(p-Hydroxyphenyl)butan-2-one Levels in Raspberries Determined by Chromatographic and Organoleptic Methods

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Different raspberry cultivars were analyzed by HPLC for (p-hydroxyphenyl)butan-2-one, the characteristic raspberry aroma component. Anthocyanin concentration, although 3 orders of magnitude higher, paralleled the (p-hydroxyphenyl)butan-2-one content of raspberry fruits of the same developmental stage with one exception. Organoleptic evaluation of the raspberry fruit was in agreement with increasing raspberry flavor and increasing (p-hydroxyphenyl)butan-2-one content of the fruits.

INTRODUCTION

The aroma of raspberries is a complex mixture of volatile compounds, of which over 230 have been identified to date (Maarse, 1989). One of the compounds having a characteristic raspberry aroma has been identified as the raspberry ketone (p-hydroxyphenyl)butan-2-one (pHPB) (Schinz and Seidel, 1957; Winter, 1961). Although modern analytical methods permitted the analysis of pHPB in fruit from different geographic origins (Braun and Hieke, 1977), no attempt has been made to date to compare the pHPB content of raspberry fruits with organoleptic evaluation methods.

In this paper we have determined the (p-hydroxyphenyl)butan-2-one content of six different raspberry cultivars and subjected them to organoleptic evaluation. We report on the similarity of results obtained between the chemical and organoleptic evaluations.

EXPERIMENTAL PROCEDURES

Solvents. All solvents used in the determination of pHPB levels were of HPLC grade from Aldrich Chemical Co. pHPB standard was obtained from Pfalz and Bauer, Inc., Waterbury, CT. Anthocyanin standards were from our laboratory collection.

Fruits. Samples of five raspberry (*Rubus idaus*) cultivars were provided by Oregon State University (N. Willamette Agricultural Experiment Station, Aurore, OR). The fruits were picked at peak maturity, frozen at −40 °C, and kept at this temperature until used. Fruits of the Royalty cultivar were obtained from Cornell University's New York State Agricultural Experimental Station, Geneva, NY, frozen, and kept at −100 °C. These cultivars were chosen to represent variations in flavor impact, although not necessarily flavor intensity.

Determination of (p-Hydroxyphenyl)butan-2-one. Raspberry fruits (100 g) were thawed and homogenized with 200 mL of distilled H_2O in the presence of 15 g of Hyflow Super Cel. The homogenate was filtered through a fine mesh nylon cloth. The filtrate was centrifuged for 15 min at 25000*g*. The supernatant was decanted and extracted with ethyl acetate (4 × 50 mL). The extract (200 mL) was filtered through a Whatman No. 1 PS filter and the filtrate evaporated to dryness. The residue was dissolved in 2 mL of 30% acetonitrile in 30 mM KH_2PO_4, pH 6.2, and loaded onto a 0.5 × 3 cm C_{18} column (Alltech Associates, Inc., Deerfield, IL). The column was eluted with 5 mL of ethyl acetate and the eluate evaporated to dryness. The residue was dissolved in 1 mL of 30% acetonitrile in 30 mM KH_2PO_4, pH 6.2. Twenty microliters of this sample was used for pHPB determination by HPLC with a Tracor 980 chromatograph using a 5 μBondapack C_{18} analytical column (Alltech C-18, 5 μm, 250 × 4.6 mm) and 20% acetonitrile in 30 mM KH_2PO_4, pH 6.2, as solvent. The flow rate was 1.0 mL/min. pHPB was detected at 280 nm and quantitated using a standard calibration curve. The retention time of pHPB was 7.85 min (±0.05 min) under the chromatographic conditions given above. The accuracy of the method was determined using 25, 50, 100, and 200 μg of authentic pHPB in 200 mL of distilled water in triplicate experiments. The standard deviation was found to be at or below the 10% level at each concentration range used.

Anthocyanin content, total soluble solids, and pH were measured as reported earlier (Hrazdina et al., 1984).

Organoleptic Evaluation. Approximately 50.0 g of slightly thawed (−4 °C) whole raspberry fruits was blended into a homogenate. This material was then used to create a 15% (w/w) dilution in distilled H_2O containing 6% (w/w) sucrose. This level of sweetness has previously been shown to be appropriate for the most objective flavor comparison. The diluted material was strained through cheesecloth to remove seeds. These final preparations were evaluated at room temperature (24 °C). An experienced panel of 11 judges evaluated the seven experimental samples. An eight-raspberry dilution, prepared from raspberries obtained at a local store, was used as a standard or reference. Each judge was told that this sample represented 50 flavor and 50 aroma on a scale of 100. The judges tasted this sample first and had access to the reference at all times. The coded samples were rated 0–100 for the intensity of raspberry flavor and aroma, and judges were allowed to make comments about specific samples or check a series of predetermined descriptions (e.g., seedy, fruity, ripe). The samples were tested individually in random sequence. The tasting room was illuminated with red lights to obscure most visual differences among the samples.

RESULTS AND DISCUSSION

(p-Hydroxyphenyl)butan-2-one Content of Raspberry Varieties. All raspberry cultivars investigated were in the fully mature stage; therefore, the data obtained are comparable. Although the fruits were in similar ripeness stages, the pH of the fruits was not uniform. ORUS 576-47 and Willamette fruits showed higher acidity than did the other cultivars. There was no detectable correlation between pH and total soluble solids of the fruits. With one exception, there seemed to be a good agreement between anthocyanin content and pHPB content of the fruits (Table I), i.e., fruits having higher anthocyanin content contained higher amounts of pHPB. The exception was the berries from the Royalty cultivar. The discrepancy shown by Royalty may be a sampling error, since in subsequent biological experiments anthocyanine and pHPB content seemed to increase in a parallel manner (data not shown). Highest anthocyanin content was found

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Table I. (*p*-Hydroxyphenyl)butan-2-one Content of the Various Raspberry Varieties

raspberry cultivar	pH	total soluble solids,[a] °Brix	anthocyanin, mg/100 g	pHPB content, μg/100 g
Canby	3.14	10.4	54.1	0.9
Meeker	2.95	10.0	74.8	5.8
ORUS 576-47	2.78	10.0	64.4	1.3
ORUS 2078	3.07	9.1	57.8	1.6
Royalty	3.15	10.1	217.7	2.2
Willamette	2.85	11.8	103.4	17.4

[a] All measurements were carried out at 24 °C.

Table II. Aroma and Flavor Scores[a] of Various Raspberry Cultivars

raspberry cultivar	aroma score	flavor score	character of flavor
Canby	41.1	49.7	fruity
Meeker	46.3	50.4	ripe, seedy
ORUS 576-47	46.5	49.8	underripe
ORUS 2078	48.1	51.4	
Royalty	38.1	50.9	
Willamette	46.0	56.2	ripe, fruity

[a] Aroma and flavor scores are averages of 11 judges' responses; character of flavor represents terms used by at least half of the judges making comments on flavor character for a particular sample.

in Royalty berries, while the highest pHPB content was observed in Willamette.

The organoleptic evaluation of the raspberry cultivars showed a general agreement with the pHPB content of the fruits (Table II). The cultivar Willamette, having the highest pHPB content of the six raspberry cultivars investigated, consistently obtained the highest flavor score.

Although the pHPB content of the different raspberry cultivars is in a similar concentration range as those of the ripening Royalty berries (data not shown), it differs by an order of magnitude from those reported by another investigator (Gallois, 1982). In that investigation, the pHPB content of the raspberry cultivars varied between 20 and 370 μg/100 g of berries fresh weight; our data show pHPB content to be between 0.9 and 17.4 μg/100 g of berries fresh weight. Therefore, our data are more in support of those by Maquin et al. (1981), reporting pHPB content of the various raspberry cultivars in the 10–70 μg/100 g of fresh weight range.

Our investigation was aimed strictly at determining the levels of free pHPB in the diverse raspberry cultivars. It was not within the scope of this investigation to determine the levels of pHPB present in the glycosidically bound form (Pabst et al., 1990). It is conceivable that various raspberry cultivars may contain different amounts of pHPB glucoside. We suggest that since conditions for the isolation of pHPB were identical with all samples, similar amounts of pHPB would have been formed by enzymatic hydrolysis from all cultivars, giving similar standard errors.

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