A simple color test was recently described by Day and Doan (1) for detecting levels of psychrophilic bacterial activity in refrigerated bottled milk which indicate incipient decomposition but precede organoleptic spoilage. The test employed neotetrazolium dye in a tube of milk evacuated, sealed and incubated at 37°C for four hours. A pink tint in such a sample preceded a recognizable off flavor by about four days.

In the study here reported, two other tetrazolium dyes were compared with neotetrazolium to ascertain whether either of them offered any advantage for the purpose in view.

The test, which previously only had been recommended for milk, was applied to bottled cream, to cream stored in bulk and to plain concentrated skim-milk. The last mentioned products are frequently held on hand by ice cream manufacturers for later use in ice cream. An attempt was also made to use the test as an indication of psychrophilic activity in creamed cottage cheese and cottage cheese curd.

**Procedure and Results**

The methods and procedures used were those reported by Day and Doan (1) except that psychrophilic plate counts were incubated at 21°C for three days and except as noted later in connection with the products held in bulk and with cottage cheese.

**Other Dyes**

Several detailed comparisons utilizing blue tetrazolium and triphenyltetrazolium, with samples of commercially pasteurized bottled milk, revealed that the former lacked sensitivity at all concentration levels. The latter, however, when used at reagent concentrations between 0.2 and 1.0 per cent gave results equally satisfactory with those obtained with neotetrazolium. Inasmuch as the results with triphenyltetrazolium showed no definite advantage and because this dye is more light sensitive than neotetrazolium (2) it was concluded that neotetrazolium is the better reagent.

Incidently, the 32 samples of milk tested in this study exhibited an average keeping period of 14 days before flavor spoilage, with a range of from three to 31 days. The tetrazolium reduction tests became positive an average of 4.4 days prior to spoilage with a range of from one to ten days. This average corresponds to 3.75 days found by Day and Doan.

**Bottled Cream**

Twenty-eight samples of commercially pasteurized and bottled single cream and 22 similar samples of whipping cream were obtained from the University Creamery and from other dealers in the State College area. These were held at approximately 40°F. in a thermostatically controlled refrigerator (39.2° to 42.8°F.) until flavor spoilage occurred. Neotetrazolium tests (1) and flavor evaluations were made at two-day intervals by withdrawing test portions aseptically. Psychrophilic bacterial counts were obtained on the day the dye reduction test became positive and again on the day the flavor was deemed unacceptable by two competent judges. A summary of the results obtained are shown in the first two lines of data in the table.

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1. Authorized for publication on February 4, 1958, as paper no. 2225 in the Journal Series of the Pennsylvania Agricultural Experiment Station.
Neotetrazolium reduction occurred in single cream an average of 4.7 days before flavor spoilage, with a range of from one day to 12 days. In whipping cream the interval was 6.5 days with a range of from two days to 13 days. These results indicate that incipient decomposition can be detected in cream farther in advance of spoilage than is the case with milk. The longer interval is attributed to the masking effect of the higher fat content and higher viscosity on flavor perception. The psychrophilic populations of the cream samples at the time of dye reduction and at the time of spoilage varied widely indicating variable ability of different psychrophiles to cause dye reduction and flavor changes. It is concluded that the neotetrazolium test can be used to detect deterioration in refrigerated bottled cream just as satisfactorily as in milk.

### Bulk Cream

To simulate the bulk holding or can holding of cream as practiced by ice cream manufacturers and others, 21 different lots of pasteurized heavy cream from the University Creamery were held in 20 qt. dispenser cans located in a large refrigerator where the temperature range was from 35°F to 45°F. The higher temperatures were during limited periods of the operating day. The cans were not filled and agitation before sampling was accomplished by manually whirling the containers. Samples were removed through the dispenser tubes after flushing about 25 ml. of cream which was discarded. Screw clamps were set near the end of the rubber tubes and the exposed portions cut off before each sampling. Some differences in the degree of recontamination of the lots of cream were obtained by washing and sanitizing the dispenser cans at various degrees of efficiency, before they were used for storing the pasteurized cream.

The third line of data in the table shows that the bulk-refrigerated lots of heavy cream reduced neotetrazolium an average of 8.5 days before flavor spoilage was detected, ranging from two to 20 days. This product exhibited the longest keeping period, the lowest psychrophilic population at the time of dye reduction and the longest interval between a positive dye test and organoleptic spoilage, of any of the products studied.

#### Concentrated Skimmilk

All samples of concentrated skimmilk were obtained from different batches, made from time to time, in the University Creamery. Some of the batches were superheated, others were not. The samples were held in quart glass milk bottles and sampled in the manner described for bottled cream.

The results obtained with concentrated skimmilk are summarized in the last line of data in the table. The samples exhibited an average keeping period of 17 days with a range of from 10 to 24 and the reduction of tetrazolium occurred on an average of 7.8 days prior to organoleptic spoilage with a range of from 3 to 14 days. This product had the highest psychrophilic population at spoilage of any of the products studied. This, however, could not be attributed to the masking effect on flavor perception of high solids because the samples were reconstituted to a fluid basis before flavor evaluation. It is more likely due to the

<table>
<thead>
<tr>
<th>Number of Samples</th>
<th>Reduction of tetrazolium</th>
<th>Days between positive test and spoilage</th>
<th>Keeping interval (Days)</th>
<th>Psychrophilic count (21°F — 3 days) (Millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Days before positive test</td>
<td></td>
<td>Day of positive test</td>
</tr>
<tr>
<td>Pasteurized bottled single cream, Fat range — 18 to 23%</td>
<td>28</td>
<td>7.4</td>
<td>8-0.13</td>
<td>4.7</td>
</tr>
<tr>
<td>Pasteurized bottled whipping cream, Fat range — 31 to 48%</td>
<td>29</td>
<td>7.0</td>
<td>7-0.13</td>
<td>6.5</td>
</tr>
<tr>
<td>Pasteurized heavy cream stored in bulk, Fat range — 37 to 43%</td>
<td>21</td>
<td>12.0</td>
<td>13-6.22</td>
<td>8.5</td>
</tr>
<tr>
<td>Concentrated skimmilk, T. S. range — 36 to 33%</td>
<td>19</td>
<td>8.8</td>
<td>10-2.13</td>
<td>7.6</td>
</tr>
</tbody>
</table>
fact that all samples exhibited a rather definite "cooked" flavor caused either by the preheating temperature (generally about 180°F) or the superheating treatment and this probably made detection of bacterial flavors more difficult.

Inasmuch as there was no significant difference in keeping ability between the superheated samples and those which were not superheated, the results of the two types of concentrated skim milk were not considered separately. The fact that no difference was noted is not particularly surprising and only serves to emphasize the fact that recontamination is the important consideration in heat treated products as far as keeping ability is concerned, rather than the bacterial numbers following the heat treatment.

A few false-positive dye tests were encountered among the samples of concentrated skim milk. These were obtained immediately after manufacture and usually became negative within 24 to 48 hours of storage. This phenomenon was mentioned by Day and Doan working with milk but in this study it only was encountered with concentrated skim milk. The samples showing initial positive tests were not confined to the superheated product and had no other distinguishing characteristics. An effort to relate dye reduction in these samples to heat-generated reducing substances (sulfides and sulfhydryls) proved inconclusive.

Cottage Cheese

When the neotetrazolium test was applied to creamed cottage cheese or cottage cheese curd of any age, in the usual manner, positive tests always resulted. This apparently is due to the fact that starter organisms occluded in the curd develop very rapidly during the four hour incubation period of the samples and reduce the dye. Consequently the test does not measure the activity of psychrophiles but merely verifies the presence of mesophilic organisms in the product.

A modification of the dye reduction test which seemed to offer some possibility for the purpose of detecting psychrophilic activity in held creamed cottage cheese or cottage cheese curd was investigated. Approximately five grams of cheese or curd, randomly removed from the container, in small portions with a sterile spatula, were placed in a sterile test tube and shaken to the bottom. Then 0.5 ml. of the 0.2 per cent aqueous solution of the dye were added, the tube plugged with cotton and held under refrigeration along with the cheese or curd. The criterion used for a positive test was the appearance of any pink or violet color in the tube. Color usually appeared as pink pinpoints, below the surface of the curd, which grew into large lavender colored areas with continued holding.

In 37 trials with creamed cottage cheese and unsalted cottage cheese curd the modified test gave positive tests an average of 11.6 days before organoleptic spoilage could be detected in the sub-surface product. The range was from zero (for 3 samples) to 25 days. In these trials, however, spoilage more frequently occurred as a surface decomposition and the modified test failed, in nearly half the trials, to give any advanced warning of this type of deterioration.

It appears that this test, if useful at all, is limited to the detection of subsurface deterioration in stored bulk cottage cheese or cottage cheese curd. Under such conditions the surface of the product could show development of slime, mold, yeast, etc., yet the product under the surface be unchanged. Inasmuch as the surface could be discarded, the dye reduction test might be used as a quality test for the sub-surface bulk of the product. When used in this way, the dye usually showed reduction well in advance of any detectable change in flavor.

Summary and Conclusions

Blue tetrazolium and triphenyltetrazolium dyes were studied as possible substitutes for neotetrazolium in the Day and Doan keeping quality test for bottled milk but it was concluded that neotetrazolium is the best of the three for the purpose.

The neotetrazolium test was found to be applicable, without modification, to bottled cream, bulk cream and concentrated skim milk for following deterioration caused by psychrophilic bacterial activity when these products are held under refrigeration. In practically all cases the test became positive (dye was reduced) several days in advance of organoleptic spoilage. It is believed that this test would prove very useful to ice cream manufacturers who hold supplies of cream and concentrated milk on hand for mix making.

Superheated concentrated milk exhibited no better keeping quality than unsuperheated, again emphasizing the critical role of recontamination as the major factor influencing the keeping quality of heat-treated products under refrigeration.

A modified neotetrazolium test for detecting psychrophilic deterioration in cottage cheese and cottage cheese curd was found to have only limited value due to the tendency for these products to exhibit surface spoilage not detected by the test.

References