cheese* mg/100g

	Cadmium (Cd)	Lead (Pb)	Nickel (Ni)	Copper (Cu)	Magnesium (Mg)	Manganese (Mn)
On dry weight	1.3732± 0.1289	2.3630± 0.4100	3.2612± 0.3082	1.5251± 0.2843	47.8440± 2.9230	6.1839± 0.8182
On wet weight	0.3351± 0.0314	0.5766± 0.1000	0.7958± o.o752	0.3722± 0.0694	11.6750± 0.7133	1.5090± 0.1997

*** The tested Kareish cheese had ash content varied from 3.67 to 5.01%**

Conclusion

In conclusion, there is a need for continuous monitoring of Egyptian kareish cheese by educating producers, distributors and retailers on good sanitary practices during processing and sale of the product and the possible danger of contaminated cheese. Also, a regular general and representative monitoring, of heavy metal contamination, of milk and milk products especially Kareish cheese, is recommended.

Further researches should be done to evaluate heavy metals in the different dairy products in Fayoum and the different Governorates in Egypt.

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سلامة الجودة فى الجبن القريش

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زاد ادراك كل من الحكومة و القائمين على الصناعة لمدى اهمية المشاكل ا المتعلقة بالتلوث.و قد بدأت في اتخاذ الخطوات اللازمة لتفادي مزيد من التدهور للبيئة.وبالتالي،تهدف هذه الدراسة لتقدير سلامة الجودة لمنتج شائع مثل الجبن القريش من خلال دراسة مدى تواجد كل من الخمائر والمعادن الثقيلة في أربعين عينة من الجبن القريش التى تم تجميعها من محافظتى الفيوم و الجيزة. و من هنا تم عد و عزل وتصنيف الخمائر بواسطة اختبار API 20 eux ، و اسفرت النتائج عن ان جميع العينات التي فحصت تحتوى على خمائر، مع قيمة متوسط ٧,٨x١٠^{٦} ± ٠,٩٥x ١٠^{٥}.

كذلك أسفرت نتائج العزل والتصنيف عن سيادة *Candida* بنسبة ٤٧,٥% حيث تم عزل الانواع الاتية من هذا الجنس

C. albican 35%; *C. lipoliticum* 7.5% ; *C. curvata* 5%; *C. tenius* 0%, و يليه جنس *Saccharomyces* ٤٢,٥% حيث تم عزل الانواع الاتية من هذا الجنس

S. cervisiae 37.5% , *S. farinosum* 10% ثم جنس *Torulopsis* بنسبة ٢٥% حيث تم عزل الانواع الاتية من هذا الجنس,*T. ernobii* 10% , *T.versatilis* 15% كذلك انواع *Trichosporon cutaneum* 25% و *Yarrowia lipolytica* 13%.

أما عن تقدير المعادن الثقيلة, فقد أسفرت النتائج عن المتوسطات الاتية للكادميوم والرصاص والنيكل والنحاس و المغنيسيوم و والمنغنيز و٠,٣٣٥١ ± ٠,٠٣١٤و ٠,٥٧٦٦ ± ٠,١٠٠٠و ٠,٧٩٥٨ ± ٠,٠٧٥٢و ٠,٣٧٢٢ ± ٠,٠٦٩٤و ١١,٦٧٥٠ ± ٠,٧١٣٣ و١,٥٠٩٠ ± ٠,١٩٩٧ ملليجم/ كجم جبن قريش وزن رطب ، على التوالي. و لقد كانت تركيزات الرصاص والكادميوم عند اختبار عينات الجبن القريش أعلى من معدلات الحدود المسموح بها فى المواصفات القياسية المصرية (٢٠٠٥) ومنظمة الصحة العالمية (١٩٩٣) .

قام بتحكيم البحث

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