MAJOR ORGANIC ACIDS IN FRUITS

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BACKGROUND

Organic acids are of great significance in plants. As intermediates in the metabolic processes of the fruit, these acids are directly involved in growth, maturation, and senescence. Fruit juices have a low pH, because they contain high levels of organic acids. The total acid content varies widely, from approximately 0.2% in pear juice to 0.8% in lime. Some of the major acids in fruits include citric, malic, and tartaric acids. Organic acids also influence the growth of microorganisms in fruit juices and therefore affect the keeping quality of the product. At proper levels certain acids are inhibitory to most bacteria. Another aspect of organic acids is their influence on the sensory properties of juice products. Acids are responsible for the fresh, tart taste of fresh fruits and their processed products. Color is also related to the type and level of acids present in the product.

PROBLEM

Identify the major organic acids in various fruits by paper chromatography. What are the major organic acids in grapes, apples, and strawberries? Do the organic acids differ between varieties of strawberries or grapes? Between varieties grown in the Midwest versus California? Between grapes grown in a warm versus a cool climate? If so, why?

MATERIALS AND METHODS

Chromatographic jar: a “one-gallon mayonnaise” jar with lid is suitable for this technique.

Chromatographic paper: the paper should be designated for “chromatography” and cut into 20 x 30 cm rectangles. Whatman No. 1 or Schleicher and Schuell No. 2043 chromatographic paper is suitable for this technique.

Graduated Cylinders: 100 and 25 ml graduated cylinders are used in measuring reagents for the chromatographic solvent.

Indicator Solution: this indicator solution is used in the chromatographic solvent and is prepared by dissolving 1 g of water-soluble bromocreasol green in 100 ml water.

Micropipettes: glass capillary tubes, I.D. 1.1 - 1.2 mm. are used to spot the wines on the chromatography paper.

Separatory Funnel: a 500 ml funnel is used in preparing the chromatographic solvent.

Solvent: to prepare the solvent for this chromatographic technique, transfer the following into the separatory funnel: 100 ml water, 100 ml reagent grade n-butanol, 10.7 ml reagent grade concentrated formic acid and 15 ml indicator solution. Then, thoroughly shake the solvent mixture and allow two layers to form. The lower layer is discarded (aqueous phase) and the upper phase is saved to develop the paper chromatogram. Although this solvent can be used more than once, it is advisable to transfer the solvent to the separatory funnel periodically to remove any additional aqueous phase.

PROCEDURE

1. Obtain the chromatography paper (20 x 30 cm) and draw a pencil line parallel to the length of the longest side of rectangle (30 cm) about 2.5 from the edge.

2. Draw into the micropipette by capillary action a sample of juice.

3. Touch the pipette to the paper on the pencil line and make a spot about 1 cm in diameter.

4. Repeat the step for each juice to be tested, about 2.5 cm apart.

5. When the spots are dry, staple the short edges of the rectangle to form a cylinder. The edges should not overlap.

6. After adding 70 ml of the solvent to the chromatography jar, place the paper cylinder into the jar with the spotted edge towards the bottom and secure the line.

7. When the solvent has ascended to near the top edge of the paper cylinder, remove the paper and place it in a well-ventilated area. With a pencil, outline or mark the distance the solvent traveled on the paper.

8. Leave undisturbed until the paper is thoroughly dry, yellow spots on a blue background.

9. In order to identify these spots as to specific organic acids, the Rf value of each acid should be determined.

10. Measure the distance that the solvent traveled, from the pencil line to the solvent front.

11. Then measure the distance between the center of each acid spot and the initial pencil line.

12. The Rf value is calculated by dividing the measured distance of the solvent front into that measured for the acid spot.

13. Each organic acid will have a different Rf value, such as succinic acid (0.78) lactic acid (0.75), malic acid (0.5), citric acid (0.45) and tartaric acid (0.26).

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