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Determinations of Organic Acids in Raspberry Cultivars Grown in Maine

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Abstract

Organic acids (oxalic, malic, citric and succinic) of different raspberry cultivars ('Boyne,' 'Festival,' 'Killarney' and 'Prestige') grown in Maine were determined using High Performance Liquid Chromatography (HPLC) during a three year study. Organic acid profiles differed significantly among the raspberries depending upon the cultivars. Weather conditions, i.e. temperature, percent of sunshine, and rainfall during growing season had also a significant effect on the organic acid.

Introduction

The demand for raspberry fruit is increasing due to its appearance in new consumer products such as yogurt and juice blends. Raspberry puree is used in the production of beverages, dairy products, jams and jellies and many specialty products. One class of raspberry components, organic acids, play a substantial part in maintaining raspberry quality. Sugars and organic acids are directly related to flavor and acceptability of fruit (5). Dietitians, nutritionists and other food related personnel need more explicit information on the individual sugars and acids in fruits and the effects of these constituents on flavor. This information might be important in interpreting and understanding fruit quality as it relates to consumer acceptance. They play an important role in maintaining the color of berries during frozen storage (11). According to Coppola and Starr (3) accurate knowledge of organic acid level (and ratio) might be useful to determine the percent juice content of juice products, and also to detect misbranding and/or adulteration. Most research conducted on compositional changes in fresh and frozen raspberries has focused on soluble solids, total sugars, reducing sugars, titratable acidity, color and pH as reliable indices for quality (2). Little research has been performed on organic acids of raspberries, since accurate quantitation of individual organic acids in fruit products has been difficult (3).

The present study was undertaken to determine the organic acids in different cultivars of raspberries grown in Maine three sampling years.

Materials and Methods

Fresh fruit of several cultivars were obtained from Whistling Wings Farm (Bidderford, Maine). Mature berries (10 pints/cultivars) were hand-picked early in the morning and refrigerated (4-5°C) immediately after picking. Berries were held under refrigeration (4-5°C) for 4 hrs. prior to transporting them to the Department of Food Science, University of Maine in coolers containing crushed ice. Extracts for organic acid analysis were made on the day of harvest. When necessary, extracts were stored overnight at 0°F before analysis. In years one and three organic acids from four cultivars ('Boyne,' 'Festival,' 'Killarney' and 'Prestige') were determined. In year two due to environmental conditions (heavy wind) only two cultivars ('Boyne' and 'Prestige') were analyzed.

Sample Preparation:

Organic acids were extracted by homogenizing 25g fruit samples in a blender (Waring Commercial Blender,

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Dynamics Crop. of America, New Hartford, CT) with 50 ml of extracting solvent (60% ethanol/40% HPLC grade water/0.2% concentrated sulfuric acid) for 5 min. The mixture was vacuum filtered using a Buchner funnel, and three 2 ml aliquots were removed via 0.45 micron Acrodiscs (VWR Scientific Co., Boston, MA) and collected in scintillation vials (1).

Organic Acid Cleanup Procedure:

Two ml aliquots obtained in the extraction procedure were evaporated to dryness under nitrogen. After redissolving the sample in 3 ml of distilled water, the pH was adjusted to 6.0 to 7.0 with 5% ammonium hydroxide. Disposable Bio-Rad Poly Prep Chromatography Columns (Bio-Rad Laboratories, Richmond, CA) were filled to 2 ml with Bio-Rad #5 anion exchange resin (chloride form, 100-200 mesh). The sample was applied to the top of the resin and washed with 3 ml of distilled water. Organic acids were eluted and collected after the application of 3.5 ml of 10% sulfuric acid and 3.5 ml of distilled water. The eluent was then passed through an activated Sep-Pak C18 cartridge (Water Chromatography Division, Milford, MA) and collected in clean scintillation vials for HPLC analysis. The Sep-Paks were activated by passing 5 ml of ethanol followed by 5 ml of distilled water through the cartridge via syringe (8).

HPLC Conditions for Organic Acid Analysis:

The HPLC system was comprised of a Waters 450 variable wavelength detector (Waters Associates, Milford, MA) set at 210 nm and a Hewlett Packard Series 1050 gradient pump (Hewlett Packard Co., Avondale, PA) with a Valco 5 μl loop injector (Valco Instruments Co., Inc., Houston, TX). A Phenomenex Rezex organic acid (300 x 7.8 mm, 0 μm particle size) and guard column (59 x 7.8 mm, 5.0 μm particle size) (Phenomenex, St. Torrance, CA) were used in conjunction with a Hewlett Packard 3396A Integrator. The flow rate was set at 0.7 ml/min., the chart speed was 0.5 cm/min. and the mobile phase consisted of 0.018 N sulfuric acid in HPLC grade water. The standards were prepared using the same cleanup procedure as the sample. Quantification of the acids employed the procedure of Bushway et al. (1).

Results and Discussion

In this research major organic acid was citric approximately 81-84% of the total of the raspberry cultivars, which was lower than the percentage of citric acid reported by Spanos and Wrolstad (8). Malic and succinic acids were higher in percentage than that has been reported in the literature (7, 8). Results from three year study are shown in Figs. 1, 2, 3, and 4. Plowman (6) reported that citric acid was the major acid found in raspberry juice at a concentration of 1.7 to 2.7% in the juice, while malic acid content was low (0.05 to 0.1%), and succinic acid was also found in small amounts in all cultivars of raspberries. According to Ryan and Dupont (7) raspberries contained 55 mg/100ml of succinic acid and 2480mg/100ml of citric acid. Nonvolatile organic acids have been identified in some raspberries. 'Malling Promise' was found to contain 29.5 and 1.2 mequiv./100g fresh weight citric and malic acids, respectively (10). Spanos and Wrolstad (8) reported that citric acid as a percent of total acid was 92.7 to 97.0% with an average of 95.5%, and malic acid was 2.3% to 6.5% with an average 3.8% in raspberry juice.

Same cultivar was higher ($p \leq 0.05$) in one season and was lower ($p \leq 0.05$) in the other growing season for organic acids. In 'Festival' and 'Killarney' oxalic acid was higher ($p \leq 0.05$) in year three than the year one. While in 'Prestige' oxalic acid was higher ($p \leq 0.05$) in year two than the year one and three. Citric acid in 'Boyne' was higher ($p \leq 0.05$) in year two when compared

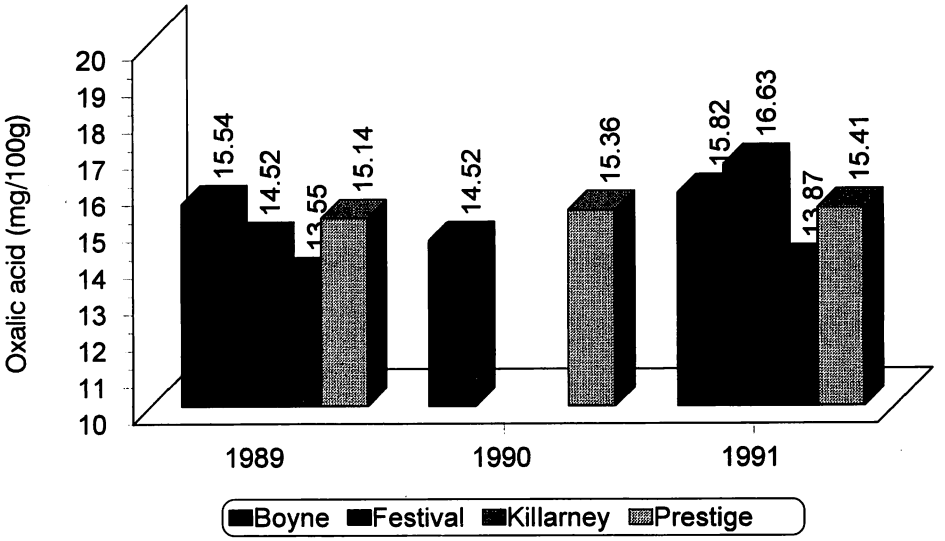

Fig. 1 Oxalic acid (mg/100g) of raspberry cultivars in different years

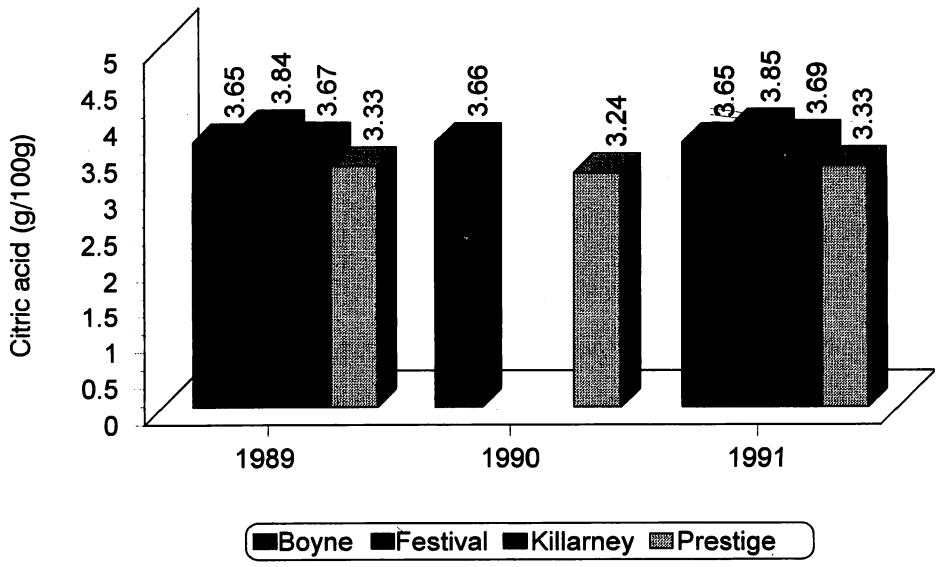

Fig.2 Citric acid (g/100g) of raspberry cultivars in different years

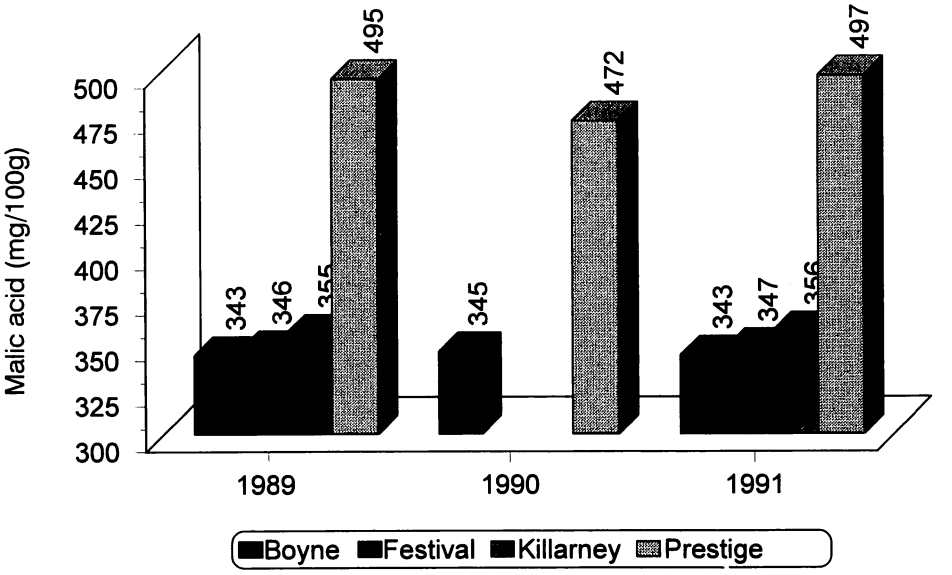

Fig. 3 Malic acid (mg/100g) of raspberry cultivars in different years

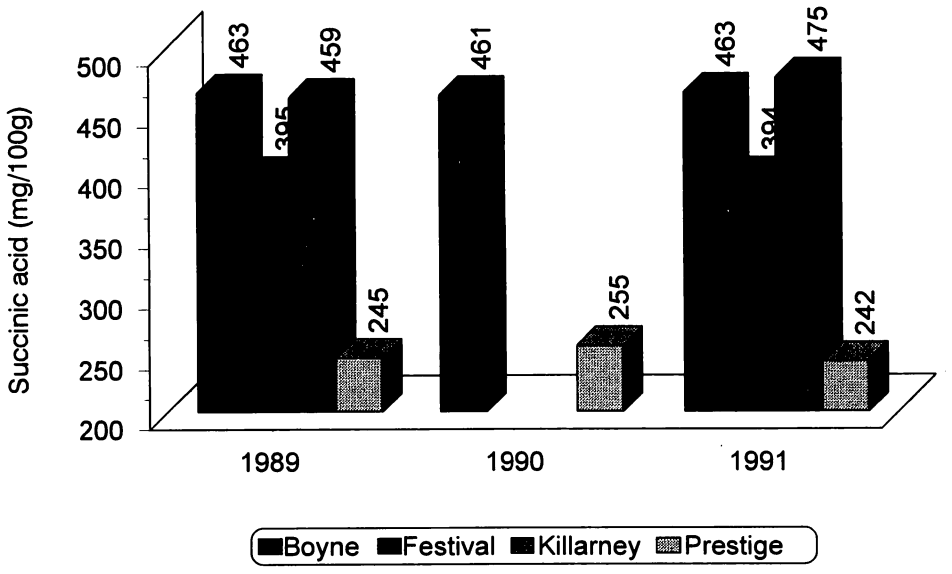

Fig.4 Succinic acid (mg/100g) of raspberry cultivars in different years

Table 1. Rainfall, temperature and sunshine during growing season of raspberries.

Month	Rainfall			Temperature			Sunshine		
	First year	Second year	Third year	First year	Second year	Third year	First year	Second year	Third year
	← in. →			← F° →			← % →		
January	1.15	3.19	2.91	26.8	30.2	23.4	55	51	65
February	2.14	2.49	1.85	31.6	25.7	29.8	58	64	53
March	2.14	1.42	6.19	31.6	34.7	37.0	58	61	40
April	2.94	5.16	6.71	41.1	44.6	45.9	59	40	51
May	8.74	5.23	3.77	55.6	51.8	58.1	44	42	59
June	4.49	4.12	1.47	63.9	62.4	65.5	50	49	54
July	2.50	3.21	2.35	69.4	70.3	70.1	64	60	61
August	1.73	1.89	15.22	58.5	69.8	69.9	62	63	58
September	4.48	3.12	5.44	60.5	59.8	58.2	65	55	69
October	4.81	7.46	2.83	49.8	52.4	50.8	59	51	52
November	3.97	7.50	4.34	37.3	41.8	40.4	50	53	36
December	2.23	7.90	4.06	14.1	33.7	26.4	73	44	51

Data supplied by National Weather Service Forecast Office, Portland International Jetport, Portland ME.

with year three. In 'Killarney' citric acid was higher ($p \leq 0.05$) in year one than the year three. 'Prestige' was significantly higher ($p \leq 0.05$) in citric acid for year three as compared to year two. Malic acid was higher ($p \leq 0.05$) in 'Prestige' for year three than the year two. Killarney' was higher ($p \leq 0.05$) in succinic acid for year three than the year one. Similar findings were reported by Sweeney et al. (9) who observed different concentration of the malic and citric acid in the same cultivar 'Chief' in different growing seasons. Lenartowicz et al. (4), reported differences between the maximal and minimal content of acids in raspberries could vary from 20 to 30% of the mean value, and considerable year to year fluctuations exist in the content of acids in the raspberries. In addition to the varietal influence on the composition of the fruit, ripening conditions may also effect the concentration of the organic acid. The possible reason for the different values could be the weather conditions during the growing season in Maine, i.e., rainfall, temperature and percentage of sunshine during the growing season. Values for the monthly rainfall, sunshine percentage and temperature during the growing season are shown in Table 1. These factors could cause an increase in sugar and a decrease in acid content (4). Experiment with ripening over a range of temperature have shown that citric acid loss is reduced at higher temperature (5).

Most of the cultivars were significantly different ($p \leq 0.05$) from each other for organic acids. In first year of the study, 'Festival' and 'Killarney' were different ($p \leq 0.05$) from each other and from the other two cultivars for the oxalic acid. 'Festival' and 'Prestige' were different ($p \leq 0.05$) from each other and from the other cultivars for citric and succinic acid. 'Killarney' and 'Prestige' were also different ($p \leq 0.05$) from each other and from the other cultivars for malic acid. In year two, oxalic, citric malic and succinic acid were different ($p \leq 0.05$) in both cultivars. 'Festival,' 'Killarney' and 'Prestige' were different ($p \leq 0.05$) from each other for oxalic acid in year three. For citric, malic and succinic acid all cultivars were different ($p \leq 0.05$) from each other.

Total organic acids were highest in 'Festival' among all the cultivars. 'Prestige' was significantly lower ($p \leq 0.05$) in succinic acid and for the total organic

acid contents when compared with the other cultivars. From this research it might be concluded that the 'Festival' cultivar is better in quality as compared to the others. 'Boyne' and 'Killarney' were in between the 'Festival' and 'Prestige.' These ranges of organic acid in different cultivars of raspberry may be helpful to detect misbranding and/or adulterations in raspberry juices and other products. Knowledge of organic acid for a particular cultivar will help fruit processor to save the surplus crop in frozen storage for next year use, keeping in mind the stability of the color.

This research also demonstrates that organic acid composition of the raspberries are more meaningful when cultivars and environmental conditions are known.

Acknowledgement

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Book Review

The Book of Apples.
By Joan Morgan and Alison Richards, with apple watercolors by Elisabeth Dowle.

The apple is the most widely grown and used fruit in the world, taking an important role in the history of mankind for over 8,000 years. The authors present, in an interesting and readable style, a very complete history of the apple. This attractive book of 304 pages contains 32 beautiful, original watercolor plates of apple varieties — the blossom cluster, the fruit, fruits growing on the tree, and fruits at maturity. The text also is supported by numerous black and white pictures and drawings.

This is a "must" book for all lovers and students of the apple, and makes fascinating reading. It is a very complete and handy reference to the history and development of the apple, and the origin and description of ancient

Reviewed by Dr. Loren D. Tukey, Professor Emeritus of Pomology, Department of Horticulture, The Pennsylvania State University.