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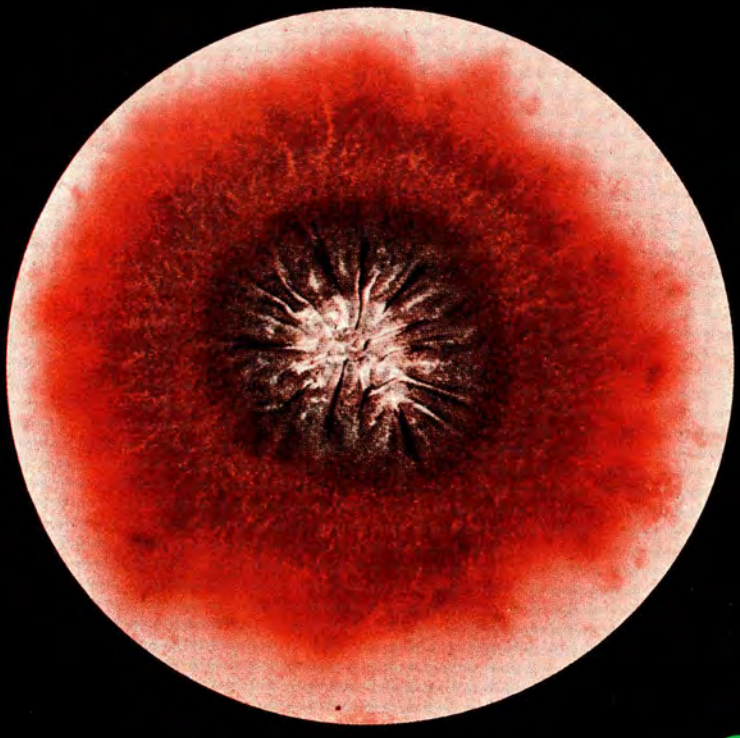

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The Yeast flora of Egyptian Butter

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Butter is an important food article and over a thousand ton is nearly consumed in this country every year (Ministry of Commerce and Industry Statistics, 1961).

There are two kinds of butter available on the market, one for the table and the other for cooking. Table butter is usually manufactured in large cities and is actually under good hygienic control, while cooking butter is the most commonly used in the country.

The method of making cooking butter in the villages consists of obtaining the cream from the milk either by skimming (shallow-pan system) or by the separator and then churned in a goat's skin bag. The product is usually shaped into medium sized loaves. Cooking butter may be partaken as such, but mostly transformed into "Ghee" (Massli).

Rahn (1931) stated that yeasts can tolerate a rather high acid medium. Hietaranta (1951) confirmed this statement and found that yeasts were not inhibited at pH 4.5; he also found that yeasts isolated from butter would tolerate 12 % salt. Moir and Russel (1937) found in export butter that the yeast count was less than 1000 per gram. Krishnaswamy (1949) isolated thirty strains of yeasts from desi- and cream butter which were asporogenous, probably belonging to Torulaceae. Ghoniem (1963) isolated from Egyptian butter yeasts belonging to Trichosporon, Candida, Endomycopsis and Saccharomyces species.

In view of the fact that the occurrence of yeast in butter may cause its spoilage and transmit certain disease to the consumer, the writers made an attempt to study the yeast flora in Egyptian butter.

Material and Methods

200 samples of cooking butter were obtained from the market at random and placed in sterile jars which were examined within one hour after collection.

One gram of the butter sample was weighed in a sterile dilution tube and melted by placing in a water-bath at 35—40° C; 9 ml warm sterile distilled water were added to make 1 : 10 dilution. Further dilutions were similarly made up to 1 : 10 000.

Isolation of yeasts

Sabouraud's medium containing 20 units of penicillin and 40 µg of streptomycin per ml was used as a selective medium for plating the serial dilutions. Two plates were used for each sample, one of which was incubated at 37° C and the other at room temperature for about 4 to 6 days.

From colonies that looked like yeast, wet preparations were made and examined microscopically for confirmation. These were subcultured on agar slopes for further identifications.

Identification of yeasts

Identification of yeasts isolated from butter was done after Lodder and Kreger-van Rij (1952), which depends on the morphological and physiological characters of yeasts. Yeast cultures may contain more than one species of bacteria or molds; cultures to be identified must be in pure form. By means of dilution method it was possible to separate different types of yeast. Bacterial

contamination could be got rid of by passing the yeast culture in Raulin's solution for 2—3 weeks. This solution killed all bacteria but not yeast. Cultures that were contaminated with molds were difficult to be separated in a pure form.

The first step to identify yeast was to examine it for building of ascospores, which is an important criteria to differentiate the family Endomycetaceae (the perfect yeasts, produce ascospores) from the family Cryptococcaceae (the imperfect yeasts, no production of ascospores). Yeasts were cultivated on Gorodkowa agar, potato slices or carrot slices which are suitable media for ascospores building.

Rice extract agar, was used for studying the morphological characters. Yeasts were examined for size, form and shape of blastospores, chlamydospores, pseudomycelium, true mycelium and arthrospores. The agar was poured in a very thin layer in plates; the yeast was inoculated in fine lines and covered with sterile cover glass, then incubated at 30° C for 2 days.

Studying the physiological characters included fermentation and assimilation of sugars. Yeasts were examined for their ability to ferment 2 % solution of glucose, galactose, sucrose, maltose, lactose and raffinose in 2 % peptone solution; the same sugars were also used for assimilation tests. Yeast nitrogen base, Difco, was added to the different sugars.

Results

Most of the isolated strains were Trichosporon species (56.9 %), while Saccharomyces species was less met with (1.1 %) as shown in **table 1.**

Table 1: Occurrence of different types of yeasts in ecoking butter

Types of yeasts		Frequency of isolation	
		at room temperature	at 37° C
Trichosporon	species	402	395
Candida	„	272	163
Endomycopsis	„	24	22
Saccharomyces	„	8	5

Table 2: Frequency Distribution of Trichosporon Species in Butter Samples

Trichosporon species	Frequency			
	Number of isolates	%	Number of Samples	%
Trich. behrendii	173	43.03	173	93.01
Trich. fermentans	93	23.13	93	50.00
Trich. capitatum	71	17.66	71	38.17
Trich. margaritiferum	55	13.43	55	28.49
Trich. cutaneum	6	1.49	6	3.22
Trich. sericeum	4	0.99	4	2.04
Total	402	100	186	100

Table 3: Frequency Distribution of Candida Species in Butter Samples

Candida species	Frequency			
	Number of isolates	%	Number of samples	%
Cand. parapsilosis	42	15.44	42	22.46
Cand. pulcherrima	25	9.19	25	13.37
Cand. pelliculosa	25	9.19	25	13.37
Cand. scottii	24	8.82	24	12.84
Cand. guilliermondii	18	6.62	18	9.63
Cand. mycoderma	16	5.88	16	8.56
Cand. claussenii	16	5.88	16	8.56
Cand. rugosa	15	5.51	15	8.02
Cand. catenulata	11	4.04	11	5.88
Cand. tenuis	10	3.68	10	5.35
Cand. intermedia	10	3.68	10	5.35
Cand. robusta	9	3.31	9	4.81
Cand. pseudotropicalis	7	2.57	7	3.74
Cand. albicans	7	2.57	7	3.74
Cand. lipolytica	6	2.21	6	3.21
Cand. curvata	6	2.21	6	3.21
Cand. macedoniensis	6	2.21	6	3.21
Cand. reukaufii	5	1.84	5	2.67
Cand. tropicalis	5	1.84	5	2.67
Cand. melinii	3	1.10	3	1.60
Cand. mesenterica	3	1.10	3	1.60
Cand. stellaloidea	2	0.74	2	1.07
Cand. humicola	1	0.37	1	0.53
Total	272	100	187	100

Discussion

It is remarkable that yeasts were isolated from all the butter samples examined. Yeast may gain access to butter from animals suffering from mycotic mastitis or from outside. The high incidence of yeasts noted night be attributed to the extensive use of antibiotics practised nowadays.

During the last 15 years several types of yeasts have been incriminated as causes of mastitis in cattle. This increase in mycotic infections seems to coincide with the introduction of antibiotics in the treatmen of udder affections. In man, before the introduction of antibiotics in treatment, yeast infections were rare and localised. Parallel to antibiotics treatment there has been an increase not only in numbers of reported cases but also in the severity of infection. In bovine mastitis, this observation was also noticed but the reported cases are rather few.

The importance of antibiotics as predisposing factor for yeast infection of the udder was proved experimentally by REDAELLI (1957), KREJAKOVIC and STOJANOVIC (1960) and HULSE (1952). Intramammary infection of antibiotics together with yeast cultures produced more severe symptoms than injection of yeast cultures alone. Experimental infection of the udder done by MEHNERT (1962) with certain species of Candida, showed that the yeast could grow in the udder and cause mild symptoms which rapidly disappeared. However, in the presence of antibiotics, invasion of the mammary gland occurred and a severe form of mastitis was observed.

Therefore, it appears that the wide use of antibiotics is responsible for the marked incidence of yeasts in milk and dairy products.

As shown in **Table 1** most of the isolated strains were Trichosporon species (56.9 %), which were isolated from 93 % of the samples. Trich. behrendii was the most frequently met with (93.01 %), while Trich. sericeum was less encountered with (2.04 %), **Table 2.**

Candida species were isolated from 187 samples; i. e. (93.5 %); Cand. parapsilosis was isolated from 22.46 %, while Cand. humicola from 0.53 % **(Table 3).**

Regarding the Endomycopsis species, were isolated from 23 butter samples, End. selenospora from 15 samples (65.22 %), while End. javenensis was isolated once (4.35 %), **Table 4.**

Table 4: Frequency Distribution of Endomycopsis Species in Butter Samples

Endomycopsis species	Frequency			
	Number of isolates	%	Number of samples	%
End. selenospora	15	62.50	15	65.22
End. fibuliger var. monospora	6	25.00	6	26.09
End. bispora	2	8.33	2	8.70
End. javanensis	1	4.17	1	4.35
Total	24	100	23	100

Table 5: Frequency Distribution of Saccharomyces species in Butter Samples

Saccharomyces species	Frequency			
	Number of isolates	%	Number of samples	%
Sacch. acidifaciens	4	50.00	4	50.00
Sacch. fermentati	3	37.50	3	37.50
Sacch. cerevisiae	1	12.50	1	12.50
Total	8	100	8	100

Table 5 shows that 8 samples were found to contain Saccharomyces species; Sacch. acidifaciens was isolated from 4 samples, while Sacch. cerevisiae was isolated from 1 sample only.

It may be noted, however, that the yeasts isolated by KRISHNASWAMY (1949) were asporogenous, probably belonging to Torulaceae. These results agree with those found by GHONIEM 1963).

Attention was called by the experiments of MEHNERT (1962) which showed that pathogenic yeasts grew only at a temperature of 37° C, while the saprophytic types failed to grow at that temperature. Accordingly, care was taken to inoculate duplicate plates for incubation at 37° C as well as at room temperature. It was interesting to note that the great majority of the yeasts isolated grew at room temperature which indicated that most of these types came in the butter accidently from outside.

Nevertheless a good number of certain facultative pathogenic yeasts have been encountered with in the samples examined. Candida albicans, for example, is known to affect the nails and hence the dairy workers would be exposed to infection. If contaminated butter is partaken it may cause thrush in the mouth and gastro-intestinal disturbance. In addition, generalisation and systemic infection particularly in children may occur. In females it often causes vulvo-vaginitis.

Unclean workers engaged in dairy products may transmit mycotic affection to the teats when milking their animals, thus setting up mycotic mastitis which is not easy to control.

Candida parapsilosis, tropicalis and pseudotropicalis may also cause such disturbance in man and animals.

The saprophytic yeasts which comprised the majority of the isolated strains, undoutedly, shortens the keeping quality of butter. Spoilage takes place by the breaking down of its components liberating thus different acids and gases which change the flavour and cause its rancidity.

Summary

All the 200 butter samples examined were found to contain yeasts. The species isolated were Trichosporon, Candida, Endomycopsis and Saccharomyces. Trichosporon species constituted most of the isolates (56.9 %), while Saccharomyces species was less met with (1.1 %).

Spoilage of butter by yeasts is caused by the breakdown of carbohydrates and fats liberating gases and acids which change the flavour and cause its rancidity.

The importance of antibiotics as predisposing factor for yeast infection of the udder was discussed.

Bibliography

GHONIEM, N. A. M. (1963): Thesis presented to the Faculty of Vet. Med., Cairo University.

HIETARANTA (1951): J. Dairy Res. **18**, 214.

HULSE, E. C. (1952): Vet. Rec., **64**, 210—211.

KREJAKOVIĆ, V. and STOJANOVIC (1960): Vet. Clasn. **14**, 25—28.

KRISHNASWAMY, M. A. (1949): Indian J. of Dairy Sci. **2**, (I) 1.

LODDER, J. and N. J. W. KREGER-VAN RIJ (1952): The Yeasts. A taxonomic study. North-Holland Publ. Co., Amsterdam.

MEHNERT, B. (1962): II. Mycological Congress, Hamburg.

MOIR and RUSSEL (1937): J. Dairy Res., **8** (3).

RAHN, O. (1931): Handbuch der Milchwirtschaft, 2 : 104—107 (cited in Bact. Rev., 5 : 337—374).

REDAELLI, G. (1957): Arch. Vet. Ital., **8**, 65, 97—120.

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