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تقييم اختبار اختزال النترات لتحديد درجة اللين

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أجريت التجارب على ٢٠٠ عينة لبن طازج ، جمعت من مزارع الالبان ببغداد وتم فحصها باختبارات الاختزال ( اختبار النترات ، الميثيلين الأزرق والريزازيسورين ) وكذلك اختبارات تقدير درجة حفظ النومية ( اختبار التخثر بالفلى وترسيب الكمبول )

دلت النتائج على وجود علاقة ارتباط عالية بين اختبارات الاختزال المختلفة كما أوضحت النتائج أن اختبار اختزال النترات والميثيلين الأزرق والريزازيسورين ( ٣ ساعات ) ذات كفاءة عالية فى تحديد عينات الالبان المرفوضة وذلك بالمقارنة باختبارات تقدير درجة حفظ النومية .

أثبتت النتائج أهمية تطبيق اختبار اختزال النترات لتقييم درجة الالبان لاغراض التصنيع .

## EVALUATION OF NITRATE REDUCTION TEST FOR GRADING MILK (WITH 4 TABLES)

BY

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### SUMMARY

Two hundred milk samples, collected from different farms in Baghdad Province, were examined by the dye reduction tests (NBT, MBRT and RRT), and the residual keeping quality tests (COB and APT). A close relationship was found between the dye reduction tests. The NRT, MBRT and RRT (3 hrs) succeeded to isolate nearly similar number of rejected samples as compared by residual keeping quality tests.

NRT proved its efficiency for grading milk intended for manufacturing purposes.

### INTRODUCTION

Reduction tests are indirect indicators of microbial populations in the milk and depends upon the relative metabolic rates of the various existing microorganisms. Contradiction in opinions has been raised about the importance of these tests for grading milk (JOHNS, 1953; SMILLE ET AL., 1958; LAGRANGE and NELSON, 1965 and ORR ET AL., 1965). Therefore, this work was planned to evaluate the nitrate reduction test in grading milk as compared with other reduction tests and the residual keeping quality tests.

### MATERIAL AND METHODS

Two hundred milk samples of morning milking, were collected from different farms in Baghdad Province, and transferred to the laboratory where they were subjected to the following tests:

- 1- Nitrate reduction test (NRT) was carried out according to CRAWLEY and TOMMEY (1969), and the nitrite detected after 4 hrs by adding 0.25 ml of Gries-Iloosky reagent (0.5 g sulphanilamide + 0.1 g  $\alpha$ -naphthalamine + 10 ml glacial acetic acid + 10ml distilled water) to the test samples. Results were interpreted as follows : No colour-good; pink-fair, and dark pink to red-poor.
- 2- Methylene blue reduction test (MBRT) and Resazurin reduction test (RRT) were performed according to the standard methods for the examination of dairy products (APHA, 1972). The milk was judged according to the reduction time.
- 3- Residual keeping quality tests:

The tests for residual keeping quality standard were performed after CHALMERS (1955).

- a- Compensated clot-on-Boiling test (COB): On arrival at the laboratory, the milk samples were placed in the atmospheric shade box and held until 4 p.m. 10 ml of the mixed sample in a test tube was held in a thermostatically controlled water bath at 22°C for a definite time calculated according to the following formula:

$$r = x - Y \frac{(t+3)^2}{(h+3)}$$

Where r is the incubation time (in hrs) at constant temperature (22°C) before applying the test, x is the residual keeping quality standard from the time the milk leaves the farm (24 hrs), Y is the period (in hrs) between the collection of the milk at the farm and the time the test is begun, t is the mean atmospheric shade temperature (°C), and h is the constant incubation temperature (25°C). The clot-on-boiling test was conducted and results were recorded.

- b- Alcohol precipitation test (APT): At the end of incubation time of samples for compensated COB test previously described, one ml of milk was mixed with equal volume of 68% (V/V) neutral ethyl alcohol containing 0.005% bromo cresol purple and thoroughly shaken. Slight clot or ppt and a yellowish green colour was recorded as positive or failed.

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Statistical analysis of results was carried according to SNEDOCOR and COCHRAN (1969).

## RESULTS

Results are represented in Table 1-4.

## DISCUSSION

It seems obvious from the findings reported in Table (1) that there is a close relationship between the reduction tests. NRT was highly correlated with RRT (one hr.) than with MBRT and RRT (2 and 3 hrs), while MBRT was highly correlated with RRT. Statistically good correlations between NRT, RRT and MBRT were reported by BORGER (1962); DARRAH ET AL. (1967) and DESAI et al. (1973). SOBECK-SKAL and BRANDL (1965) found good agreement between the results of NRT and RRT but both tests were influenced by milk cooling, especially in the case of low-count milk.

Comparing the results for grading milk according to dye reduction tests by using chi-square test, a significant difference between the tests existed (Table 2). RRT (one hr) could isolate the highest number of acceptable samples than the other tests, while it failed to detect poor grades. On the other hand NRT, MBRT and RRT (3 hrs) succeeded to isolate nearly similar numbers of rejected samples.

Comparing the results of dye reduction tests with those obtained by residual keeping quality tests (Table 3), it is evident that there was a good agreement between residual keeping quality test and NRT, MBRT and RRT (3 hrs) for detection of poor quality milk samples. Out of 151 milk samples satisfactory graded by RRT (one hr), 34 samples failed APT and 3 samples clotted on boiling after 24 hrs residual keeping quality standard. While all acceptable milk samples by NRT, MBRT, RRT (2 and 3 hrs), satisfied the residual keeping quality standard. It was also observed that nearly similar number of milk samples were graded fair and poor on using NRT, MBRT and RRT (2 hrs).

The results reported in Table (4) point out that there is a significant difference between COB and APT tests and that unsatisfactory samples showed the highest difference. Compensated clot-on boiling test is considered ideal to indicate the sanitary condition of milk production.

Some investigators concluded that the resazurin test was of no value in measuring bulk-milk quality (ATHERTON, 1958 and ORR ET AL., 1965). The RRT (3 hrs), because of its longer incubation period, has advantages over RRT (one hr.). The triple reading resazurin test is comparable to the MBRT in its agreement with the standard plate count, and is considered an advisory test for determination of bulk milk quality (THOMAS & MACKINSON, 1964). The MBRT is used to grade raw milk specially for manufacturing purposes (KOWALEVESKA ET AL., 1973).

In recent years the interest in the NRT as a means of assessing milk quality has increased. As NRT is not affected by lactic-acid bacteria, therefore it outstand MBRT for detection of poor quality milk (KANDLER, 1961; BOCKLEMAN, 1962 and TWOMEY ET AL., 1969).

It could be concluded that, NRT and MBRT are highly correlated with residual keeping quality standards for detection of acceptable milk samples for manufacturing purposes. While RRT (one hr.) can be used as a screening test and should be supplemented by other quality control tests.

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TABLE (1)

Linear correlation coefficient between  
dye reduction tests<sup>≡</sup>

Test	MBRT	RRT 1 hr	RRT 2 hr	RRT 3 hr
MRT	0.615	0.688	0.596	0.546
MBRT		0.842	0.843	0.894
RRT 1 hr			0.947	0.915
RRT 2 hr				0.693

<sup>≡</sup> Significant correlation at 0.01 level ( $r > 0.181$ )

TABLE ( 2 )

Comparison between the dye reduction tests  
using Chi-square test.

Test		Grade			Total	Chi <sup>2</sup>
		Good	Fair	Poor		
NRT	Observed	120	33	47	200	32.13
	Expected	112.2	62.2	25.6		
MBRT	Observed	113	47	40	200	11.82
	Expected	112.2	62.2	25.6		
RRT 1 hr	Observed	151	49	0	200	41.82
	Expected	112.2	62.2	25.6		
RRT 2 hr	Observed	125	71	4	200	20.93
	Expected	112.2	62.2	25.6		
RRT 3 hr	Observed	52	111	37	200	75.66
	Expected	112.2	62.2	25.6		
Total		561	311	128	1000	
Chi <sup>2</sup>		47.72	59.75	74.89		182.36 <sup>*</sup>

<sup>\*</sup>Significant difference at 0.01 level.

## NITRATE REDUCTION TEST FOR GRADING MILK

TABLE (3)  
Comparison between the results of dye reduction tests and residual keeping  
quality tests.

Test	Degree	COB		APT	
		-ve	+ve	-ve	+ve
NRT	Good	120	0	98	22
	Fair	29	4	20	13
	Poor	2	45	1	46
MBRT	Good	113	0	94	19
	Fair	38	9	25	22
	Poor	0	40	0	40
RRT 1 hr	Good	148	3	117	34
	Fair	3	46	2	47
	Poor	0	0	0	0
RRT 2 hr	Good	125	0	104	21
	Fair	26	45	15	56
	Poor	0	4	0	4
RRT 3 hr	Good	52	0	45	7
	Fair	99	12	74	37
	Poor	0	37	0	37

TABLE (4)  
Comparison between the residual keeping quality tests using Chi-square test.

Test		Results		Total	Chi <sup>2</sup>
		-ve	+ve		
COB	Observed	151	49	200	5.83
	Expected	135	65		
APT	Observed	119	81	200	5.83
	Expected	135	65		
Total		270	130	400	
Chi <sup>2</sup>		3.78	7.68		11.66*

\* Significant difference at 0.01 level.