C-12

11th EUROPEAN MEETING OF MEAT RESEARCH WORKERS.

Belgrade. 16th - 21st August, 1965.

INFLUENCE OF MUSCLE EXTRACTS ON THE EVALUATION OF MICROBIOLOGICAL QUALITY.

A. B. Talbot

Denny Group Central Laboratory, Portadown, N. Ireland

SUMMARY

The beneficial effect of using an unheated muscle extract preparation as a diluent has been studied. Originally found to prevent bactericidal action during serial dilution in the enumeration of viable bacteria in bacon curing brines, the muscle extract preparation has been shown to give substantially higher counts in milk, but only marginal advantages in raw minced beef, bacon and fresh English-style sausage. Gram negative bacteria were found to be the principal group contributing to the increased counts.

The protectant effect of the muscle preparation was heat sensitive, and was not replaceable by peptone, lyophilized pork liver, meat extract, or bovine albumin. The addition of catalase to quarter-strength Ringers diluent was without effect.

Possible further extensions of this work are discussed.

July, 1965.

L'influence des extraits de muscle sur l'évaluation de la qualité microbielle.

RÉSUMÉ.

L'effet avantageux de faire usage d'une préparation, pas chauffée, d'extrait de muscle comme délayant fut etudié. Cette préparation, qu'on a trouvé originellement propre à prévenir l'action bactéricide pendant des séries de délayements en énumerant les bactéries vivantes dans les saumures de jambon, donne des numérations beaucoup plus grandes dans le lait, mais ses avantages ne sont que marginals dans le boeuf cru haché, le jambon et les saucissons anglais.

La groupe principal contribuant aux énumerations augmentées fut constitué de microbes gramnegatives.

L'effet protectif de la préparation de muscle fut résistant à la chaleur, et la préparation ne pût etre remplacée par la peptone, les reins de porc lyophilisés, l'extrait de viande ou l'albumine de boeuf. L'addition de catalase au délayant de Ringer, $\frac{1}{4}$ concentration, fut sans effet.

Les extensions possibles en outre de ce travail sont discutées.

Der Einfluss von Muskelextrakten auf die Bewertung der mikrobiologischen Qualität.

ZUSAMMENFASSONG.

Der güstige Einfluss eines ungehitzten als Verdünnungsmittel gebrauchten Muskelextraktpräparats wurde studiert. Dieses Präparat, welches sich ursprünglich als fähig zeigte, eine bakterizide Wirkung während Serienverdünnung bei der Lebendkeimzahlung in Schinkenpökellaken zu verhindern, liefert bedeutend höhere Zahlen in Milch, bietet aber nur Randvorteile bei rohem, zerkleinertem Rindfleisch, Schinken und frischer Wurst nach englischer Art. Die wichtigste zu den zugenommenen Zahlen beitragende Gruppe bestand aus gramnegativen Bakterien.

Die schützende Wirkung des Muskelpräparats war hitzeempfindlich und das Präparat war nicht durch Pepton, lyophilisierte Schweinelende, Fleischextrakt oder Rindereiweiss zu ersetzen. Ein Katalasezusatz zu ¼ Stärke Ringersverdünnungsmittel hatte hier keine Wirkung.

Die Möglichkeiten weiterer Ausdehnungen dieser Arbeit werden erörtert.

INFLUENCE OF MUSCLE EXTRACTS ON THE EVALUATION OF MICROBIOLOGICAL QUALITY.

A. B. Talbot

INTRODUCTION

Since the demonstration by Straka & Stokes (1957) of the value of peptone water as a diluent in the enumeration of bacteria in poultry pies many workers have adopted the use of a 0.1% peptone solution as a basis for a diluent in enumeration techniques in food microbiology. The contributors to a discussion on the effect of the diluent on the recovery of bacteria, as reported by Jane-Williams (1963), drew evidence from a wide range of circumstances, and were in general agreement on the suitability of adding 0.1% peptone to the diluent except in certain specific cases, (1) when counts are done on low dilutions of proteinaceous foods (2) when counts are made of osmophilic yeasts (3) when counts are made of oxygen sensitive bacteria in which circumstances the addition of a 0.05% (w/v) cysteine HCl to the diluent was recommended.

However, Patterson & Cassells (1963) examined the value of adding peptone to diluents used in the bacteriological testing of bacon curing brines, and found that although the addition of 0.1% peptone aided survival it did not completely remove a bactericidal effect which they were able to demonstrate in quarter-strength Ringers, 4% and 10% NaCl solutions.

In this laboratory the use of a muscle extract solution as the basis for the preparation of diluents has been used for the enumeration of bacteria in bacon curing brines, and has been found not only to prevent any bactericidal effects in the dilution stage but also to lead to higher recoveries than the equivalent salt concentration in distilled water, or this with the addition of 0.1% (w/v) peptone.

The possible extension of the use of a muscle extract solution as a diluent in other fields of food bacteriology has also been studied and is

the subject of this interim report which is with special reference to milk.

THE EFFECT OF A MUSCLE EXTRACT AS A DILUENT IN THE BACTERIOLOGICAL TESTING OF MILK.

(i) Materials and Methods.

Samples of milk were taken as distributed to the consumer from a number of different retail sources in the area of the laboratory. Most of the work was carried out on pasteurised milk but some samples of unpasteurised milk were also examined.

The reference method for the examination of the samples for total plate count was that of the Ministry of Health (1937) in which samples are serially diluted in quarter-strength Ringers solution, and subsequently 1 ml. portions are inoculated into Yeastrel milk agar using a standard pour plate technique. The plates are incubated at 37°C. for 48 hours.

The basic test diluent was prepared as follows: psoas major muscles, taken from cooled (5°C.) pig carcases 24 hours post mortem, were freed from fat and ligamentous tissue. 350 gm. minced psoas muscle was mixed with 200 ml. tap water (at 50°C.) and kept on a water-bath at 50°C. for 1 hour, and stirred frequently. The mixture was stored overnight at 5°C. and the liquor separated by pressing through fine muslin. The pH was adjusted and the final volume made up to 300 ml. with water. This was filtered through a No. 54 Whatman paper to clarify and then sterilised by passing it through a sterile Seitz filter fitted with a sterilising pad (type SB/A - Ford's Sterimat) at a pressure not exceeding 5 lbs. p.s.i. The pH adjustment was designed to give a final pH 7.5 . 9 ml. quantities of the diluent were transferred aseptically into glass-stoppered Pyrex reagent bottles (25 ml. capacity) and held in an ice-bath to reduce the temperature below 5°C. The method of preparation of this muscle extract is based on the method used by Jespersen and Riemann (1958) for the preparation of pork-juice agar.

A streak plate technique was also used as an alternative to the pour plate method. In this, standard Yestrel milk agar (YMA) plates were poured and dried. Approximately 0.1 ml. portions of the appropriate serial dilution were delivered onto the surface of prepared plates by means of a calibrated glass, platinum-tipped, dropping pipette and spread carefully over the surface of the medium with the aid of a platinum wire bent at a suitable angle to provide a flat streaking portion approximately one inch long. The inoculated plates were sealed by wrapping with Parafilm (Messrs. A.Gallenkamp & Co. Ltd., London.).

In all cases, plates were inoculated within an interval of not greater than 3 minutes after the serial dilution had been prepared, and all counts reported are the average of triplicate plates.

(ii) Results and Discussion.

The study of the comparative performance of quarter-strength Ringers and muscle extract as diluents was extended to include the effect of altering the aerobic character of the environment during colony growth by comparing pour plates and surface streak plates. The results are summarised in Tables 1 - 2 and show the counts obtained by the various test methods expressed as a percentage of the count obtained by the standard method.

A wide variation in behavior is noted between different samples of milk, possibly associated with the presence or absence of bacteria of more strongly aerobic character. Very generally, however, two main effects can be noted, (1) at an incubation temperature of 37°C. the muscle extract as a diluent raised the counts by pour plate to approximately the same level as streak plates inoculated from quarter-strength Ringers diluent, (2) the use of muscle extract as a diluent in the streak plate technique resulted in even higher plate counts. At 25°C. the first effect was very much less noticeable and the second effect completely absent.

TABLE 1.

THE EFFECT OF MUSCLE EXTRACT AS A DILUENT IN THE ENUMERATION OF BACTERIA IN MILK USING POUR PLATE AND STREAK PLATE TECHNIQUES, WITH INCUBATION AT 37°C. FOR 48 HOURS.

Diluent	Quarter-strength Ringers (Oxoid)		Muscle Extract	
Plating Technique	Pour	Streak	Pour	Streak
Sample No.		as a % of (1)	as a % of (1)	as a % of (1)
1.		102.3	394.3	216.6
2.		162.4	131.7	219.1
3.		98.8	146.0	187.7
4.		135.0	225.8	177.2
5.		198.6	260.3	434.9
6.	Counts	261.8	324.9	356.5
7.	taken	166.0	247.3	282.7
8.	as	133.3	109.9	121.0
9.	100%	371.4	126.5	428.6
10.		12.8	166.7	41.0
11.		85.8	108.0	167.2
12.		121.6	117.2	153.1
13.		444.4	216.7	972.2
14.		230.5	223.7	250.7
15.		187.5	184.4	328.1
16.		168.8	155.8	649.3
Mean (arithmetic)	100	180.1	196.2	311.6
Mean (log)	100	145.2	182.1	247.3

TABLE 2.

THE EFFECT OF MUSCLE EXTRACT AS A DILUENT ON THE ENUMERATION OF BACTERIA IN MILK USING POUR PLATE AND STREAK PLATE TECHNIQUES, WITH INCUBATION AT 25°C. for 72 HOURS.

Diluent	Quarter-strength Ringers (Oxoid)		Muscle Extract	
Plating Technique	Pour	Streak	Pour	Streak
Sample No.1		as a % of (1)	as a % of (1)	as a % of (1)
1.		224.8	131.2	245.0
2.		113.8	128.1	128.6
3.	Counts	128.8	87.5	136.4
4.	taken	288.1	150.0	313.5
5.	as	122.7	113.5	126.2
6.	100%	242.7	74.5	244.8
7.		133.9	120.9	110.4
8.		164.9	224.9	130.1
Mean (arithmetic)	100	177.5	128.8	179.4
Mean (log)	100	167.7	122.5	166.7

Consideration was given to the possibility of the increased counts being due to a carry through of growth promoting properties to the medium from the muscle extract in the diluent rather than to a protective effect in the dilution stage itself. In Table 3 are given the results in which (1) 0.1 ml. of sterile muscle extract was dropped onto poured YMA plates, streaked out and allowed to dry before inoculating by streaking with serial test dilutions in quarter-strength Ringers and (2) a batch of YMA was

prepared to contain 1% muscle extract added to the cooled media just prior to pouring.

TABLE 3.

THE EFFECT OF MUSCLE EXTRACT ADDED TO THE PLATING MEDIUM IN RELATION TO ITS USE AS A DILUENT.

Medium	YMA	YMA and Muscle Extract	YMA
Diluent	Quarter-strength Ringers	Quarter-strength Ringers	Muscle extract
Streak plates	143%	120%	170%
Pour plates	100%	101%	137%

(mean of 7 tests incubated at 37°C. for 48 hours.)

The results indicate that the muscle extract would appear to act as a protectant in the dilution stage, and no carry over effect could be demonstrated. The effect of heat on the protectant action of muscle extract is shown in Table 4 which indicates a heat lability at 80°C. and complete destruction of the effect at 100°C.

TABLE 4.

THE ACTION OF HEAT ON THE PROTECTANT EFFECT OF MUSCLE EXTRACT AS A DILUENT USING STREAK PLATES INCUBATED AT 37°C. FOR 48 HOURS.

Diluents			
Quarter-strength Ringers (Oxoid)	Muscle extract		
	Heated at 80°C. for 5 mins.	Heated at 100°C. for 5 mins.	Normal
(taken as) 100%	105%	150%	252%

Further experiments were carried out to determine if any of the following protein sources could substitute for muscle extract. (1) Bacto Beef Extract, Difco Laboratories, U.S.A., used at a concentration of 6% (w/v), (2) Pork Liver, lyophilized, Nutritional Biochemical Corporation, U.S.A., at 8% (w/v), (3) Bovine Albumin, Nutritional Biochemical Corporation, U.S.A., at 2% and 16% (w/v), (4) Bacto Peptone, Difco Laboratories, U.S.A., at 1% and 5% (w/v). The test concentrations were prepared in quarter-strength Ringers solution, adjusted to give a final pH of 7.5 after sterilisation by Seitz filtration. As Table 5 indicates, none of these protein sources was as effective as muscle extract, although the pork liver preparation showed an appreciable beneficial effect.

It has been reported (Report, 1962) that during the routine determination of the numbers of Staphlococci (Miles and Misra method) undiluted milk, or low dilutions, either failed to produce colonies or gave very much reduced numbers as compared with the higher dilutions. An interaction between milk xanthine oxidase and xanthine from the medium meat extract was demonstrated to produce inhibitory hydrogen peroxide. The use of catalase in aerobically incubated media was recommended. This argues against the use of meat extract as a diluent giving increased counts but it is possible that although fresh muscle extract will contain xanthine the fact that it also contains catalase could avoid the hydrogen peroxide reaction mentioned above. In Table 6 is shown the effect of using catalase in quarter-strength Ringers solution as a diluent and demonstrates that it does not act as a replacement for muscle extract.

In order to establish if the protectant effect of the muscle extract was associated with particular groups of bacteria the colonies from six samples of milk showing large increases in streak plate count when muscle extract was used as a diluent were examined and grouped according to Gram reaction and shape. The results are given in Table 7 from which it can be seen that the main increase in count associated with the use of muscle extract diluent occurs in the gram negative rods.

It is interesting to note that Kitchell, reported by Jayne-Williams (1963), indicated that in the comparison of blender and bead methods of disintegration of samples of bacon and beef, lower counts obtained using the bead method were associated with a loss of certain gram negative rods.

TABLE 5.

COMPARISON OF THE EFFECT OF OTHER DILUENTS IN RELATION TO THE PROTECTANT EFFECT OF MUSCLE EXTRACT.

(Streak plate counts incubated at 37°C. for 48 hours)

Test Diluent	Number of Samples	Mean of tests. (expressed as a percentage of the count using quarter-strength Ringers as diluent)		
		Quarter-strength Ringers	Test Diluent	Muscle Extract
Beef Extract, Difco (6%)	5		106	138
Liver, pork (8%)	4	Count	190	213
Albumin, bovine (2%)	3	taken	78	161
Albumin, bovine (16%)	3	as	104	145
Peptone, Bacto (1%)	6	100%	110	172
Peptone, Bacto (5%)	6		108	178

TABLE 6.

COMPARISON OF THE EFFECT OF CATALASE IN QUARTER-STRENGTH RINGERS DILUENT IN RELATION TO THE PROTECTANT EFFECT OF MUSCLE EXTRACT.

(Streak plate counts incubated at 37°C. for 48 hours)

Test Diluent	Number of Samples	Mean of tests. (expressed as a percentage of the count using quarter-strength Ringers as diluent)		
		Quarter-strength Ringers	Quarter-strength Ringers + Catalase	Muscle Extract
(1) Catalase, crude (0.1%)	8	Count taken as 100%	110	147
(1) Catalase, crude (1.0%	8		117	147

(1) Catalase, crude (Beef-liver) - Nutritional Biochemical Corporation, U.S.A.

THE EFFECT OF MUSCLE EXTRACT AS A DILUENT IN THE BACTERIOLOGICAL TESTING OF MINCED BEEF, BACON, AND FRESH ENGLISH SAUSAGE.

(i) Methods.

In the case of minced beef and fresh English Sausage three diluents were tested, (1) quarter-strength Ringers (Oxoid), (2) quarter-strength Ringers + 0.1% (w/v) peptone, and (3) muscle extract as prepared previously. Streak plates were inoculated as described under milk using a Nutrient Agar medium (Oxoid) containing Lab-Lemco Beef Extract 1 gm., Yeast Extract 2 gms., Peptone 5 gms., Sodium Chloride 5 gms., Agar 15 gms. per litre. Final pH 7.5 . As a further extension of these tests trials were carried out in which 4% and 10% (w/v) NaCl was added to diluents and media respectively.

Similar diluents, with and without added salt were used in the tests on bacon but a special medium was employed containing Bacto Beef Extract (Difco) 3 gms., Bacto peptone (Difco) 5 gms., Tri-Sodium citrate (A.R.) 3 gms., Potassium chloride (A.R.) 2 gms., Magnesium Sulphate, heptahydrate (A.R.) 10 gms., Ferrous Sulphate (A.R.) 5 ml. of 0.01% solution, Agar (Oxoid) 15 gms. per litre. Final pH 7.5 . To this medium was added salt at the various levels stated.

The initial samples were prepared using a blender method in all the experiments reported here. Counts are based on the means of triplicate plates.

(ii) Results and Discussion.

The results are summarised in Tables 8, 9 and 10.

The protectant effect of muscle extract in the diluent was not as noticeable in the three types of meat food chosen, and although the increases in count were considerable in particular samples, the overall trend was marginal. The anomalous behaviour of peptone added to the quarter-strength Ringer diluent is noted but cannot be explained.

TABLE 8.

THE EFFECT OF VARIOUS DILUENTS ON THE COUNTS OF FRESH SAUSAGE ON A MEDIUM CONTAINING 0, 4 and 10% (w/v) NaCl.

Diluent	Salt content of medium					
	0% NaCl		4% NaCl		10% NaCl	
	37°C. for 2 days	25°C. for 3 days	37°C. for 4 days	25°C. for 4 days	37°C. for 7 days	25°C. for 7 days
Quarter-strength Ringers	100	100				
Quarter-strength Ringers + 0.1% peptone	143	77				
Muscle extract	183	114				
Quarter-strength Ringers + 4% NaCl			100	100		
Quarter-strength Ringers + 4% NaCl + 0.1% peptone			84	82		
Muscle extract + 4% NaCl			126	121		
Quarter-strength Ringers + 10% NaCl					100	100
Quarter-strength Ringers + 10% NaCl + 0.1% peptone					100	86
Muscle extract + 10% NaCl					123	143

(results are the mean of three samples expressed as a percentage of the reference diluent)

TABLE 9.

THE EFFECT OF VARIOUS DILUENTS ON THE COUNTS OF BACON ON A MEDIUM CONTAINING 0, 4 and 10% (w/v) NaCl.

Diluent	Salt content of medium					
	0% NaCl		4% NaCl		10% NaCl	
	37°C. for 2 days	25°C. for 3 days	37°C. for 4 days	25°C. for 4 days	37°C. for 7 days	25°C. for 7 days
Quarter-strength Ringers	100	100				
Quarter-strength Ringers + 0.1% peptone	73	40				
Muscle extract	117	109				
Quarter-strength Ringers + 4% NaCl			100	100		
Quarter-strength Ringers + 4% NaCl + 0.1% peptone			69	100		
Muscle extract + 4% NaCl			187	233		
Quarter-strength Ringers + 10% NaCl					100	100
Quarter-strength Ringers + 10% NaCl + 0.1% peptone					65	147
Muscle extract + 10% NaCl					93	218

(results are the mean of three samples expressed as a percentage of the reference diluent)

TABLE 10.

THE EFFECT OF VARIOUS DILUENTS ON THE COUNTS OF MINCED BEEF ON A MEDIUM CONTAINING 0, 4 and 10% (w/v) NaCl.

Diluent	Salt content of medium					
	0% NaCl		4% NaCl		10% NaCl	
	37°C. for 2 days	25°C. for 3 days	37°C. for 4 days	25°C. for 4 days	37°C. for 7 days	25°C. for 7days
Quarter-strength Ringers	100	100				
Quarter-strength Ringers + 0.1% peptone	84	98				
Muscle extract	119	121				
Quarter-strength Ringers + 4% NaCl			100	100		
Quarter-strength Ringers + 4% NaCl + 0.1% peptone			84	78		
Muscle extract + 4% NaCl			107	111		
Quarter-strength Ringers + 10% NaCl					100	100
Quarter-strength Ringers + 10% NaCl + 0.1% peptone					84	74
Muscle extract + 10% NaCl					125	119

(results are the mean of three samples expressed as a percentage of the reference diluent)

(iii) Conclusions.

Since the meat foods chosen for examination could be said to produce their own meat extractant during preparation of the initial sample, the lower response of added muscle extract is presumably to be expected. This accords with the remarks of Mossel, as reported by Jane-Williams (1963), in as much as the term "proteinaceous foods" applies to pork and beef, but not in the case of milk under the conditions of test reported here.

The magnitude of the effect of muscle extract in the diluent on the plate counts of milk is surprisingly large and requires further investigation, particularly in relation to the role played by the gram negative rods present in the milk flora.

However an important corollary to this is the possible protectant effect of the muscle extractants on the flora of the meat itself and this aspect is being currently investigated.

REFERENCES.

1. JANE-WILLIAMS D.J. (1963). Report of a discussion on the effect of the diluent on the recovery of bacteria. J. Appl. Bact. 26 (3) 398-404.

2. JESPERSEN N.J.T. and RIEMANN H. (1958). The numbers of salt tolerant bacteria in curing brines and in bacon. 2nd Int. Symp. Fd. Microbiol. Cambridge p.177-182. H.M.S.O. London.

3. MINISTRY OF HEALTH (1937). Bacteriological Tests for Graded Milk, Memo. 139/Foods. H.M.S.O. London.

4. PATTERSON J.T. and CASSELLS J.A. (1963). An examination of the value of adding peptone to diluents used in the bacteriological testing of bacon curing brines. J. Appl. Bact. 26 (3) 493-497.

5. REPORT (1962). Inhibition of Staphylococci on solid media. Annual Report. National Inst. for Research in Dairying, Reading, England.

6. STRAKA R.P. and STOKES J.L. (1957). Rapid Destruction of Bacteria in Commonly used Diluents and its Elimination. Appl. Microbiol. 5 (1) 21-25.