ARTICLE
JOURNAL OF UNIVERSITY OF BABYLON
For Pure and Applied Sciences (JUBPAS)
Vol.30; No.1.| 2022

Prevalence, Isolation and Identification of Microorganisms from Homemade Cheese in Babylon Province

Sabreen A. Kamal[1], Abeer F. Murad[1], Ruia Khudhair Abbas[1]

[1]College of Science for Women, University of Babylon, Kamal_sabreen@yahoo.com Babylon, Iraq.

*Corresponding author email: Kamal_sabreen@yahoo.com

Received:	13/1/2021	Accepted:	23/3/2022	Published:	31/3/2022

ABSTRACT

Forty samples of Gibin al Arab cheese were collected from local market at Babylon province for studying the microbial contamination (bacterial and fungal growth). first the study revealed that there was negative samples for each culture media used for bacterial detection, in MSA there was 27+ve samples and 13-ve samples, EMB with 24+ve samples and 16-ve samples, MRS with 32+ve samples and 8-ve samples finally S-S agar with 31+ve samples and 9 –ve samples. MSA record 17 strain of *Staphylococcus aureus*, 10 strain of *Staphylococcus epidermidis*, EMB record 12 strain of *Escherichia coli*, 18 strain of *Klebsiella*, MRS record 32 strain of *Lactobacillus*, S-S agar record 10 strain of *Salmonella*, 29 strain of *Shigella*. while fungi isolated on PDA was : *Penicillium digitatum* (74.7%), *Penicillium italicum* (13.2%), *Penicillium citrinum* (6.02 %), *Penisillium chrysogenum* (2.40%), *Aspergellus nedulans* (3.7 %), *Alternaria* (0%), *Candida spp.* with more isolates, also *Rhodotorella* more. MEA media used for fungi isolation record : *Penicillium digitatum*(80%), *Penicillium italecum* (3.1%), *Penicillium citrinm* (0%), *Penicillium chrysogenum* (6.2%), *Aspergillus nidulans* (7.69%), *Alternaria* (3.1%), *Candida spp*. with more isolates, *Rhodotorella* more like on PDA.

Key words: bacterial and fungal growth, homemade cheese

INTRODUCTION

Local cheese in Iraq which called (Gibin Al- Arab) a type of milk products are being favorable nutrition food for human in Iraq, because of it is rich with high protein, fat, calcium, phosphate, Vitamins and minerals [1]. In addition to its Nutritive value with it is a distinctive flavor and it is price make people consumed it and cannot dispensed it as a meal at any time [2]. This local produced cheese is traditionally made from milk of cow, milk of buffalo, milk of goat. In adequate temperature using pots special for making it. All condition from milk collection, cheese making and marketing give a good chance for microbial contamination [2]. Cheese polluted depend on the microbial quality of milk, milk heat treatment, manufactured temperature [3]. Therefore, it is contamination with microorganisms causes economic losses and health risk for a consumer as a result of affecting it with microbial toxins [4].

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

Regard as a good media for the growth of various pathogenic microbes like bacteria, yeast and mould [5]. Microbial content of milk products is influenced by an initial flora of raw milk, product condition and post heat treatment contamination[6] bacteria that can cause cheese spoilage include Gram negative, Coliform, Lactic acid bacteria in addition to salmonella sp., Listeria monocytogenes, pathogenic strain of E.coli, Enterotoxin strain of staphylococcus aureus, Bacillus Species that can found in milk product[7].These microbes can contaminate milk and it is products when it was a raw material, manufactured, storage and distribution[8]. Fungi have the ability to secret mycotoxins which are fungal secondary mitabolites that made fungi connected with scope of pathologies went from gastroenteritis to cancer, because these toxins are highly toxic, mutagenic and carcinogenic[9]. Yeasts play two roles in cheese : positive one it gave cheese a good flavour and texture during Preparation process and negative one it can regarded as spoilage organism in fermented milk and cheese[10] that because sources of yeast infections continue during the whole chain of production. The aim of this study was to isolation and determination of microorganisms (fungi, bacteria, yeast) from local produced cheese and which genus was dominance in it.

Materials and Methods

1 -Collection Sample : Total of 40 samples of locally made cheese (50 gm) was randomly collected from the market of Babylon province. Samples were collected in February to March, in dry, clean, sterile glass container preserved freeze in refrigerator until transport to Microbiology Laboratory in Biology department in college of Science for Women in Babylon University.

2-Cheese samples Preparation : from each sample (10 gm) were mixes with 90 ml sterile 0.2 % sodium citrate solution in mortar. from the main sample one ml homoginate then added to a test tube containing 9ml 0.1% sterile peptone water to bring dilution of 10-2.(11)

3 -Culture media :

1 .Mannitol salt agar (MSI) :

Get ready as indicated by guidelines on the Hi Media Laboratories By thawed (111gm) in (1L) and sanitizing via autoclave apparatus, it was utilized to analyze Staph. aureus microscopic organisms.

2 .Eosin methylene blue (EMB) :

Get ready as per guidelines on the Hi Media company Laboratories by melted (35.96) gm in (1L) and cleaning via autoclave, it was utilized to analyze Klebsiella, E. coli.

3 .Salmonella-Shigella agar(S-Sagar):

Plan as per directions on the Hi Media Laboratories by dissolving (63) gm in (1L) and sanitizing via autoclave, it was utilized to analyze Salmonella and Shigella microbes.

4 .Lactobacillus MRS agar (MRS) :

Get ready as indicated by directions on the Hi Media Laboratories by dissolves 67.15 gm in (1L) and cleaning via autoclave apparatus, it was utilized to analyze Lactobacillus.

6 .Sabouraud dextrose agar (SDA) :

Get ready as per directions on the company Hi Media Laboratories by dissolves (65) gm in (1L) and cleaning via autoclave, in the wake of adding Chloramphenicol, use to isolate fungi & yeasts from samples.

4 -Diagnosis of isolated microorganisms :

1 - Diagnosis isolated bacteria: The nutritional media utilized as a differentiation and demonstrative of microscopic organisms, MSA is utilized to isolate Staphylococcus aureus microbes, EMB to analyze Escherichia coli, klebsella and S-S agar to isolate Salmonella and Shigella microbes, MRS to analyze Lactobacillus.

2 - Diagnosis of fungi: Was analyzed relying upon an appearance of plainly visible characters (shading - surface - appearance - the measurement of the settlement) and microscopic characters (spores and mycillium).(12)

Results and Discussion

Manufactured dairy products from raw milk show a wide range of pathogenic microorganisms (bacteria, fungi) (13). First we discussed bacterial contamination isolate from 40 cheese samples, results revealed that Gibin Al- Arab locally manufactured by people at home and consumed by customers from markets was contaminated by Staph. aureus, staph. epidermids, E.coli, Klebsiella, lactobacillus, Salmonella, Shigella according to method depend on differentiation culture media specific for each genus.

Table -1- indicate the presence or absence of bacterial growth according to the culture media used.

Culture media	Positive growth	Negative growth
Mannitol salt agar (MSA)	27	13
Eosin methylene blue(EMB)	24	16
Lactobacillus MRS agar (MRS)	32	8
Salmonella –Shigella agar (S-S)agar	31	9

ISSN: 2312-8135 | Print ISSN: 1992-0652 | www.journalofbabylon.com | jub@itnet.uobabylon.edu.iq | info@journalofbabylon.com

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

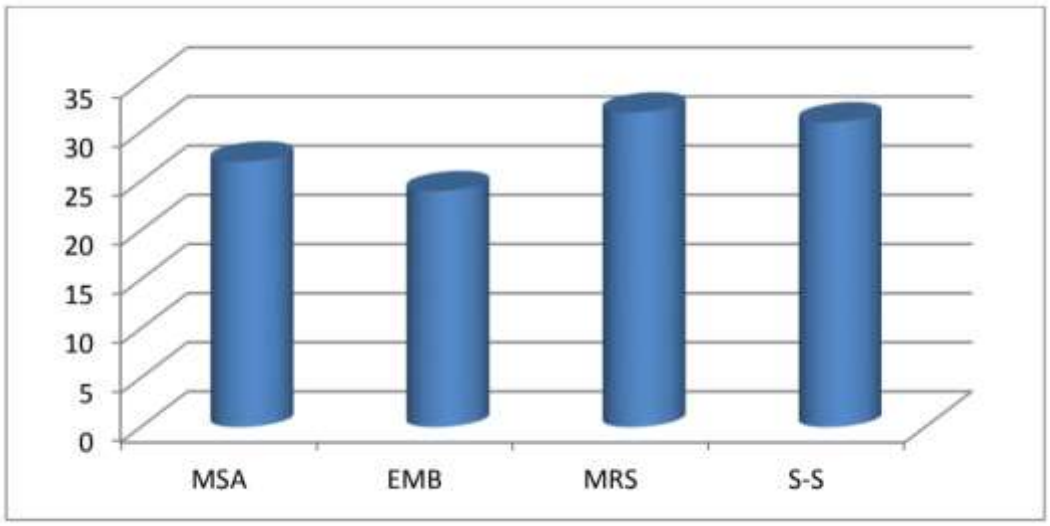

Figure -1- indicate the presence of bacterial growth according to the culture media used .

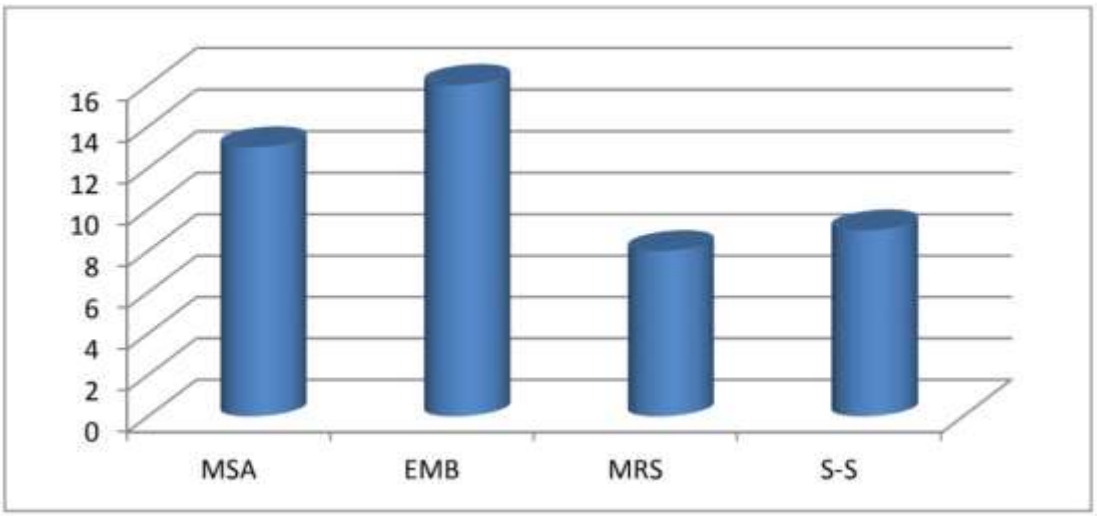

Figure -2- indicate the absence of bacterial growth according to the culture media used.

Table -1- revealed that there was not bacterial growth in some samples mean that may be it was not contaminated or people producing follow safety condition during production, heat treatment of cheese, short imported time good storage condition (14). So for decreasing bacterial growth in these products, which by direct solicitation with consumer hygiene it should, adapted strict hygiene conditions during manufacturing, handling, marketing (15). Cheese samples

collected from different sources of markets in Babylon province, table -2- revealed bacterial species detected from cheese samples by differential culture media.

Table -2- Bacterial growth in gibin al arab cheese samples according to differential media .

Culture media	Bacteria	+ve samples	%	-ve samples	%
MSA	*Staphylococcus aureus*	17	42.5	23	57.5
MSA	*Staphylococcus epidermidis*	10	25	30	75
EMB	*Escherichia coli*	12	30	28	70
EMB	*Klebsiella*	18	45	22	55
MRS	*Lactobacillus*	32	80	8	20
S-S Agar	*Salmonella*	10	25	30	75
S-S Agar	*Shigella*	29	72.5	11	27.5

MSA revealed that 17 samples (42.5 %) are contain Staph. aureus and 10 samples (25%) are contain Staph. epidermidis while (23,30) samples (57.5 %, 75 %)respectively didn't contain bacterial growth.

EMB media revealed that 12 samples (30 %)were gave positive results for E.coli and 28 samples (70 %) were negative, also gave 18 samples (45%) the results for klebsiella isolation and 22 samples(55%)were negative for it .

MRS media which was specific for Lactobacillus species revealed that (32) samples (80%) positive for it with growth gradients from little to many. While the other (8) samples (20%) were negative results.

Finally S-S agar showed (10)samples (25%) positive for Salmonella and (30)samples (75%) were negative and (29) samples (72.5%) positive for Shigella while (11)samples (27.5 %)were negative for it. according to table -2- presence of these bacteria and cause milk product contamination may be due to study period, the milk may be not pasteurized,the cured is handling by cheese maker when don`t follow hygiene rules, animal skin, container cleaners present of Coliform bacter as high rate in samples as known it is high rate in human and animal faeces that infected food.(16)

ISSN: 2312-8135 | Print ISSN: 1992-0652 | www.journalofbabylon.com | jub@itnet.uobabylon.edu.iq | info@journalofbabylon.com

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

Table-3- show the fungi species that isolate from cheese samples ,by using PDA , MEA media .

Fungi species	PDA		MEA	
	No. of isolates	%	No. of isolates	%
Penicillium digitatum	**62**	**74.7**	**52**	**80**
Penicillium italicum	**11**	**13.2**	**2**	**3.1**
Penicillium citrinum	**5**	**6.02**	**0**	**0**
Penicillium chrysogenum	**2**	**2.40**	**4**	**6.2**
Aspergillus nidulans	**3**	**3.7**	**5**	**7.69**
Alternaria	**0**	**0**	**2**	**3.1**
Candida spp.	**more**	**more**	**more**	**more**
Rhodotorella	**more**	**more**	**more**	**more**
Total	**83**		**65**	

Samples tested with (17) who detected that Penicillium is prevalent type contamination in Iraq reported by Erdogan et. Al. (2001) as the major contaminant of Turkish cheese also (19) revealed that Penicillium spp is responsible for cheese contaminant in Argentinean cheese previous studies have proved that Penicillium spp, make cheese surface soft (20) with an unwanted flavour because of fungi growth and mycotoxin production (12), consumed of contaminant cheese may associated with pulmonary infection, urinary tract infection.(11)

Finally presence of Candida spp. Is indicate that cheese quality was not good because yeast secrets toxic metabolites (14) with pathogenicity in gastrointestinal parcel is connected with grip factors that intercede yeast restricting to cell surface and creation lipase, phospholipase, proteinase which help yeast in invasion.(9)

Conclusion

From the obtained result we can conclude that all cheese samples were contaminated with different types of bacteria and fungi and yeast this show that there was contamination during production steps starting from : milk collection, preparation, production and marketing, therefore more attention should be paid to public health condition during production for the health of consumers.

ISSN: 2312-8135 | Print ISSN: 1992-0652 | www.journalofbabylon.com | jub@itnet.uobabylon.edu.iq | info@journalofbabylon.com

Conflict of interests.

There are non-conflicts of interest.

References

1 -Hameed,KG. Fungaldiversity in different types of cheese and the effect of natamycin on their survival during Feta cheese manufacture and storage. Journal of Advanced Veterinary and Animal Research. Vol 3No.3,pp:214-220 .(2016) .

2 -Fadihl,SJ.Determination of some virulence factors of Candida spp. Isolatedfrom locally produced cheese in Diyala Governorate- Iraq. Al-Mustansiriyah Journal of Science. Vol. 27, No.4.pp:1-6 .(2016) .

3 -Torkar GK, Teger GS. The presence of some pathogenic microorganisms, yeasts and moulds in cheese samples produced at small dairy processing plants. Acta Agriculture Slovenia Journal, 88: 37-51 . .(2006)

4 -Taniwaki, M.H. and Van DenderP, A.G.F. Occurrence of toxigenic moulds in Brazilian cheese. J. Food Protection 3:187-191 .(1992) .

5 -Yousef A. Effect of technological factors on the manufacture of karish cheese. Egyptian J. Dairy Sci., 31(1): 161-165 .(2007) .

6 -Richter, R. C, Ledford, Ail and Murphy, S.C. Compendium of methods for the microbiological examination of foods. America Public Health Association, washinton.D. C,chapter 45.pp.837-856 . .(2005)

7 -Tatini,S.R;Kauppi,K.L.Encyclopedia of diary sciences.Roginski,H; Fuquay,J.W.(eds).Vol.(Academic pressand Elsevier science, Amsterdam,Boston,London.pp.74- 79. (2003 .(

8 -Kure, C.F., Skaar, I and Brendehaug, JMould contamination in production of semi-hard cheese. Int. J. Food Microbiology. 2004.

9 -ELbagory,AM ;Amal,M E ; Hammad,AM ;Salwa,AD. Prevalence of fungi in locally produced cheese and molecular characterization of isolated toxigenic molds. BENHA VETERINARY MEDICAL JOURNAL.Vol. 27,No.2. pp:9-20 .(2014).

10 -Fleet GH. Yeasts in dairy products: a review. J. Appl. Bacteriol., 68(1): 199–211 .(1990) .

11 -APHA (American Public Health association). Compendium of methods for the microbiological examination of foods. Third Ed. (Vanderzant, C and SPlittoesser, D. Eds) Washington DC, USA, 675-8000.(2003) .

12 -Barnett, J.L. and Hunter, B.B. Illustrated Genera of Imperfect Fungi, Burgess Publishing company, Minneapolis, Minn, USA .(1972) .

13 -Melville PA, Benites NR, Ruz-peres M, Yokoya E. Proteinase and phospholipase activities and development at different temperatures of yeasts isolated from bovine milk. Journal of Dairy Research, 78: 385-390.(2011) .

-14 Kapoor,R.and Metzger,L.E.Processed cheese :scientific and technological aspects A-review.Comp.Rev.Food.Sci. and Safety. 7:194-214 .(2008) .

15 -Mohammed, SJ. Detection of microbial contamination in some types of processed cheeses available in local market. J Fac Med Baghdad. Vol. 58,No. 1 .(2016) .

16 -Alper,S and Nesrin, C.Bacterial contamination in fresh white cheeses sold in bazaars Canakkale, Turkey. International Food Research Journal. Vol. 20(3). Pp:1469-1472.(2013) .

17 -Alborzi, S. Fungal contamination in a cheese factory in Iran. 14th European congress of clinical microbiology and infectious disease. Prage,Czech Republic. May 1-4, 2004 (Abstract) .(2004) .

18 -Erdogan, A., Gurses, M., Turkogllu, H.and Sert, S. The determination of mould Flora of some Turkish cheese types (Kasar, Civil, Lor, and Tulum). Pak.J.Biol.Sci. 4:884-885.(2001) .

19 -Basilico, J.C.; de Basilico, M.Z;.Chiericatti C and J.F.P Vinderole, C.E. Characterization and control of threat mould in cheese. Letters in Applied Microbiology 32:419-423.(2001) .

20 -Minervini, F.; Montagna, M.T.; Spilotron, G.; Monaci, L.; Santacroce, M.P. and Visconti, A. Survey on mycoflora of cow and buffalo dairy products from southern Italy.Int.J. Food Microbiology. 19(69):141- 146 .(2001) .

21 -Lavoie K, Touchette M, St-gelais D, Labrie S. Characterization of the fungal microflora in raw milk and specialty cheeses of the province of Quebec. Dairy Science & Technology, 92: 455-468 .(2012) .

الخلاصة

تم جمع أربعون عينة من جبن العرب (الجبن المصنع محليا") من السوق المحلي في محافظة بابل لدراسة التلوث الجرثومي (النمو البكتيري والفطري). كشفت الدراسة اولا" عن وجود عينات سالبة لكل وسط زرعي استخدم للكشف عن البكتيريا، حيث وجد في وسط MSA كان هناك 27 عينة موجبة و 13 عينة سالبة, على وسط EMB ظهرت 24 عينة موجبة و 16 عينة سالبة، وعلى الوسط MRS ظهرت 32 عينة موجبة و 8 عينات سالبة أخيرا" وسط S–S أكار ظهرت 31 عينة موجبة و 9 عينات سالبة. تم عزل 17 سلالة من Staphylococcus aureus على وسط MSA, و 10 سلالات من Staphylococcus epidermidis. وعلى الوسط EMB تم عزل 12 سلالة من Escherichia coli و 18 سلالة من Klebsiella. وسط MRS سجل 32 سلالة من Lactobacillus واخيرا وسط S–S agar سجل 10 سلالات من Salmonella و 29 سلالة من Shigella . اما الفطريات التي تم عزلها على وسط PDA فقد كانت : Penicillium digitatum (74.7 %), Penicillium italicum (13.2 %), Penicillium citrinum (6.02 %), Penisillium chrysogenum (2.40 %), Aspergellus nedulans (3.7 %), Alternaria (0 %) وعدد كبير من مستعمرات ال Candida و Rhodotorella . وعلى وسط MEA تم عزل الفطريات الاتية وكالاتي : Penicillium digitatum(80%), Penicillium italecum (3.1 %), Penicillium citrinm (0%), Penicillium chrysogenum (6.2%), Aspergillus nidulans (7.69%), Alternaria (3.1) وكذلك عدد كبير من مستعمرات الCandida و Rhodotorella كما في وسط PDA .

ISSN: 2312-8135 | Print ISSN: 1992-0652 | www.journalofbabylon.com | jub@itnet.uobabylon.edu.iq | info@journalofbabylon.com