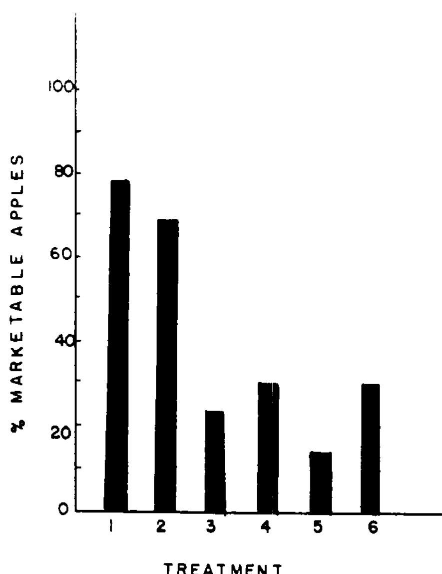

Figure 3. Comparison of alkaline permanganate and activated carbon in maintaining marketability of Grimes apples during 95 days' storage

1. Alkaline permanganate plus poststorage aeration
2. Alkaline permanganote
3. Activated carbon plus poststorage a eration
4. Activated carbon
5. Control plus poststorage aeration
6. Control

were spread out on a table to provide aeration during the 3 days prior to counting.

The final scald count was conducted 95 days after storage with 20 bushels of Grimes apples from each of the test rooms. Ten bushels from each room were wrapped with paper and stacked; the apples from the remaining 10 bushels were spread out singly in a large vacant room. The apples were kept at room temperature for 4 days, and then inspected for the incidence and severity of scald. Slight scald was judged as that injury which would not appreciably detract from the market value of the fruit, medium scald that injury which would definitely result in a drop in market value, and severe scald as that which would render the fruit essentially unsalable.

Results and Discussion

Pressure tests with the Magness and Taylor pressure tester showed no consistent differences in softness between apples in the control room and those in the permanganate room; however, they did show a slightly softer fruit in the activated carbon room. The air temperature in the carbon room rose to 42° F. during a 5-hour period on the 17th day of storage because of necessary repairs on the cooling system. This temperature rise may account in part for the slightly increased comparative softness of the fruit in this storage room.

The incidence and severity of scald on apples stored in the permanganate room were substantially lower than those on apples in the other two rooms (Table I). The percentage of marketable apples was, therefore, much greater in the permanganate room (Figure 3). Most of the scald in this room was slight and would result in little reduction of the market value of the apples. On the other hand, apples in the control and carbon rooms had a considerably lower percentage of marketability (Figure 3).

Aerating after removal from cold storage generally proved beneficial in reducing scald on apples from the permanganate room but not on apples from the other rooms.

Although perfect control of apple scald was not attained in this experiment, the method shows considerable promise. Since the apples were stored in tight wooden boxes, lack of proper aeration during storage was undoubtedly an important limiting factor. Apples in the center of the box consistently exhibited more scald injury than those on top or around the sides. Slatted field crates would undoubtedly allow greater aeration, and with respect to scald control, would be more suitable. It is probable that most orchardists would pick apples at a more advanced stage of maturity and therefore less susceptible to scald than those used in this experiment. The conditions imposed by this experiment have provided a severe trial for the prevention of scald.

Certain improvements can be made in the air-scrubbing apparatus. The apparatus was out of operation for short periods because of mechanical failure. During the last 10 days of the experiment the circulating pump ceased to operate and only the blower was functioning. Three shutdowns of approximately 4 hours each were experienced while the solution was being changed. Minor changes in mechanical design and the use of a continuous-action circulating pump of larger capacity should correct these defects.

The total cost of operating the air scrubber for 95 days was $31.00, $10.00 of which was for chemicals and $21.00 for electrical power to operate the pump and blower. This work is preliminary in nature. Further experiments using an improved air scrubber and other varieties of apples are planned.

Summary

At the end of a 95-day storage test with Grimes apples 68% of the apples were marketable when stored in a room equipped with an alkaline permanganate air-scrubbing unit, as compared with 29% in a room equipped with an activated carbon unit, and 29% in a room without air treatment. When apples were aerated after removal from storage, the percentages of marketability were 78, 22, and 14%, respectively.

Experimental equipment using an alkaline permanganate solution for the removal of apple storage volatiles is described.

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Received for review August 24, 1953. Accepted September 28, 1953. Journal Paper No. 744, Purdue Agricultural Experiment Station, Lafayette, Ind. Part of a comprehensive interdepartmental project on storage volatiles supported jointly by Purdue University Agricultural Experiment Station, Research and Marketing Act, and the Refrigeration Research Foundation.

FOOD ANALYSIS

Identification of Enzyme-Desugarized Egg Solids

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A NEW METHOD FOR REMOVING GLUCOSE FROM EGG ALBUMEN and whole egg prior to drying has been adopted by producers of egg solids in the past year (*1*, *2*, *4*). The glucose is converted to gluconic acid through the use of glucose oxidase and catalase with the concomitant addition of hydrogen peroxide as a source of oxygen. When properly prepared, their blandness and freedom from odor distinguish these products from egg solids prepared by other com-

Egg solids prepared through the use of glucose oxidase and catalase for desugarization are identified by the presence of gluconic acid in significant quantities. The glucose acid is extracted from the egg solids by aqueous methanol, in which the fat and protein remain essentially insoluble. Gluconic acid in the extract is identified by spot test and confirmed by paper chromatography. A new color reaction between gluconic acid and a buffered ferric chloride solution is presented.

mercial desugarization methods. A means of identification not subject to the vagaries of human sensory perception was needed.

The method presented is based on the detection of gluconic acid in the egg solids. It is assumed that the presence of gluconic acid, in significant quantities, is indicative of the fact that the glucose oxidase–catalase system was used in the desugarization in the course of preparation of the egg solids. Gluconic acid is not normally formed in significant quantities in eggs desugarized by any other method, including the natural and "controlled" fermentation methods practiced in the industry.

Method

Ten grams of egg solids (yolk, albumen, or whole egg) are suspended in 50 ml. of aqueous methanol and adjusted to pH 7.5 to 8.0 by the dropwise addition of 2*M* sodium hydroxide, using a glass electrode pH meter. (If the pH is above 8.0 prior to any adjustment, it need not be adjusted downward.) The suspension is then transferred to a glass-stoppered 125-ml. Erlenmeyer flask, allowed to remain with occasional shaking for 30 minutes. Then filtered through a Whatman No. 12 (or Schleicher and Schuell No. 588) fluted paper. Approximately 20 ml. of the filtrate is evaporated to dryness on a steam bath. In the case of albumen solids, the residue in the evaporating dish is redissolved in 2 ml. of distilled water; in the case of whole egg and yolk solids, the residue is redissolved in 1 ml. of distilled water.

Presumptive Test. One drop of the redissolved residue from the evaporating dish is added to 4 drops of the ferric chloride reagent in a spot plate. The persistence of an orange color after the addition of the drop of redissolved residue is a definite indication that enzyme processing was not used. If the color turns to a bright yellow, it may be presumed that the material being tested was processed by the enzyme method. A faint yellow-white is indicative of the addition of citric acid.

Confirmed Test. If a pale or bright yellow is obtained, it is desirable to confirm the presence of gluconic acid. This is best done by spotting 0.01 ml. of the redissolved residue from the evaporating dish on a strip of 1/2-inch wide Whatman No. 1 paper.

After drying, this is chromatographed by the descending technique, using aqueous methanol as the solvent. After removal from the chromatography jar, the strips are sprayed with the ferric chloride reagent and the location of yellow spots, appearing against a mauve background, is recorded. The presence of gluconic acid is confirmed by a bright yellow spot possessing an R_f of 0.35 to 0.5. Citric acid gives a blue-white spot at R_f 0.3 and is easily differentiated from the gluconic acid. Other common fermentation acids do not show up.

Reagents

Aqueous Methanol. Mix one part of distilled water with 3 parts of methanol. Merck's C.P. methanol has been found satisfactory without any further purification.

Ferric Chloride Reagent. Mix 50 ml. of 2*M* sodium propionate with 50 ml. of 0.4*M* hydrochloric acid and dissolve 1 gram of ferric chloride hexahydrate in the mixture.

An inexpensive chromatography setup can be purchased from Schaar & Co., 754 West Lexington St., Chicago, Ill., for under $35, including a 600-foot roll of Whatman No. 1 paper and an indicator spray bottle.

Table I. Effect of Addition of Water on Extraction of Gluconate from Egg Solids with Methanol

Sample Methanol-Water	Aqueous Methanol Suspension	Filtrate	Chromatogramed Spot
Enzyme albumen			
10:0	Very fluid, handled well	Colorless	None
9:1	Very fluid, handled well	Light yellow	Showed up well
5:1	Fluid, handled well	Yellow	Showed up well
3:1	Fluid, handled well	Yellow	Showed up well
2:1	Fairly viscous, lumped, handled poorly	Yellow	Not run
1:1	All clumped		
Enzyme yolk			
10:0	Very fluid, handled well	Deep yellow	None
9:1	Very fluid, handled well	Yellow	None
5:1	Very fluid, handled well	Light yellow	None
3:1	Very fluid, handled well	Pale yellow	Showed up well
2:1	Very fluid, handled well	Very pale yellow	Showed up well

Discussion

Aqueous methanol was found superior to aqueous 2-propanol in the extraction of the gluconate. Some water was required in the solvent to extract the gluconate, but too much water would cause the suspension of egg solids to gum up, making the extraction difficult. Methanol water in a 3 to 1 ratio was found to give good extraction yet filter and handle easily (see Table I).

Table II. Effect of Acidic and Basic Solutions for Spotting on R_f of Spots

Sample	R_f: H_2O	R_f: 2N HCl	R_f: 0.1M Na_2HPO_4
Sodium gluconate	0.50	0.59	0.46
Gluconic acid	0.62	0.66	0.57
Citric acid	0.65	0.80	0.73
Enzyme albumen	0.36	0.72	0.36
Enzyme yolk	0.50	0.63 (faint)	0.39
Enzyme whole egg	0.46	0.66 (faint)	0.40

Because of the difference in R_f, between gluconic acid (glucono-δ-lactone) and sodium gluconate, it was necessary to convert all the gluconate to one form or the other to avoid the possibility of confusion resulting from two spots.

Without adjusting the pH of the original egg solids suspension in aqueous methanol, water, 2*N* hydrochloric acid, and 0.1M disodium hydrogen phosphate were compared for redissolving the residue in the evaporating dish. The results are presented in Table II. It is apparent from Table II that acidification at this stage resulted in poor spots; although disodium phosphate solution gave better results than acidification, it too was unsatisfactory.

The investigation then turned to the

Table III. Effect of Acidic and Basic Solutions for Chromatographing on R_f of Spots

	R_f		
Sample	0.1M KOH	1N HCl	1.5N NH_4OH[a]
Sodium gluconate	0.45	0.70	0.38–0.55[b]
Gluconic acid	0.65	0.79	0.42–0.56[b]
Citric acid	0.73	0.79	0.24–0.31[b] to no spot
Enzyme albumen	0.34	0.65	0.35[b]
Enzyme yolk	0.33 (faint)	0.62	0.35[b]
Enzyme whole egg	0.36	0.52	0.37[b]

[a] Results varied, depending on age of ammoniacal methanol.
[b] Results obtained with solvent several days old. Lower values were obtained with freshly prepared solvent.

use of different solvents for the chromatography. This involved the use of 3 to 1 methanol-water made 1N in hydrochloric acid, 0.1N in potassium hydroxide, and 1.5N in ammonium hydroxide. Results are presented in Table III.

An examination of the preceding data indicates that solvents for chromatography containing acids or bases are not satisfactory. However, because alkaline solvents for extracting the residue in the evaporating dish and for chromatographing seemed to give the best results thus far, it indicated that a complete conversion to sodium gluconate was the best way of getting a single spot that would show up well. Therefore adjustment of the pH of the aqueous methanol suspension of the egg solids was tried, using 2N sodium hydroxide and a glass electrode pH meter to adjust to pH 7.5 to 8.0 (pH papers such as Hydrion paper were found to give false low apparent pH "readings," probably because of the high methanol content). Results of these trials are in Table IV.

Whatman No. 1 paper was chosen over Whatman No. 3 because it gave clearer, more definite spots, despite the fact that three times as much material could be spotted on the No. 3 paper.

The ferric chloride reagent was developed after it was found that 0.01 ml. of 2% solutions of various acids spotted on Whatman No. 1 paper and sprayed with 1% ferric chloride hexahydrate in 5% acetic acid gave the colors indicated in Table V.

Table V. Colors Given by Common Fermentation Acids with Ferric Chloride Reagent

Acid	Color of Spot
Gluconic	Yellow against mauve background
Citric	Pale yellow against mauve background
Pyruvic	Red-brown against mauve background
Tartaric	Same as background
Lactic	Same as background
Formic	Same as background
Fumaric	Same as background
Succinic	Same as background

Ferric chloride was tried in an attempt to take advantage of the strong chelating action of this acid. One molar solutions of acetic, lactic, propionic, boric, and tartaric acids were prepared at varying pH values by adding varying quantities of 2M sodium hydroxide to 5 ml. of 2M acids and making up to 10 ml. Then 0.5 ml. of 1% ferric chloride in distilled water was added to 10 ml. of each of the above in a Klett tube, mixed, and allowed to remain for 15 minutes before reading in the Klett at 4200 A. with the instrument set to read zero with distilled water. After the Klett reading 0.5 ml. of a 2% gluconic acid solution was added to each tube and the Klett reading was again obtained. With acetate buffer there was a clear optimum at pH 5.1 for maximum yellow color development. With propionate buffer, the pH optimum was slightly higher, at pH 5.3. The color of the tartrate and lactate buffers remained essentially unchanged after the addition of the gluconic acid. Unlike all of the preceding, which gave a definite color reading prior to the addition of gluconic acid, the boric acid solution was colorless until the addition of the gluconic acid, whereupon it developed a very deep yellow color. However, the borate was such a weak buffer that because of this and the potential hazard in introducing a boric acid mist into the laboratory air, borate was eliminated.

Acetate and propionate buffers gave the best results in the Klett testing. They were used as the base for ferric chloride solutions used to spray actual spots. In these tests the propionate buffer showed up as the better one and was therefore chosen in this work.

Concentrations of ferric chloride hexahydrate higher than 1% tended to give such dark background colors that the spots were obscured.

Various other salts were tried, using the propionate buffer base, and spraying spots on Whatman No. 1 paper (Table VI). Of the salts tried, ferric chloride is the best.

Table IV. Application of Presumptive and Confirmed Test to Various Egg Solid Samples

Sample	Spot Plate Color	R_f of Chromatogramed Spot
Sodium gluconate	Deep yellow	0.47
Gluconic acid	Deep yellow	0.50
Citric acid	Faintly yellow	0.29 (blue-white spot)
Enzyme albumen	Deep yellow	0.40
Enzyme yolk	Deep yellow	0.45
Enzyme whole egg	Deep yellow	0.40
Naturally fermented albumen	Orange (unchanged)	None
Controlled bacterial fermentation	Orange (unchanged)	None
Yeast albumen (3)	Orange (unchanged)	None
Yeast whole egg (3)	Orange (unchanged)	None

Table VI. Color Development of Gluconate Spot by Metal Salts

Salt in Propionate, 1%	Color of Gluconic Acid Spot	Background Color
$FeSO_4$	None	None
$FeCl_3.6H_2O$	Yellow	Mauve
$Fe_2(NO_3)_3$	Faint yellow	Pale pink
Ferric ammonium sulfate	Yellow	None
Nickelous chloride	None	None
Cobalt chloride	None	None
Chromium chloride	None	Pale green
Zinc chloride	None	None

Acknowledgment

The author wishes to thank Frank Hammer for his very valuable assistance in this work.

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Received for review August 20, 1953. Accepted October 23, 1953. Presented before the Division of Agricultural and Food Chemistry at the 124th Meeting of the AMERICAN CHEMICAL SOCIETY, *Chicago, Ill.*