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Differences in Glutamic Acid and 5′-Ribonucleotide Contents between Flesh and Pulp of Tomatoes and the Relationship with Umami Taste

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A difference in taste characteristics between the outer flesh and the inner pulp of tomatoes has been observed; in particular the pulp, which contains the seeds, had more umami taste. Analysis of the free amino acids and 5′-ribonucleotides in the different parts of 13 varieties of tomatoes showed that in all cases the pulp contained higher levels of glutamic acid, 5′-adenosine monophosphate (AMP), 5′-guanosine monophosphate, 5′-uridine monophosphate, and 5′-cytidine monophosphate. The mean concentration of glutamic acid in the flesh was 1.26 g/kg and that in the pulp 4.56 g/kg but in some varieties the difference between pulp and flesh was more than 6-fold. For AMP, the mean concentration in the flesh was 80 mg/kg and that in the pulp was 295 mg/kg with one variety showing an 11-fold difference between pulp and flesh. These differences in concentration of these compounds, which are known to possess umami characteristics, provide an explanation for the perceived difference in umami taste between the flesh and pulp of tomatoes.

KEYWORDS: Tomato; glutamic acid; 5′-ribonucleotides; umami.

INTRODUCTION

The tomato (*Lycopersicon esculentum*) is a member of the *Solanaceae* family and, although botanically is a berry fruit, it is cultivated and used as a vegetable. Nutritionally, the tomato is a good source of vitamin A and C; however, composition varies depending species, stage of ripeness, soil, fertilization, irrigation, climatic conditions, and other conditions of cultivation, handling and storage (*1*). The flavor of tomato results principally from a combination of volatile aroma compounds (over 400 volatiles have been found), sugars, organic acids, free amino acids, and salts (*2*, *3*).

Sweet, bitter, salty, and sour were considered to be the four taste qualities upon which human sense of taste was based until Ikeda, in 1909, discovered a fifth taste (*4*). Ikeda's discovery was based on an observation concerning the dominant taste of dashi, a Japanese soup base, which had a taste clearly distinct from that of the four basic tastes. It was found to be due to glutamate and its taste was named "umami", which is usually described as meaty, broth-like, or savory (*5*). Much later, 5′-nucleotides, in particular, 5′-guanosine monophosphate (GMP), and 5′-inosine monophosphate (IMP), were found to be also important umami compounds and a synergism between glutamate and nucleotides was also reported (*6*). Glutamic acid is one of the most abundant amino acids in nature and the free amino acid is particularly abundant in milk and many vegetables, such as tomatoes and mushrooms, as well as certain seaweeds used in Japanese cooking. IMP is primarily associated with meat and fish, whereas GMP is more abundant in plant-based foods. 5-Adenosine monophosphate (AMP), which has a lower taste intensity than IMP or GMP, is another important umami substance that is widely distributed in natural food, especially seafood (*5*).

The concentration of free glutamic acid is higher in tomato compared with many vegetables and it increases in ripened and cooked tomatoes. The ratio of glutamate to aspartate has been shown to have an effect on the taste of ripening tomatoes. In a recent review, Ninomiya (*7*) cites a Japanese patent by Okumura in 1968 in which the ratio and the coexistence of both amino acids were the most important factors in reproducing tomato taste; the taste of synthetic extracts, without added glutamate, was similar to that of green tomato or citrus. The major nucleotide in tomato is AMP and its concentration also increases with ripening (*6*). It is present at a higher concentration compared with meat and poultry and contributes to a fuller, rounder flavor in foods, such as meat and fish, which are cooked with tomato (*8*).

The effect of umami substances on the taste of foods is a very important issue for food scientists and chefs engaged in developing new foods products. Monosodium glutamate (MSG) and 5′-ribonucleotides are flavor enhancers and they have been used effectively for nearly a century to optimize flavor in meat

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dishes and other foods. But, even before the formal identification of umami as a separate taste, glutamate-rich foods and ingredients were used in many civilizations (*9*). It is well-known that certain seaweeds and bonito make tastier soups, that combining meat (or fish) with vegetables make more flavorful stocks, and that cheese or tomato cooked with meat or seafood produces a tastier dish.

Although tomato is eaten raw, significant amounts are consumed in the form of processed products such as tomato juice, paste, purée, ketchup, sauce, and salsa. During tomato processing a byproduct, known as tomato pomace, is generated which consists mainly of peel and seeds (*10*). Various authors have studied the nutritional potential value of tomato pomace, showing a high fiber content as well as protein, fat, and minerals (*10*, *11*), suggesting that tomato pomace could be added to different traditional foods such as flours and other cereals products to improve their nutritional quality. It could also reduce waste, which is an increasing disposal problem for food industry.

Recently, it has been observed that there appeared to be a sensory difference in the umami character between the pomace and the flesh of raw tomatoes (H. Blumenthal, unpublished results). There are no reports of comparisons of the taste components of different parts of the tomato fruit. Neither has variation of umami compounds between different varieties of tomatoes been examined previously. Therefore, the objective of this paper is to evaluate the nonvolatile compounds, namely glutamic acid and 5′-nucleotides, present in the flesh and the pulp (center) of the tomato, and to relate this to the perceived taste characteristics.

MATERIALS AND METHODS

Chemicals. For capillary electrophoresis, purine (7*H*-imidazo-[4,5-*d*]pyrimidine) and nucleotide reference compounds, were purchased from Sigma-Aldrich Co. Ltd., Dorset, U.K., and were ≥99% purity. Reagent grade sodium tetraborate decahydrate and sodium hydroxide were also from Sigma-Aldrich Co. Ltd., Dorset, U.K. Reagent grade dipotassium hydrogen orthophosphate was from BDH, Merck, Dorset, U.K. For the sensory studies, food grade sodium chloride and MSG were purchased from a local food retailer.

Tomatoes. Fourteen commercial tomato varieties were purchased from a local food retailer, comprising four salad varieties, six cherry varieties, three plum varieties and one beefsteak variety. The salad varieties were grown in Canary Islands (Sainsbury's basic), U.K. (Carousel Flavoripe and Elegance Vine-ripened), and Italy (Elegance Vine-ripened). The cherry varieties were grown in Portugal (Conchita), France (Piccolo), Holland (Santa Pomodorino), and the U.K. (Dulce Supersweet and Sungold). All plum varieties were of U.K. origin, but the origin of the beefsteak variety was not known.

After blanching and removing the skins, the tomatoes were separated into flesh and pulp (center of the tomato containing the seeds and the aqueous phase surrounding them) and they were blended separately. The material was frozen in aluminum trays in a blast freezer and freeze-dried. The resulting freeze-dried extract was then vacuum-packed in oxygen-impermeable film and stored in the refrigerator at 2 °C prior to extraction.

Analysis of Nucleotides. Freeze-dried tomato samples (0.300 ± 0.005 g for the flesh and 0.200 ± 0.005 g for the pulp) were weighed into 14 mL screw-top vials. Hydrochloric acid (10 mL, 0.01 N) was added to the vial together with 200 μL of purine solution (1 mg/mL in water) as internal standard. The sample was stirred for 20 min at room temperature, and the mixture was allowed to settle for another 20 min. An aliquot of the supernatant (1.5 mL) was centrifuged at 7200*g* for 30 min.

The extracts were analyzed by capillary electrophoresis using a HP^{3D} CE (Agilent, Palo Alto, CA) with diode-array detection and a HP^{3D} Chemstation for instrument control. A modification of the method described by Uhrova (*12*) was used for the determination of nucleotides in the tomato samples. Electrophoretic separation was performed by constant pressure (50 mbar) with a 5 s injection of the sample onto an extended light path capillary of 64.5 cm total length (56 cm to detector) × 75 μm i.d. × 2.7 bubble factor, maintained at 25 °C. A 0.02 mol/L phosphate−borate buffer adjusted to pH 9.2 was used for the separation (the buffer was changed for every analysis). Preconditioning consisted of 5 min with 0.1 M NaOH followed by 5 min with buffer. A constant voltage of 20 kV was applied with positive to negative polarity. Detection was at 254 nm for 30 min and full spectra collection between 195 and 600 nm.

Analysis of Free Amino Acids by GC−MS. An aliquot of the centrifuged supernatant (100 μL) was derivatized using the EZ-Faast amino acid derivatization technique (Phenomenex, Torrance, CA). GC−MS analysis of the derivatized samples was carried out using an Agilent 5975 instrument (Agilent, Palo Alto, CA) as described by Elmore et al. (*13*).

Screening of the Sensory Panel. Thirteen assessors (12 female, 1 male) who were all experienced in the sensory evaluation of food products, were screened for their ability to detect and recognize MSG. Because MSG contains sodium, care was taken to compare individual sensitivities to MSG relative to sodium chloride (NaCl). In a previous study the group threshold for MSG was 0.32 ± 0.35 mM and that of NaCl was 0.82 ± 1.05 mM (*14*). In this present study solutions were prepared of MSG and NaCl in mineral water at concentrations ranging from 0.015 to 32 mM in a geometric progression of ratio 2. Assessors were asked to taste the solutions in order (from low to high concentration) until they noted a difference from the reference (mineral water), this was taken as their estimated detection threshold. All tasting was carried out in well-ventilated sensory booths, solutions were at room temperature. The geometric mean of the concentration at which an assessor detected the solution and the previous concentration was calculated and taken as an estimate of the individual's detection threshold. The group detection threshold for MSG was 0.63 ± 0.46 mM and for NaCl was 1.74 ± 0.75 mM (geometric mean ± SE). Eight out of 13 assessors demonstrated a higher sensitivity for MSG than for NaCl and were, therefore, considered to be glutamate tasters. A further 4 assessors appeared to have the same sensitivity to MSG as NaCl and were considered to be hypotasters (*14*). One assessor was considered a nontaster due to their higher sensitivity for NaCl than for MSG.

Sensory Evaluation. Tomatoes (5 kg) were blanched and skinned. The flesh and pulp were separated and blended using a domestic food processor. The mixtures were centrifuged at 10000*g* for 30 min, and the supernatants from each were separated to yield clear aqueous solutions. The panel of 13 assessors was used to develop a sensory profile to describe the sensory characteristics of the aqueous solutions and the characteristics were estimated quantitatively. Aliquots (20 mL) of the solutions from the pulp and flesh were presented to each assessor at room temperature in clear polypropylene tasting cups. During the development of the sensory profile the assessors were asked to provide as many descriptive terms as seemed appropriate. These terms were discussed, by the assessors as a group, with the help of the panel leader, and this led to an agreed aroma profile comprising 5 taste terms, 4 flavor terms, 3 mouthfeel

Table 1. Mean Panel Scores for Sensory Attributes of Aqueous Extracts of Flesh and Pulp from Elegance Vine Ripened Tomatoes

attribute[a]	flesh.[b]	pulp.[b]	sign.[c] samples	sign.[c] assessors	sign.[c] interaction
taste					
salty	23.0	34.3	*	***	ns
umami	36.4	45.8	ns ($p = 0.17$)	*	***
sour	29.5	41.0	**	***	ns
mouthfeel					
tongue tingling	14.5	29.2	*	*	**
astringent	25.9	37.1	*	***	ns
viscosity	23.2	40.5	***	*	ns
aftertaste					
salty	18.0	23.3	ns ($p = 0.13$)	**	ns
umami	28.8	40.1	*	**	*
sour	23.5	33.5	**	***	ns

[a] Only attributes where significant (or nearly significant) differences were found between samples are shown. Other nonsignificant attributes were appearance (color strength, bubbles), other taste and aftertaste (sweet, bitter), flavor (green, purity, tomato intensity) and flavor development (build up, persistence). [b] Mean values from a 100 point unstructured line scale. [c] Significance of difference as shown by ANOVA: 0.1% confidence level (***), 1% confidence level (**), 5% confidence level (*), not significant (ns); F-ratios for sample and assessor were calculated by comparing the mean square of the effect with the mean square of the sample x assessor interaction.

terms, and 4 aftertaste terms (Table 1). The qualitative sensory assessment took place under red lighting in the sensory booths, each equipped with computer screen and a mouse. Assessors wore nose clips to evaluate taste attributes and removed the noseclips to assess all other attributes. The interactive profiling option in the Taste software package (Reading Scientific Services Ltd., Reading, U.K.) was used to acquire the sensory data. The intensity of each attribute for each sample was recorded by the assessors on a 100-point unstructured line scale. Each sample was assessed twice and the order of presentation was randomized.

Analysis of Sodium Chloride and pH. Aliquots of the centrifuged supernatant (5 g) from the pulp and flesh of tomatoes prepared for sensory were analyzed in triplicate for chloride ions in the presence of acid by the Volhard procedure. This involved the addition of silver nitrate and back-titration with potassium thiocyananate, as described by Egan et al. (*15*). The solutions were also measured for pH using a calibrated Mettler Toledo 320 pH meter.

Statistical Analysis. Analysis of variance (ANOVA) and Fisher's Least Significant Difference (LSD) test were used to indicate significant differences ($p \leq 0.05$) in the levels of glutamic acid and 5′-nucleotides between the flesh and the pulp of the different varieties of tomato. The sensory data were processed using two-way ANOVA.

RESULTS AND DISCUSSION

Tomato is often included in meat dishes to enhance the savory taste characteristic and it believed that umami compounds in tomatoes, such as glutamate and AMP, act synergistically with IMP and other ribonucleotides in meat. During the preparation of dishes containing tomato it was observed that the center of tomato containing the seeds and the sounding pulp appeared to possess a higher umami character than the outer flesh (H. Blumenthal, unpublished results). This was interesting because the center is often discarded during the preparation of tomato-containing dishes. This observation has now been confirmed using a sensory panel who were asked to assess the quality attributes of aqueous extracts from the center and the pulp of a batch of Elegance Vine salad tomatoes. Taste and aftertaste attributes were scored higher in the tomato pulp than in the flesh (Table 1) confirming the earlier observation about the taste of flesh and pulp. Although umami taste and after taste received the highest mean scores of all the attributes, the panel scores for salty and sour also showed significant differences between the pulp and the flesh. However, chloride and pH determinations showed that the differences perceived between the pulp and the flesh were not related to actual salt or acidity differences. The concentrations of sodium chloride in the pulp and flesh were $0.03 \pm 0.01\%$ and $0.06 \pm 0.01\%$ respectively. This small difference between the pulp and flesh, with the flesh showing the higher concentration, cannot explain the higher perceived saltiness in the pulp compared with the flesh. The pH of the pulp and flesh extracts were 4.22 and 4.18, respectively; hence the difference in sour perceived by the sensory panel also cannot be explained by pH. Although all the assessors were trained and were shown to be able to taste glutamate, some assessors may have confused their perception of umami with that of salty or sour. In the initial threshold tests 6 assessors described the glutamate solutions as salty and 3 assessors used the descriptors sour or tangy. Umami compounds are known to have flavor enhancement properties and this could contribute to the overall taste differences between the two parts of the tomato. The assessors in this study certainly found the tomato pulp samples to have more overall taste even if they were not necessarily consistent in their interpretation and scoring for umami (Table 2 shows a significant sample−assessor interaction for the term umami). Previous studies have found umami-taste compounds to impart sour taste or enhance sour taste (*16*). The tomato pulp imparted greater mouthfeel characteristics than the flesh in terms of tongue tingling sensation and astringency. Umami compounds are typically known to increase saliva flow rather than cause astringency. In previous studies, foods with substantial umami characteristics have been found to contain compounds causing mouth drying (e.g., γ-aminobutyric acid in morel mushrooms) in addition to the umami contributing compounds (*16*). It is possible that constituents in the tomato, other than the amino acids and ribonucleotides quantified in this study, were responsible for the astringency.

A total of 14 varieties of tomatoes from four different types of commercial tomato available in retail stores were examined for amino acids and ribonucleotides: standard salad tomatoes, smaller cherry tomatoes, plum tomatoes, one variety of large beefsteak tomato. Analysis of free amino acids showed that the major amino acids in the tomatoes were glutamic acid, aspartic acid, glutamine, and asparagine (Figure 1). The pulp contained much higher concentrations of glutamic acid in all 14 varieties of tomato that were examined (Table 2), with overall mean concentrations of 4.56 and 1.26 g/kg in the pulp and the flesh, respectively. For all varieties, the concentrations in the pulp and flesh were significantly different statistically at $p < 0.001$. Highly significant differences were also found between varieties; in the flesh concentrations ranged from 0.66 to 3.51 g/kg whereas in the pulp they ranged from 2.03 to 16.5 g/kg. Although the levels of glutamic acid in the both the flesh and the pulp varied widely over the tomato varieties, the levels in the pulp were always higher than in the flesh. Varieties showing the greatest differences between pulp and flesh were cherry Dulce Supersweet (ratio = 5.7), a vine-ripened plum (ratio = 6.7), and salad Elegance vine-ripened from U.K. (where the pulp:flesh ratio was 6.4), although another sample of the same variety from Italy did not show such large differences. Over all

Table 2. Concentrations[a] of Glutamic Acid, Aspartic Acid, and 5′-Ribonucleotides in the Flesh and Pulp of Different Varieties of Tomatoes

	Glu (g/kg)			Asp (g/kg)			AMP (mg/kg)			CMP (mg/kg)			GMP (mg/kg)			UMP (mg/kg)		
variety	flesh	pulp	ratio	flesh	pulp	ratio	flesh	pulp	ratio	flesh	pulp	ratio	flesh	pulp	ratio	flesh	pulp	ratio
salad:																		
Sainsbury's Basics	1.40	2.03	*1.4*	0.57	0.28	*0.5*	55	282	*5.1*	4.7	13.2	*2.8*	6.7	18.8	*2.8*	51	111	*2.2*
Carousel Flavoripe	1.25	4.00	*3.2*	0.24	0.22	*0.9*	65	268	*4.1*	3.8	12.4	*3.3*	6.3	16.2	*2.6*	47	106	*2.2*
Elegance Vine UK	0.88	5.59	*6.4*	0.25	0.40	*1.6*	38	437	*11.6*	4.7	14.8	*3.2*	2.7	24.8	*9.2*	28	125	*4.5*
Elegance Vine Italy	1.32	2.63	*2.0*	0.3	0.38	*1.2*	101	252	*2.5*	4	8	*1.8*	8.2	16.0	*2.0*	45	63	*1.4*
mean salad	***1.21***	***3.56***	*2.9*	***0.35***	***0.32***	*0.9*	***65***	***310***	*4.8*	*4.3*	***12.0***	*2.8*	***6.0***	***18.9***	*3.2*	*43*	***101***	*2.4*
cherry:																		
Conchita	1.70	5.66	*3.3*	0.60	0.85	*1.4*	108	365	*3.4*	7.5	22.0	*2.9*	11.6	34.3	*3.0*	67	155	*2.3*
Dulce Supersweet	0.66	3.80	*5.7*	0.26	0.91	*3.5*	126	284	*2.3*	10.6	14.7	*1.4*	15.2	24.6	*1.6*	75	143	*1.9*
Piccolo	3.51	16.5	*4.7*	1.22	1.82	*1.5*	146	561	*3.8*	6.5	21.0	*3.2*	15.8	37.9	*2.4*	96	183	*1.9*
Sungold	1.14	6.06	*5.3*	0.34	0.59	*1.8*	197	317	*1.6*	13.8	21.3	*1.5*	23.2	27.9	*1.2*	100	147	*1.5*
Santa Pomodorino	0.98	5.30	*5.4*	0.31	0.44	*1.4*	128	238	*1.9*	9.0	19.3	*2.1*	15.4	23.5	*1.5*	73	139	*1.9*
Tiger	1.57	4.18	*2.7*	0.64	0.38	*0.6*	106	521	*4.9*	4.1	18.2	*4.4*	9.5	37.6	*4.0*	49	134	*2.7*
mean cherry	***1.59***	***6.92***	*4.3*	***0.56***	***0.83***	*1.5*	***135***	***381***	*2.8*	***8.6***	***19.4***	*2.3*	***15.1***	***31.0***	*2.0*	***77***	***150***	*2.0*
plum:																		
Celine	1.70	2.83	*1.7*	0.42	0.46	*1.1*	57	251	*4.4*	3.0	11.7	*3.9*	6.1	13.1	*2.1*	49	105	*2.1*
Flavorino Vine	1.73	4.78	*2.8*	0.72	0.94	*1.3*	94	213	*2.3*	5.8	13.3	*2.3*	9.3	12.8	*1.4*	65	88	*1.3*
Vine Ripened	1.23	8.25	*6.7*	0.35	0.54	*1.5*	141	288	*2.0*	7.4	16.5	*2.2*	13.2	13.2	*1.0*	71	116	*1.6*
mean plum	***1.55***	***5.28***	*3.4*	***0.50***	***0.65***	*1.3*	***97***	***251***	*2.6*	***5.4***	***13.8***	*2.6*	***9.5***	***13.0***	*1.4*	***62***	***103***	*1.7*
beefsteak:																		
Growdena	0.69	2.47	*3.6*	0.22	0.28	*1.3*	24	238	*9.8*	2.6	11.7	*4.5*	3.3	18.2	*5.6*	38	118	*3.1*
overall mean	***1.26***	***4.56***	*3.6*	***0.41***	***0.52***	*1.3*	***80***	***295***	*3.7*	***5.2***	***14.2***	*2.7*	***8.5***	***20.3***	*2.4*	***55***	***118***	*2.2*
LSD[b]	0.39	1.28		0.09	*0.16*		6.32	31.7		1.46	3.64		1.03	2.90		4.42	12.32	

[a] Values are the mean of four replicates and are on a wet weigh basis. [b] Least significant difference for variety; for all compounds ANOVA showed a highly significant effect of variety ($p < 0.001$).

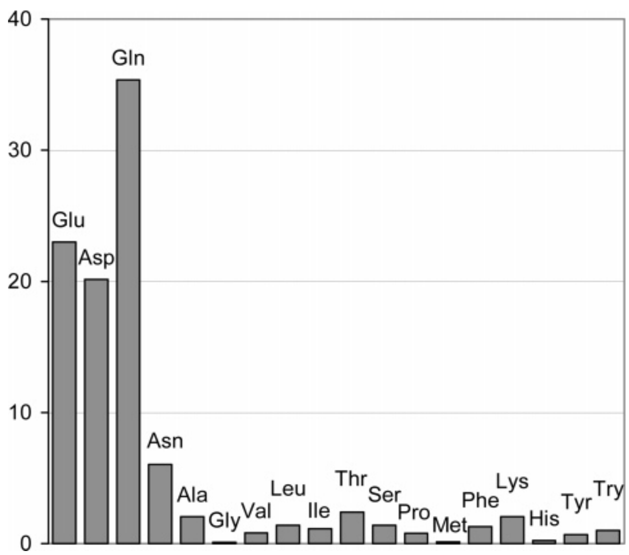

Figure 1. Free amino acid composition (shown as % of total) in the flesh of a typical salad tomato (variety Elegance vine-ripened Italy).

the varieties the mean ratio for pulp:flesh was 3.6. Although differences between varieties within the tomato types were large, there appeared to be a trend for the cherry tomatoes to show higher levels of glutamic acid in both parts of the fruit. Glutamic acid concentration increases progressively with ripening and the content in full ripened red tomato can be more than 8 times higher than in green tomato (*6*). Although visually all the tomatoes were at similar stages of ripeness, no other measurements of ripeness were made; this could contribute to the differences between the tomato varieties. The other free amino acid, which may contribute to taste characteristics of tomatoes, is aspartic acid, but the differences between the flesh and pulp were small with some varieties showing less aspartic acid in the pulp than in the flesh, although overall the ratio of pulp: flesh was 1.3.

The major nucleotide in all the tomatoes was AMP, which is in agreement with observations by Ninomiya (*6*). Other ribonucleotides also present in tomato, although at lower concentrations, were GMP, 5′-uridine monophosphate (UMP), and 5′-cytidine monophosphate (CMP). They all showed patterns similar to those of the glutamic acid with higher concentrations in the pulp compared with the flesh. The effect was largest with AMP, where concentrations in the flesh ranged from 24 to 197 mg/kg and in the pulp from 213 to 561 mg/g. The average pulp:flesh ratio for AMP over all the varieties was 3.7; salad Elegance vine-ripened from the U.K. had a pulp:flesh ratio of 11.6, and for Growdena beefsteak it was 9.8. Interestingly, the differences in all the ribonucleotides between flesh and pulp were generally less pronounced for plum and cherry types. This could be due to the physiological structure of plum tomatoes, which have firm flesh and less juicy centers (*1*).

It was not the intention of this work to undertake a comprehensive study of the effect of variety on levels of taste compounds; such a study would require larger trials in which plants are all grown under similar conditions in the same location and would need to include examination of agronomic effects. However, the work shows, for the first time, that large differences in glutamic acid and AMP concentrations occur between the center pulp and the outside flesh of tomatoes and this is common to all varieties. These differences offer an explanation for the higher umami and other taste characteristics observed by the sensory panel in the pulp compared with the flesh.

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