logical properties of that outer region and it will be responsible for the experimental facts described in this report. However, this question will require further detailed study, chiefly in order to observe if other pentoses have the same effects.

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SUMMARY

Experiments were carried out in an attempt to determine the influence of arabinose on the fermentation of glucose by *Saccharomyces cerevisiae* NRRL Y-684.

The presence of arabinose increases the rate of CO_2 production and the optimum pH interval of the yeast. The alcohol yields, with and without arabinose, are the same. The reduction of the medium pH during fermentation in the presence of arabinose is smaller than that observed on culture medium containing only glucose. Arabinose concentration decreases when the yeast ferment glucose. Arabinose is not fermented by the yeast studied; the inoculation of media containing only arabinose as a major carbon source results in an almost complete destruction of the yeast cells.

REFERENCES

BORZANI, W. AND FALCONE, M. 1952 Estudo sôbre os processos de dosagem de álcool em vinhos resultantes da fermentação de mosto de melaço. Bol. assoc. brasil. quím., **10**, 5–9.

BORZANI, W. AND FALCONE, M. 1953 Control of starter preparation in fermentation of molasses. J. Agr. Food Chem., **1**, 1070–1071.

BORZANI, W., DOLES, J. M., ITINOSE, S., AND ALBUQUERQUE, L. M. C. 1956 Estudo da hidrólise ácida do pinhão. Engenharia, **14**, 276–278.

BROWNE, C. A. AND ZERBAN, F. W. 1941 *Physical and chemical methods of sugar analysis*, Ed. 3, pp. 915. John Wiley & Sons, Inc., New York, New York.

CONWAY, E. J. AND DOWNEY, M. 1950 An outer metabolic region of the yeast cell. Biochem. J., **47**, 347–355.

WHITE, J. 1954 *Yeast technology*, p. 135. Chapman & Hall, London, England.

Isolation of Salmonellae from Food Samples

II. The Effect of Added Food Samples upon the Performance of Enrichment Broths

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Many of the differences that exist between the needs of the clinical bacteriologist and the food analyst have been defined and discussed previously (Taylor *et al.*, 1958). Nowhere are these differences any more pronounced than in the need for the food bacteriologist to quantify the salmonellae in foods. The small numbers which characterize the occurrence of salmonellae in foods, often less than one per gram, prohibit the use of direct counting methods. The problem posed by the analysis of large amounts of food sample for small numbers of organisms has been attacked traditionally by resorting to a modification of the most probable number (MPN) technique (Hoskins, 1934; Ayres, 1949). As routinely performed, this technique involves the inoculation of triplicate 90 ml aliquots of selenite or tetrathionate enrichment broths with 10, 1 and 0.1 g amounts of food material. Successively greater dilutions are used when the food is known or thought to be more heavily contaminated. It was particularly in these latter cases during the course of analysis of a great many food samples that a recurring anomaly was observed. It was found quite frequently that *Salmonella* type organisms were not isolated from enrichment broth aliquots representing the greatest amounts of inocula, but were detected with ease in those which had received much smaller quantities of samples. The levels at which these "skips" occurred were usually the 10, 1 and 0.1 g replicates. Since the MPN technique is in effect a series of qualitative determinations from which quantitative data may be obtained from probabilities of frequency of occurrence, the underlying assumption is that the introduction of a single viable *Salmonella* type cell into a tube of enrichment broth will ultimately manifest itself as a *Salmonella*-positive test upon subsequent plating. The frequency and the extent of the skips belied this assumption. If, as we began to suspect, the salmonellae were undetectable at the aforementioned levels, this was a severe limitation of the method, because those were the most frequently used concentrations. Also, if the salmonellae did not occur in numbers great enough to show positives in subsequent dilutions, one would be led to believe that

there were none present when actually their presence was being concealed by factors as yet unknown.

The primary non-salmonella organisms occurring in the enrichment broths are coliform bacteria. The appearance of the plates which logically should have been positive was one of great coliform overgrowth. These results suggested two possible factors: that (1) in the event of great numerical superiority of coliforms over salmonellae, the selectivity of the enrichment broth is overwhelmed and no salmonellae are detected, or (2) the performance of the enrichment broth is negated by the added food material. The import of this analytical anomaly led to this investigation.

Materials and Methods

The effect of an unfavorable imbalance of coliforms to salmonellae was determined by establishing varying ratios of coliform to salmonellae and inoculating enrichment broths. Pure cultures of each of three strains of coliforms and salmonellae newly isolated from food samples were mixed in known proportions and 1 ml of each of the mixtures were added to 99 ml of cystine-selenite F (North and Bartram, 1953) or tetrathionate broths. After 18 hr incubation at 37 C, brilliant green agar (BG) plates received 0.1 ml of a dilution calculated to produce 100 to 300 well distributed colonies by spreader bar or "hockey stick" technique so that the percentage of salmonella colonies appearing on the plates could be used as an index of performance for the media.

Since it was quite possible that the effect of an unfavorable ratio might be entirely different when the absolute numbers of salmonellae were small rather than large, both conditions were investigated.

The ability of added food to alter performance of enrichment broths was tested with a variety of dried, powdered foods. The food samples were sealed in 404 x 700 (46 oz) tin cans and autoclaved for 40 min at 15 lb. Sterile 11 g samples were weighed into 99 ml of enrichment broths in 150 ml capacity amber round glass jars containing broken glass. The coliform-salmonella mixtures in varying ratios were added and, after 10 min of mechanical shaking, incubated at 37 C for 18 hr. BG plates inoculated by spreading were counted for salmonella percentages.

The separation of bacteria from soluble food sample by centrifugation employed the addition of the 10 per cent food material and the organisms to sterile buffered water, shaking, centrifuging and resuspending the sediment in 99 ml of enrichment broth. Centrifugation for 10 min at 1100 *g* in an angle head Servall[1] centrifuge secured the desired removal of bacteria. Each food sample of approximately 110 ml was equitably distributed in 3 to 50 ml polyethylene centrifuge tubes, which had been covered with crimped aluminum foil, and steamed for 10 min. (Since the completion of this work, high temperature polyethylene tubes which will permit autoclaving have been made available.)

[1] Ivan Sorvall, Inc., Norwalk, Connecticut.

Results and Discussion

The effect of unfavorable coliform-salmonella ratios on the performance of enrichment broths is shown in table 1. In view of the overwhelming preponderance of coliforms in the most extreme ratios, the selectivity of these broths for salmonellae is undeniable. When, for example, only 10 salmonellae are inoculated concomitantly with 10 million coliforms, salmonellae comprise 15 and 22 per cent of the population on BG plates after incubation in selenite and tetrathionate broth, respectively. From these data it was concluded that the performance of the enrichment broths in the absence of food samples was not likely to be responsible for the "skips" in the MPN analysis, regardless of the coliform-salmonella ratios involved.

The alternative possibility, negation of the efficacy of the enrichment broths through adulteration of the media with large food samples, was examined with the results shown in columns A and B of tables 2 and 3. In these experiments, we have used 10 per cent food

TABLE 1

Selectivity of enrichment broths for Salmonellae

Percentage of Salmonellae in Broth after Incubation					
Large inoculum			Small inoculum		
C:S	Cystine-selenite F broth	Tetrathionate broth	C:S	Cystine-selenite F broth	Tetrathionate broth
10^9:10^7	63	100	10^4:10	100	67
10^9:10^6	43	97	10^5:10	83	69
10^9:10^5	25	100	10^6:10	20	97
10^9:10^4	3	100	10^7:10	15	22

* C:S = Coliform:Salmonellae inoculum per 100 ml broth.

TABLE 2

The effect of centrifugation of food samples upon salmonella recovery

Ratio, C:S*	Per Cent of Salmonella Colonies Developing on BG Plates after Enrichment in Tetrathionate Broth														
	Albumen			Whole egg			Yolk			Gelatin			Dried beef		
	A†	B	C	A	B	C	A	B	C	A	B	C	A	B	C
10^3:1	98	77	95	—	—	—	—	—	—	—	—	—	—	—	—
10^4:1	96	43	93	71	76	81	—	—	—	—	—	—	—	—	—
10^5:1	96	7	96	68	18	64	76	60	28	100	44	55	33	7	10
10^6:1	77	4	82	25	2	52	61	70	3	96	31	86	19	2	9
10^7:1	63	1	63	13	0	37	36	44	2	95	18	0	0	1	4
10^8:1	—	—	—	—	—	—	43	10	7	93	1	95	6	0	2

* C:S = ratio of numbers of coliforms:salmonellae.

† A = control—no food sample added; B = test—10 per cent food sample added; C = centrifuged, food sample added, centrifuged, and sediment used as inoculum.

samples throughout because the skips were most pronounced at that level of inoculum; however we had observed that with many foods the effect was noticeable even at levels of 0.01 per cent inocula. It could be seen also that the performance of the enrichment broth varied with the type of food added. In table 3, the loss of selectivity of selenite broth for salmonellae after the addition of gelatin is marked to the extreme. One recovers almost a pure culture of salmonellae, 95 per cent, when coliforms outnumber them 1000 to 1 in the original inoculum, whereas the addition of 10 per cent gelatin to an identical aliquot results subsequently in failure to find even one salmonella colony on the BG plate. In table 2, the same appearance is presented with the 100 million to 1 ratio of coliforms to salmonellae after the addition of gelatin to tetrathionate. Albumen is shown to be very similar to gelatin in its effect on salmonella recovery percentages, whereas dried beef in both tables affects the performance of the enrichment broths far less, and egg yolk has almost no effect. Since these tables represent composites of different experiments using different sized inocula and different organisms from one food sample to another, the data shown are representative of many trials and were chosen largely because they exhibit the minimum of aberrent recovery percentages. Erratic results are more apt to be seen in the area of greatest coliform-salmonella inocula ratios, because it was felt that the number of salmonellae should be kept minimal in order to be truly representative of the actual numbers of organisms in food samples. In many experiments, therefore, the average salmonella inoculum is as few as five organisms, the coliform being adjusted accordingly for the ratios desired.

The import of detection of salmonella at low recovery percentages may be better appreciated if it is known that both streaked plates and spread plates were compared in all experiments to visualize the results which would be obtained in the routine performance of the MPN method. We observed that when the levels of salmonella recovery were between 5 to 10 per cent on the spread plate, their detection on streaked plates was uncertain; at levels below 5 per cent, detection was unlikely.

TABLE 3

The effect of centrifugation of food samples upon salmonella recovery

Ratio, C:S*	Per Cent of Salmonella Colonies Developing on BG Plates after Enrichment in Cystine-Selenite F Broth								
	Albumen			Gelatin			Dried beef		
	A†	B	C	A	B	C	A	B	C
10^2:1	63	2	81	—	—	—	—	—	—
10^3:1	43	1	45	92	0	32	100	60	91
10^4:1	25	0	11	32	0	6	83	21	61
10^5:1	3	0	2	0	0	0	20	14	29
10^6:1	—	—	—	0	0	2	15	18	33

* C:S = ratio of numbers of coliforms:salmonellae.

† A = Control—no food sample added; B = test—10 per cent food sample added; C = centrifuged, food sample added, centrifuged, and sediment used as inoculum.

The difference in the results obtainable by the two methods accrues largely to the advantages of the spread plate technique. We have found that, when as many as 500 colonies are on a spread plate, the even distribution which characterizes the method makes it possible for individual colonies to display their distinctive colonial attributes on BG plates. One finds but rarely a streaked plate on which even 100 colonies are so well distributed, indeed, 50 such are considered excellent for the technique. The limitations of both techniques are determined by the maximum number of colonies which one may be able to distinguish upon a plate and recognize as single salmonella colonies. The clear superiority of the spread plate dictated its choice. The data shown, therefore, are from the more sensitive technique and the low recovery percentages all too frequently did not result in positive detections in the streaked plates from those same samples.

By both plating methods the deleterious impact of the addition of certain food materials was unequivocally demonstrated. It was apparent that the diminution of salmonella numbers was effected more by the kind of food material added than by either the strains inoculated or the enrichment broth employed. Other authors have noted that in the presence of 20 to 40 per cent whole egg, *Salmonella pullorum* was inhibited less by selenite F broth (Hurley and Ayers, 1953), and that dried albumen diminished the number of *S. pullorum* isolations in tetrathionate broth (Schneider, 1946).

Inasmuch as the selectivity of salmonella enrichment broths is measurably impaired by the addition of as little as 0.01 per cent of certain foods, it is obviously impractical to consider the solution to this problem through the use of sufficiently large volumes of media to dilute out the effect. A more fruitful approach seemed to lie in the separation of the bacteria from the food materials, or at least from those components of the food which are responsible for decreased efficiency of the media. It was found that this could be accomplished by centrifugation of suspensions or solutions of the food. In initial studies 10 per cent solutions of egg albumen were intentionally contaminated with coliform organisms. These solutions were then centrifuged and the supernatant fluid was assayed for bacteria after various periods. As seen in table 4, 99.9 per cent of the organisms were removed from the supernatant fluid in 10 min; virtually all of the bacteria could be found in the sediment.

Experiments were conducted in which the effect of separation of the bacteria from the food sample by

centrifugation on subsequent enrichment could be ascertained. In tables 2 and 3, the percentages of recovered salmonellae at varying inoculum ratios are shown in the absence of food sample (column A), in the presence of 10 per cent food samples (column B), and after removal of the food material by centrifugation and incubation of the sediment in the enrichment media (column C). It is seen that almost without exception the recovery of salmonellae from sedimented samples was as good as from control cultures. This proves beyond a reasonable doubt that, when food materials destroy the selectivity of otherwise highly selective enrichment media, sedimented inocula offer the means to circumvent that undesirable result.

As previously noted, the degree to which foods affect the efficiency of the media varies with the nature of the materials added. Obviously, the effect of centrifugation is to separate the water soluble components of the food from the bacteria and water insoluble fraction. Reference to table 5 confirms the inference that it is, in fact, water soluble materials which are most deleterious to the efficiency of the enrichment media. Quite expected, then, is the observation that the most dramatic effects of centrifugations are seen with foods like gelatin and egg albumen, and the least with the almost insoluble egg yolks.

The foregoing experiments were conducted in a population consisting of only two groups of organisms; the ultimate usefulness of centrifugation could be determined only empirically. To this end, duplicate aliquots of dried albumen and whole egg samples, many of which had shown no salmonellae by conventional MPN determinations, were analyzed by routine and centrifugation techniques with both enrichment broths. The results are shown in table 6. The first five samples were 9-tube MPN's, the remainder were the three 10 g sample replicates only. For the purpose of statistical analysis MPN conversions were not made, but the total number of positive *Salmonella* isolations was tabulated and subjected to Chi-square analysis using a 4-fold table including Yate's correction. The Chi-square value obtained, 10.463, indicated that the probability of the difference between 20/126 and 47/126 occurring through chance alone was less than 1 per cent. Analysis of the difference observed between the responses of tetrathionate and selenite yielded a Chi-square value of 0.123, with a probability greater than 70 per cent that those observations were due to chance. We conclude, therefore, that both enrichment broths are affected by the food adulterant to approximately the same degree.

It has been shown, in the analysis of food products for salmonellae, that, when the expected levels of contamination necessitate the examination of relatively large amounts of food material, the loss of selectivity of the enrichment media will frequently result in failure to isolate salmonellae when they are, in fact, present. This should be anticipated if one examines the basic principles underlying the application of the enrichment techniques. An enrichment broth constitutes, essentially, an attempt to create an environment which favors the growth of one particular group of organisms to the exclusion of other types which may or may not be closely related. Consider the effect of adding 10 per cent food solids to what is in essence a 2.3 per cent

TABLE 4

Removal of bacteria from 10 per cent albumen by centrifugation at 1100 g

Centrifuged	Per Cent Bacteria Remaining in Suspension				
	I	II	III	IV	Average
min					
0	100	100	100	100	100
10	0.005	—	0.2	0.06	0.088
20	0.004	—	0.07	0.02	0.031
30	—	0.07	0.06	0.005	0.045
60	—	0.002	0.04	0.02	0.021

TABLE 5

Removal of nitrogen and sugar from foods

Food*	Per Cent Discarded in Supernatant	
	Nitrogen	Sugar
Gelatin	99.9	—
Albumen	97.6	—
Dried milk	60.8	85.9
Egg, 30% sucrose	57.4	86.3
Whole egg	41.9	65.1
Dried beef	24.2	—
Egg yolk	7.5	—

* 10 per cent solutions centrifuged at 1100 g for 10 min.

TABLE 6

The effect of centrifugation of albumen and egg samples upon the number of salmonella isolations

Sample	Cystine-Selenite F Broth		Tetrathionate Broth	
	Control	Centrifuged	Control	Centrifuged
Albumen 76	0/9*	4/9	0/9	3/9
68	1/9	5/9	1/9	4/9
60	2/9	3/9	0/9	2/9
61a	0/9	0/9	0/9	1/9
79	7/9	6/9	4/9	4/9
20	0/3	2/3	3/3	3/3
21	1/3	1/3	0/3	3/3
42	0/3	1/3	0/3	0/3
49	1/3	1/3	0/3	0/3
19	2/3	1/3	1/3	2/3
Whole egg reference	0/3	1/3	0/3	0/3
Total	11/63 (22.2%)	25/63 (39.7%)	9/63 (14.3%)	22/63 (34.9%)

* Number *Salmonella* positive/total number.

broth (selenite) or a 4.6 per cent solution (tetrathionate) and it ceases to be a source of wonderment that the performance of these broths are affected. Centrifugation is a means by which the bacteria may be freed of the food adulterant. It is by no means the only possible way of securing freedom from the loss of enrichment performance; we are investigating other means. But in a larger sense these phenomena point up a fundamental principle involved, to wit: the adaptation of a method borrowed from one discipline for use in another without consideration of the method's intent cannot be made. The failure of the enrichment method in the regimen of the food analyst is so abject, however, that it leads us to suspect that even in the clinical laboratory it may have shortcomings which are not fully recognized. The detection of intermittent carriers, for example, might be enhanced if large fecal samples were centrifuged and resuspended in adequate amounts of enrichment media. We know of many laboratories that use greater than 10 per cent inocula of fecal material for enrichment purposes. The effect of adding blood to enrichment broths is a severely debilitating factor in enrichment broth performance; lysing the red cells by the addition of distilled water, centrifuging, and suspending the sediment is a possible improvement.

In the food and dairy laboratories, the methods borrowed from water analysis are equally abused. In brilliant green bile lactose broth used for coliform analyses, one is expected to discern the fermentation of 1 per cent lactose in the presence of ice cream, orange juice, and similar products which often superimpose 1 to 2 per cent sucrose concentrations in the fermentation tube. Many of these situations are amenable to centrifugation techniques. In these instances, re-evaluation of intent and applicability of analytical methods is not merely profitable but necessary if one is to disabuse himself of the faulty information which he may obtain.

SUMMARY

Neither overwhelming numbers of coliform organisms nor an unfavorable imbalance in the ratio of coliforms to salmonellae materially affected the selectivity of selenite or tetrathionate enrichment broths for salmonellae.

The performance of these broths were, however, greatly diminished by the addition of many kinds of food materials; gelatin and albumen caused the most striking diminution of salmonella recoveries; egg yolk, the least.

Separation of the bacteria from the soluble food material by centrifugation proved to be an effective means of restoring the function of enrichment media.

The degree to which the selectivity of enrichment broths was altered was shown to be directly proportional to the amount of soluble material in the sample and was in no manner influenced by either the strains of organisms employed or the differences between the enrichment broths.

The use of centrifugation in the analysis of naturally contaminated albumen and whole egg samples demonstrated a statistically significant ability to detect low levels of salmonella contamination hitherto undetectable.

REFERENCES

AYRES, J. C. 1949 A procedure for the quantitative estimation of *Salmonella* in dried egg products. Food Technol., **3**, 172–176.

HOSKINS, J. K. 1934 Most probable numbers for evaluation of *coli-aerogenes* tests by fermentation tube method. Public Health Repts., **49**, 393–405.

HURLEY, N. A. AND AYRES, J. C. 1953 A comparison of six enrichment media for isolating *Salmonella pullorum* from egg products. Appl. Microbiol., **1**, 302–306.

NORTH, W. R. AND BARTRAM, M. T. 1953 The efficiency of selenite broth of different compositions in the isolation of *Salmonella*. Appl. Microbiol., **1**, 130–134.

SCHNEIDER, M. D. 1946 Investigation of *Salmonella* content of powdered whole egg with not more than two percent moisture content. II. General survey on occurrence of species of *Salmonella* in high quality egg powder. Food Research, **11**, 313–318.

TAYLOR, W. I., SILLIKER, J. H., AND ANDREWS, H. P. 1958 Isolation of salmonellae from food samples. I. Factors affecting the choice of media for the detection and enumeration of *Salmonella*. Appl. Microbiol. **6**, 189–193.