Investigation of Marion Blackberry, Strawberry, and Plum Fruit for the Presence of Saponins

Extracts of Marion blackberry, strawberry, and plum fruit were tested for their ability to form a stable foam, induce hemolysis of erythrocytes, and produce colors characteristic of triterpenes or sterols with Liebermann-Buchard reagent. There is strong evidence for the presence of saponins in blackberries as positive reactions were obtained for all tests. Thin-layer chromatography indicates the presence of four saponins in blackberry juice. While strawberries contain compounds that will produce a stable foam, the presence of saponins is not substantiated by other tests. There is no evidence for presence of saponins in plum fruit.

Saponins are sterol or triterpene glycosides possessing foaming properties and hemolytic activity. Their biological and pharmacological properties (Shibata, 1977), toxic properties (George, 1965), and nutritional and physiological properties (Cheeke, 1976) have been reviewed.

Of particular interest is the finding by Malinow et al. (1977) that dietary saponins reduce plasma cholesterol levels in primates; thus they may lower the risk of coronary heart disease in man (Potter et al., 1980). While saponins are widely distributed in higher plants and produced by some marine organisms (George, 1965; Shibata, 1977), their occurrence in food plants is limited. In a recent review, Oakenfull (1981) listed 28 food plants that contain saponins, only 17 of which are used in quantity in any of the world's cuisines.

In previous investigations (Wrolstad andHeatherbell, 1974; Wrolstad and Struthers, 1971) we have observed that certain fruits contain compounds with foaming, solubility, and chromatographic properties similar to those ascribed to saponins. A major objective of this investigation was to examine dietary fruits—blackberries, plums, and strawberries—to see if the number of saponin-containing food plants is more extensive. While the possible role of saponins in lowering levels of blood cholesterol is of primary interest, food processors have a practical concern in identifying the compounds responsible for the foaming activity of fruits. The fact that some saponins possess antifungal properties (Tschesche and Wulff, 1965; Schlosser, 1975) has important agricultural implications as well. In this study, the presence of saponins in fruit extracts was determined through a combination of tests—the ability to form a stable foam in an aqueous system, formation of colors characteristic of sterols or triterpenes with Liebermann-Buchard reagent, and the ability to induce hemolysis of enthyrocytes.

EXPERIMENTAL SECTION

Plant Materials. Fruits of Marion blackberries (*Rubus* ssp. *Hyb*), strawberries (*Fragaria ananassa* Duch. var. Totem), and Italian plums (*Prunus domestica*) were obtained from the OSU North Willamette Experiment Station and Lewis Brown farm. Fruits were washed, the plums were halved and pitted, and all were quick frozen at −34 °C. They were packed in polyethylene bags, placed in tins, and stored at −10 °C.

Extraction. Thawed fruit (500 g) and sufficient 95% ethanol to cover the fruit were homogenized in a Waring blender. The homogenate was combined with 700 mL of 80% ethanol and heated under reflux on a steam bath for 4 h; the material was filtered and the residue reextracted in the same manner 2 successive times. Filtrates were combined and concentrated with a rotary evaporator (water bath = 50 °C; vacuum = 23 mmHg). The concentrate (ca. 300 mL) was extracted with diethyl ether and the ether extract discarded. The defatted extract was extracted with 500-mL portions of water-saturated 1-butanol 4 successive times; the water phase was discarded and the butanol extract concentrated on a rotary evaporator. The concentrate was dissolved in 60% ethanol (ca. 300 mL). The solution was combined with approximately 50 g of insoluble polyvinylpyrrolidone (PVPP) (Polyclar AT, GAF Corp., New York, NY) that had been hydrated overnight with distilled water. The mixture was filtered through a Büchner funnel lined with Whatman No. 1 filter paper coated with a 2-cm layer of hydrated PVPP (Wrolstad and Struthers, 1971).

Filtering through PVPP was repeated until the filtrate was colorless (5 times for blackberries; 3 times for plum and strawberries). The PVPP-treated solutions were combined and concentrated to 100 mL on a rotary evaporator.

In addition to analysis of whole Marion blackberry fruit, the juice and seeds were extracted separately.

Froth Test. The methods of O'Dell et al. (1959) and Farnwsorth (1966) were modified as follows: 1 mL of the sample extract was combined with 4 mL of distilled water in a 10-mL test tube, heated for 15 min at 80 °C, and shaken by hand for 1 min, and the stability of the foam was observed. Persistence of the honeycomb froth for 10 min or longer is regarded as a positive test.

Liebermann-Buchard Test. Five milliliters of sample solution was evaporated on a watch glass and the residue treated with acetic anhydride and sulfuric acid (Liebermann-Buchard reagent) according to the method of Webb (1955). Red, pink, and purple colors are regarded as positive tests for triterpenoids whereas steroids give a blue or blue-green color.

Hemolytic Tests. Hemolytic activity tests of the extracts were carried out with a modification of the procedure by von Kartnig et al. (1964). Filter paper disks (5 mm) were wetted with the sample solution and embedded in a blood–gelatin suspension. The blood–gelatin suspension was prepared by combining 5 mL of citrated cow's blood and 5 g of gelatin in 100 mL of isotonic phosphate buffer, pH 7.4, and pouring on a glass plate (Kazerovskis, 1962). The plate was maintained at 100% relative humidity at room temperature for 16 h (Fenwick and Oakenfull, 1981). Formation of a clear zone around the paper disks was evidence for hemolysis. A 0.01% digitonin solution in 95% ethanol was used as a reference standard.

Hemolytic activity of spots separated by thin-layer chromatography (TLC) was tested by the procedure of Smoczkiewicz et al. (1977). The developed TLC plate was coated with the blood–gelatin suspension and held at room temperature for 16 h. The presence of hemolytic components on the plates were revealed as white spots against a red background.

TLC. Repeated 1-μL applications of sample extracts were made on 5 × 20 cm × 0.20 mm silica gel plates (E. Merck, Kieselgel 60). A total of 5 μL was applied for

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Table I. Results of Detection of Saponin

	froth test	Liebermann-Buchard test	hemolytic test
blackberry			
juice	+	purple	+
seeds	+	purple	−
strawberry	+	yellow-brown	−
plum	−	yellow-brown	−

detection with H_2SO_4 and the Liebermann-Buchard reagents, and 10 μL was used for hemolysis tests and the silver nitrate–NaOH reagent. The plates were developed 10 cm with $CHCl_3$–methanol–water (25:17:3) (Higuchi and Kawasaki, 1976) and $CHCl_3$–methanol–water (65:35:10, bottom layer) (Oakenfull, 1981). The following visualization reagent sprays were used: general organics, 50% H_2SO_4 and 15-min heating at 110 °C.

Liebermann-Buchard reagent for triterpenoids and steroids, 30% acetic anhydride in 52% sulfuric acid and 10-min heating at 90 °C (Oakenfull, 1981); free sugars and glycosides, silver nitrate in acetone followed by 0.5 N NaOH (Trevelyan et al., 1950); phenolics, 2% $FeCl_3$ in 80% ethanol.

RESULTS AND DISCUSSION

Table I summarizes the results of the froth, Liebermann-Buchard, and hemolytic tests for the sample extracts. While none of these tests alone can be considered an unequivocal indication for the saponins, positive responses to all three can be regarded as a reliable index for their presence. Blackberry juice extracts showed the purple color in the Liebermann-Buchard test which is characteristic of triterpenoids, formed a stable honeycomb froth, and produced a clear zone around the paper disk on the blood–gelatin plate. The amount of hemolysis was comparable to that produced by an equal volume of 0.01% digitonin solution. The blackberry seed extract gave similar results to blackberry juice except for the hemolysis test. The seed extract seemed to clot the blood–gelatin media rather than cause hemolysis. While strawberry extract formed a stable foam, hemolysis did not occur and the yellow-brown color in the Liebermann-Buchard test is a generalized color reaction of organic compounds and not characteristic of triterpenoids or steroids. All tests for plum extract were negative. The plum extract produced a small froth that was stable for only a few seconds and the other tests were negative for saponins as well.

Figure 1 presents composite thin-layer chromatograms of blackberry (juice and seed), strawberry, and plum extracts that have been tested for hemolytic activity and treated with Liebermann-Buchard reagent (triterpenoid and steroids), sulfuric acid (organic compounds), and silver nitrate–NaOH (free sugars and glycosides). In the blackberry juice extract, from eight (solvent system I) to nine (solvent system II) spots reacted positively to Liebermann-Buchard reagent. At least four of these spots possessed hemolytic activity. From Seven (solvent system I) to twelve (solvent system II) spots in the blackberry seed extract gave a positive saponin response to Liebermann-Buchard reagent. There was weak hemolytic activity for some of the spots separated in solvent system I, but no hemolysis was evident in the chromatogram from solvent system II. The conflicting results in these two plates and the blackberry seed extract (Table I) suggest that some substance must be present that interferes with the test for hemolysis. It is known that tannin and other phenolics can interfere with saponin-induced hemolysis of erythrocytes (Segelman et al., 1969; Segelman and Farnsworth, 1969). Insoluble PVPP has been used to effectively remove

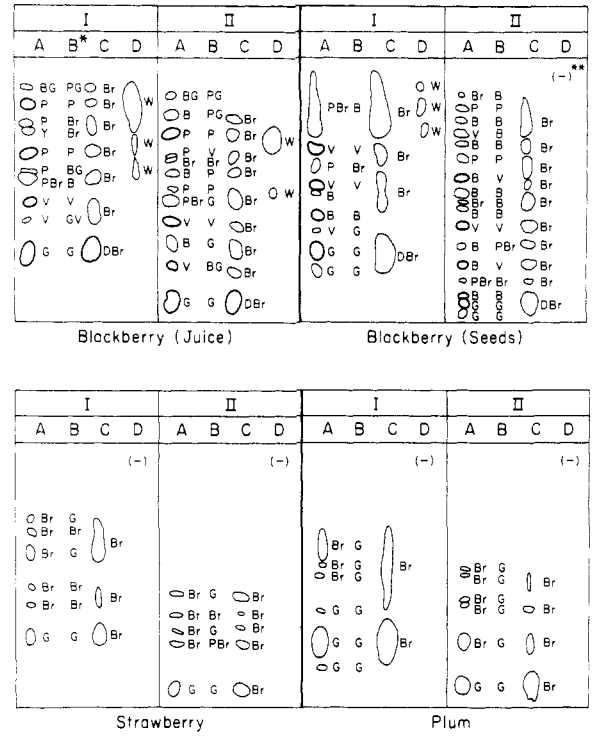

Figure 1. Composite thin-layer chromatograms of 1-butanol extracts of blackberry (juice and seeds), strawberry, and plum fruit. I = $CHCl_3$–MeOH–water (25:17:3); II = $CHCl_3$–MeOH–water (65:35:10, bottom layer); A = treated with Liebermann-Buchard reagent (90 °C, 10 min); B= treated with 50% H_2SO_4 (110 °C, 15 min); C = treated with $AgNO_3$–NaOH (room temperature); D = treated with a blood–gelatin suspension. *The chromatographic separation for B was similar to that of A; the spots are not reproduced but the colors of the spots are indicated. **(−) indicates no hemolysis occurred. Abbreviations for colors: B, blue; BG, blue-gray; Br, brown; DBr, dark brown; G, gray; P, purple; PBr, purple-brown; PG, purple-gray; V, violet; W, white; Y, yellow.

plant phenolics (Loomis and Battaile, 1966; Sanderson and Perera, 1966; Andersen and Sowers, 1968; Wrolstad and Struthers, 1971) and was incorporated into the isolation procedure because of the fruits' high phenolic content. Ferric chloride spray reagent indicated the absence of phenolics in the blackberry seed extract. None of the spots in the TLC chromatogram of the strawberry extract possessed hemolytic activity. While the silver nitrate–NaOH spray gave evidence for the presence of glycosides, Liebermann-Buchard and ferric chloride reagent sprays gave negative responses for saponins and phenolics, respectively. One can speculate that glycopeptides, glycoproteins, or sugar esters of organic acids may be responsible for the extract's foaming activity. There is evidence for presence of glycosides and free sugars in the plum extract. None of these compounds showed hemolytic activity and none gave a response characteristic of saponins with Liebermann-Buchard reagent.

In this investigation, strong evidence is presented for the presence of saponins in blackberry fruit, particularly the juice. This may have nutritional significance as it extends the somewhat limited list of foods that have been reported to contain saponins; perhaps their occurrence in other dietary fruits is more widespread. Removal of phenolics with PVPP may be a critical experimental step as phenolics can interfere with hemolytic detection.

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