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Effect of Amino Acid Composition on the Taste of 'Hakuho' Peaches (*Prunus persica* Batsch) Grown under Different Fertilizer Levels

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Summary

The effect of amino acids on the taste of the peach fruit was investigated by sensory evaluation tests. Fruits were harvested from 'Hakuho' trees that were grown under three levels of complete liquid fertilizers, containing nitrogen at 40 ppm (L), 80 ppm (M), and 160 ppm (H). Major amino acids analyzed were asparagine (ASN), serine (SER), threonine (THR), arginine (ARG), and aspartic acid (ASP), which accumulated at significantly higher concentrations with increased fertilizer levels. Sensory evaluations showed that juice from the H treatment fruit was more sour and had a bitter taste, whereas that from the L treatment was equally sweet as the juice from the M treatment; the overall evaluation gave the highest mark to the M treatment. After removing amino acids from juice using ion exchange resins, the major amino acids were added again individually or cumulatively to the original concentrations of each treatment juice. SER, ARG, and ASN increased sweetness, whereas ASP increased sourness of each case. Such amino acids improved "umami" (deliciousness or deepness of the taste) and overall taste at the concentrations of those in the M treatment juice but they increased bitterness and, thereby, lowered the overall evaluation of the H treatment juice. These results indicate that amino acids improve the taste of peach fruit, but excessively high levels of ASN and ARG lower the sensory quality.

Key Words: amino acids, peach, sensory evaluation, taste.

Introduction

Our previous work (Jia et al., 1999) showed that excessive fertilizer application for peach trees not only reduced the accumulation of fruit sugar, red pigment, and aromatic components, but greatly increased amino acid concentration, which resulted in poorer fruit taste.

Taste is the sum of the sensory attributes arising from the stimulation of the gustatory receptors on the tongue. It has been suggested that the soluble solids/titratable acid ratio (Cooper, 1955; Proebsting et al., 1957), total sugar, or total acid content (Beutel, 1988; Carter et al., 1958; Schneider et al., 1958; Stembridge et al., 1962; Robertson and Meredith, 1988) are important for peach flavor. It is common knowledge that amino acids have a significant effect on food taste. However, the importance of amino acids in peach flavor has not been firmly established.

The purpose of this research is to investigate the effect of amino acid composition on the flavor of 'Hakuho' peaches grown under different fertilizer levels by various chemical analyses and sensory evaluations.

Materials and Methods

Detailed descriptions of experimental variables and sampling procedures have been published previously (Jia et al., 1999). In 1998, we utilized three-year-old 'Hakuho' (*P. persica*) peach trees, planted in 75 liter buried containers and trained to a central leader system in the Okayama University orchard. Complete liquid fertilizer (OHTSUKA HOUSE) were applied at 160ppm-N (H), 80ppm-N (M), and 40 ppm-N (L) twice a week from bud break to the end of Stage II (stone hardening stage); the levels were then lowered to half in each plot. Fruits were harvested at the shipping mature stage (Aoyagi, et al., 1976) on July 9 in the L treatment, July 13 in the M treatment, and July 15 in the H treatment. Fruits were weighed and stored at room temperature for 3 days. Fruit were separated into two groups for sensory and chemical analyses.

Juice analysis

Total soluble solids (TSS), titratable acidity, and concentrations of amino acids were analyzed according to Jia, et al. (1999).

Sensory test of juice

Juice, squeezed from the fruits in each treatment, was evaluated for aroma, juice color, sweetness, sourness, bitterness, and overall taste by sensory test as follows. The 20-ml sample juice was evaluated by a tasting panel consisting of 10 laboratory stuffs, four men and

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six women. The evaluation was conducted at room temperature (25 ± 2 ℃) under a fluorescent light. Panelists were asked to take a sip of water between each sample tasting and were also instructed not to eat, drink, chew gum, or use toothpaste during 30 minutes before each session. The significance of data was determined by the use of rank total tables (Kramer, 1956).

Sensory test of individual amino acid in water solution

Four systems of water solution were compared using a two-sample difference test. Three systems contained amino acids with the same concentrations as those in the L, M, and H treatment fruits, and the fourth had 20 mM of each amino acid. Distilled water was used as a blank. The significance of data was determined by the two-sample difference test (Haga, 1973). The panel was given the reference standards for sweetness (as 5% and 10% sucrose), sourness (as 0.5% and 1.0% citric acid), bitterness (as 0.5% and 1.0% quinine HCI), and "umami" (as 20 mM glutamic acid).

Preparation of base juice

The experimental base juice was prepared from fruit in L, M, and H treatments. After removing amino acids by using ion-exchange resins, Amberlite CG 120, the pH value of the sample juice was adjusted by micro-titration with 10% NaOH to 4.32, 4.42, and 4.17, which are the pH of the original juice of L, M, and H treatments, respectively.

Sensory evaluation for the contribution of amino acids to juice taste

The seven amino acids, supposed to contribute to 'Hakuho' peach taste, and other minor amino acids were added either individually or cumulatively to the base juice equivalent to those of L, M, and H treatments. Twelve trained panelists rated the modified juice samples for sweetness, sourness, bitterness, "umami", and overall taste. The order of presentation of the samples was randomized. The scores were numerically converted as follows: +2 (increased the taste strongly), +1 (increased the taste weakly), 0 (taste unchanged), -1 (reduced the taste weakly), -2 (reduced the taste strongly). The Duncan's multiple range test was used to analyze data. Environmental conditions in the evaluation were similar to those in sensory test of juice.

Results

Fruit quality

Different fertilizer levels led to fruit of equal weight among treatments but the harvest date was delayed with increased fertilizer N (Table 1). Juice TSS was higher in the M and L treatments than in the H treatment, whereas titratable acidity was highest in the H treatment. Eighteen amino acids, aspartic acid (ASP), glutamic acid (GLU), serine (SER), asparagine (ASN), glutamine (GLN), glycine (GLY), histidine (HIS), threonine (THR), arginine (ARG), alanine (ALA), γ-aminobutyric acid (GABA), proline (PRO), tyrosine (TYR), valine (VAL), isoleucine (ILEU), leucine (LEU), lysine

Table 1. Effect of fertilizer levels on fresh weight and qualities of mature 'Hakuho' peach fruits[z].

Treatment[y]	Harvest date	Weight (g/fruit)	TSS (° Brix)	Titratable acidity[x] (mg/100ml)
L	Jul. 9	169.0 ± 14.1	14.8 ± 1.3	0.56 ± 0.03
M	Jul. 13	190.0 ± 11.5	14.9 ± 0.9	0.48 ± 0.01
H	Jul. 15	187.8 ± 31.4	12.0 ± 1.6	0.82 ± 0.01

[z] Fruit weight (mean ± SD) measured at harvest day. TSS and acidity measured after 3 days of storage at room temperature.
[y] Applied with 3 levels of complete liquid fertilizer containing 40 ppm (L), 80 ppm (M), and 160 ppm (H) of N. The levels were lowered to half of each at the end of stone hardening stage.
[x] Represented as malic acid.

Table 2. Effect of fertilizer levels on amino acid[z] concentration ($\mu mol \cdot ml^{-1}$) in 'Hakuho' peach fruits at harvest.

Treatment[y]	ASN	SER	THR	ARG	ASP	ALA	GLY	PRO	GABA	GLU	Others	Total
L	1.44c[x]	0.83c	0.70b	1.10c	1.06c	0.30b	0.18b	0.17b	0.08b	0.07b	0.10c	6.01c
M	9.06b	3.13b	0.80b	2.87b	2.02b	0.37b	0.28ab	0.31a	0.11ab	0.08b	0.22b	19.27b
H	18.73a	6.93a	3.73a	3.49a	2.71a	0.52a	0.47a	0.26a	0.15a	0.11a	0.59a	37.70a

[z] ASN: asparagine, SER: serine, THR: threonine, ARG: arginine, ASP: aspartic acid, ALA: alanine, GLY: glycine, PRO: proline, GABA: γ-aminobutyric acid, GLU: glutamic acid, Others: valine (VAL)+isoleucine (ILEU)+phenylalanine (PHE)+histidine (HIS)+leucine (LEU)+tyrosine (TYR)+lysine (LYS)+glutamine (GLN).
[y] See Table 1.
[x] Values with the same letter within a column significantly different by Duncan's multiple range test (p<0.05).

(LYS), and phenylalanine (PHE), were detected. The major amino acids, ASN, SER, THR, ARG, and ASP (Table 2) accounted for about 90% of total amino acids and increased significantly with increased fertilizer levels. ASN, SER, THR, and ARG in the H treatment fruits were several times greater than those in the L treatments.

Juice sensory quality

Sensory evaluations of the 'Hakuho' peach juice rated the H treatment juice to be most sour, bitter, and of lightest color (Table 3). The L treatment juice was sweetest but had a dark color. Although no significant difference in juice aroma was found among treatments, the overall evaluation ranked the M treatment fruit above those of the L and H treatment juices.

Taste characteristics of amino acids in water solution

To evaluate the influence of amino acids on fruit taste, each amino acid in a water solution was tested as a preliminary test. Twenty mM of each amino acid produced a distinct taste (Table 4). ASN and ARG were described as very bitter and slightly sweet, whereas ASP and GABA tasted very sour, but ASP also had a slightly bitter taste. GLU and GLN tasted "umami". SER, ALA, and PRO tasted sweet, whereas VAL, HIS, THR, LEU, LYS, and PHE tasted bitter (Table 4). At the concentration of the L treatment juice, ARG and ASP were sweet and sour, respectively; other compounds were judged tasteless. Seven amino acids, ASN, ARG, ASP, SER, THR, ALA, and GABA, at the concentrations of the M and H treatment juices, had the taste similar to those at 20 mM. Amino acids at the concentration of the H treatment were rated as bitter.

Effect of the addition with each amino acid on juice taste

The addition with each individual amino acid changed the taste of base juices from each treatment (Table 5). Base juices of L and M treatments were rated as sour but

Table 3. Subjectively determined rank sum test for color, taste, and aroma of 'Hakuho' peach juice grown under different fertilizer levels[z].

Treatment[y]	Juice color	Sweetness	Sourness	Bitternsee	Aroma	Overall taste
L	30a[x]	24a	21b	18b	22a	21b
M	16b	22a	14b	16b	22a	25a
H	14c	14b	25a	26a	16a	14c

[z] Higher values indicate darker color or stronger taste for each given character.
[y] See Table 1.
[x] Rank sums with the same letter within a column are not significantly different (p<0.05).

Table 4. Taste of each amino acid in water solution at different concentrations[z].

Amino acid	Sweetness				Sourness				Bitterness				"Umami"[y]			
	L	M	H	S	L	M	H	S	L	M	H	S	L	M	H	S
ASN		*[x]	*	*							**	**				
ARG	*	*	*	**							*	**				
ASP					*	*	*	**			*	*				
SER		*	*	**												
THR											*	**				
ALA		*	*	**								*				
GABA						*	*	**								
GLU				*												**
GLN				*												*
PRO				*												
VAL				*								**				
HIS								*				*				
ILEU												*				
LEU												*				
PHE												**				
LYS				*								**				

[z] L, M, and H: at the concentrations in each treatment (see Table 1) juice, S: at 20 mM.
[y] Deliciousness or deepness represented by the taste of glutamic acid.
[x] ** and *: strength the taste significantly at 5% and 1% level by t–test, respectively, blank: taste similar to distilled water.

Table 5. Effect of the addition with individal amino acids on base juice[z] taste of 'Hakuho' peaches grown under three levels of fertilizer.

Amino acids added	Sweetness			Sourness			Bitterness			"Umami"[y]			Overall taste		
	L[x]	M	H	L	M	H	L	M	H	L	M	H	L	M	H
ASP				+	+	+							↑	↑	↓
THR									++						↓
SER		+	+			-							↑	↑	↑
ASN	+	+	+	-	-	-			+		+		↑	↑	↓
GABA					+									↑	
ARG	+	+	+	-	-	-		+	++		+			↑	↓
ALA			+												↑
Others[w]													↑	↑	↑

[z] Base juice was obtained by removing amino acids from the original juice. Each mark shows that the taste of each base juice changed significantly at 5% level by Duncan's new multiple range test. −: taste weakly reduced, +: taste weakly increased, ++: taste strongly increased, blank: no change, ↑ : improved taste, ↓ : deteriorated taste.

[y] See Table 4.

[x] Concentrations of amino acids are equal to the original juice in L, M, and H treatment, respectively.

[w] Others: the sum of LYS, TYR, LEU, PHE, HIS, ILEU, VAL, GLN, GLU, and GLY.

the overall taste when ASP was added. The addition of THR increased bitterness of the H treatment base juice and lowered the overall rating, whereas the addition of ASN and ARG increased sweetness and reduced sourness of each base juice from the three treatments. These two amino acids increased the bitterness of the H treatment juice and decreased the overall taste rating but increased "umami" of the M treatment juice and improved the flavor. The addition of SER and ALA at the concentrations of the H treatment increased the sweetness and improved the overall taste of the juice.

Effects of the cumulative addition of amino acids on juice taste

The addition to the base juice with the combination of the following minor amino acids, LYS, TYR, LEU, PHE, HIS, ILEU, VAL, GLU, GLN, and GLY increased sweetness, decreased sourness, and improved the overall taste (Table 6). The cumulative addition with GABA, ALA, and THR in that order did not change the taste of each treatment juice. However, the addition of SER, ARG, and ASN increased juice sweetness, whereas ASP increased sourness. Those four amino acids increased "umami" at the concentration of the M treatment juice and improved overall taste at the concentration of the L and M treatment juices. However, they increased bitterness and lowered the overall taste at the concentration of the H treatment juice.

Discussion

More than 17 amino acid compounds have been identified in peach fruit (Miura and Araki, 1988; Moing et al., 1998). ASN is the major amino acid in peach flesh as in other *Rosaceae* fruit e.g. apricot (Nigam and Sharma, 1988) and apple (Blanco Gomis et al., 1990). ASN, ARG, ASP, and SER are the major amino acids in 'Hakuho' peach fruits and their concentrations significantly increased with higher levels of fertilizer

Table 6. Effect of the cumulative addition with amino acids on juice taste of 'Hakuho' peaches grown under three levels of fertilizer[z].

Amino acid sadded	Sweetness			Sourness			Bitterness			"Umami"[v]			Overall taste		
	L[y]	M	H	L	M	H	L	M	H	L	M	H	L	M	H
1. Base juice[x]															
2. 1+Others[w]	+	+	+	-	-	-							↑	↑	↑
3. 2+GABA															
4. 3+ALA															
5. 4+THR															
6. 5+SER	+	+	+						+		+		↑	↑	↑
7. 6+ASP				+	+	+			+		+		↑	↑	↓
8. 7+ARG	+	+	+						+		+		↑	↑	↓
9. 8+ASN	+	+	+						+		+		↑	↑	↓

[z,y,x,w] See Table 5.

[v] See Table 4.

application. These results agree with the work of Cummings and Reeves (1971).

As expected higher levels of N application increased amino acid concentrations because N an essential element of amino acids. The variations in leaf amino acid contents that result from a variable nutrient supply as noted by Steinberg (1953), became evident in the fruit. Cummings and Reeves (1971) indicated that the levels of amino acids in peach fruit might be influenced significantly by excessive or insufficient levels of certain elements. For example, the application with a high level of K reduced total amino acids of 'Redhaven' peaches, whereas an excessive Mg application increased total amino acids.

Our sensory evaluations demonstrated that fruit taste is closely related to the composition and concentration of amino acids. According to panelists, low concentrations of amino acids as in the L treatment made the juice tasteless for sourness and "umami" but only sweet. However, the panelists also rated peach juice with high concentrations of amino acids as in the H treatment, to be unsavory. This rejection is attributed to high concentrations of ASN and ARG that lowered the overall peach flavor. Most amino acids in water solution at 20 mM taste bitter, but they increase sweetness when they are added to the base juice at the concentrations of L and M treatment fruits. The mechanism of such complicated effects of amino acids on the taste are not yet known.

Our study indicates that amino acids are an important contributory factor to peach taste. Fruit containing a lower concentration of several major amino acids taste flat but does not detract from the overall taste. In contrast, a high concentration of amino acids, particularly ASN and ARG, makes fruit taste unsavory.

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異なる施肥濃度で栽培されたモモ‘白鳳’の果実に含まれるアミノ酸含量が食味に及ぼす影響

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摘　要

モモ‘白鳳’果実におけるアミノ酸含量と食味の関係を明らかにするため，窒素を40ppm (L区)，80ppm (M区)，および160ppm (H区)含む総合液肥で栽培した果実について，果汁の成分分析と味覚テストを行った．H区の成熟果実の果汁は，他の区よりもTSS含量が低く，滴定酸含量が高かった．アミノ酸は，アスパラギン，セリン，スレオニン，アルギニン，アスパラギン酸が主要成分であり，施肥濃度の高い区ほど濃度が高かった．果汁の食味試験の結果，H区では甘味が劣り，苦味と酸味が強かった．L区はM区と同程度に甘かったが，総合的食味はM区で最も優れた．各処理区の果汁と同じ濃度のアミノ酸水溶液の食味を官能評価した結果，アスパラギンとアルギニンは甘味と苦味を，セリンとアラニンは甘味を，アスパラギン酸とγ-アミノ酪酸は酸味を，スレオニンは苦味を示した．イオン交換樹脂によりアミノ酸を除去した果汁に，各アミノ酸を各区の濃度で個別または累積的に添加した結果，セリンおよびアルギニン，アスパラギンは各区の甘味とM区の旨味を強めたが，H区では苦味も強めた．アスパラギン酸は各区の酸味とM区の旨味を強めたが，H区では苦味も強め，総合的な食味を低下させた．以上の結果から，アミノ酸はモモ果実の食味を改善するが，高濃度の施肥をするとアスパラギンやアルギニンが高くなりすぎ，苦味が強くなるために食味が劣ると考えられる．