JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Umami Compounds Are a Determinant of the Flavor of Potato (*Solanum tuberosum* L.)

WAYNE L. MORRIS, HEATHER A. ROSS, LAURENCE J. M. DUCREUX, JOHN E. BRADSHAW, GLENN J. BRYAN, AND MARK A. TAYLOR*

Quality, Health and Nutrition, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, United Kingdom

Vegetable flavor is an important factor in consumer choice but a trait that is difficult to assess quantitatively. The purpose of this study was to assess the levels of the major umami compounds in boiled potato tubers, in cultivars previously assessed for sensory quality. The free levels of the major umami amino acids, glutamate and aspartate, and the 5′-nucleotides, GMP and AMP, were measured in potato samples during the cooking process. Tubers were sampled at several time points during the growing season. The levels of both glutamate and 5′-nucleotides were significantly higher in mature tubers of two *Solanum phureja* cultivars compared with two *Solanum tuberosum* cultivars. The equivalent umami concentration was calculated for five cultivars, and there were strong positive correlations with flavor attributes and acceptability scores from a trained evaluation panel, suggesting that umami is an important component of potato flavor.

KEYWORDS: Aspartate; glutamate; flavor; 5′-nucleotide; potato; *Solanum tuberosum*; *Solanum phureja*; umami

INTRODUCTION

Increasingly, potato tuber quality traits are assuming a greater importance in breeding programs, as consumers demand greater variety and retailers wish to market cultivars that have distinctive commercial advantages. However, as with many food crops, potato flavor is difficult to assess in breeding programs. Assessments are highly subjective and require trained sensory panels. These have a low sample throughput and are consequently expensive. As a result, flavor is generally assessed only in the later stages of a breeding program after selection for more easily quantifiable traits. In fact, most of the potential flavor improvements are likely to be discarded, and to a large extent the marketplace determines whether a new cultivar is acceptable to consumers (*1*).

The volatiles produced by raw and cooked potatoes have been studied extensively (*2*–*4*), and over 250 compounds have been identified in potato volatile fractions. Attempts have been made to discriminate which of these components are important for potato flavor, which are specific to the method of cooking, cultivar differences, the effects of agronomic conditions, and the effects of storage (*5*–*9*). Overall, there is no clear-cut identification of which volatiles (if any) are the key contributors to cooked potato flavor and taste.

In addition to the volatile compounds produced on cooking of potato tubers, soluble cellular constituents are likely to be important in flavor also (*10*). Interactions between tastants and aroma compounds probably give rise to the overall sensory quality of the cooked tuber. The soluble, matrix-associated compounds define the basic taste parameters, sweet, sour, salty, or bitter and umami (a Japanese word meaning delicious). Compounds including monosodium glutamate (MSG), several process-derived glutamate glycoconjugates, adenosine 5′-monophosphate (5′-AMP), inosine 5′-monophosphate (5′-IMP), and guanosine 5′-monophosphate (5′-GMP), are well-known to show umami-like sensory characteristics (*11, 12*). Umami compounds generally enhance flavor and mouthfeel, giving the impression of creaminess and viscosity to savory dishes (*10*). The umami taste intensity increases exponentially when glutamate interacts with 5′-ribonucleotides (*13*). The synergistic effect between certain free amino acids and 5′-nucleotides can be measured using the equivalent umami calculation [see Materials and Methods (*13*)]. Glutamate is the most potent umami amino acid, with aspartate showing only 7% of the taste activity of glutamate. The taste activity of 5′-GMP is the most potent common 5′-nucleotide, having a 2.3-fold greater effect than 5′-IMP (*13*). The intensity of the umami taste may be enhanced by a range of salts including sodium, potassium, and magnesium (*14*) as well as certain organic acids such as succinate (*15*).

As early as 1971 it was suggested that boiled potato taste was largely due to the natural mixture of glutamic acid and other amino acids in combination with the 5′-GMP and other 5′-nucleotides produced on cooking (*16*). Indeed, some authors claim that there is only a small contribution from volatile (olfactory) components and that chemicals representing the so-called sweet, sour, salty, and bitter tastes do not provide a

* Corresponding author (e-mail mtaylo@scri.sari.ac.uk; telephone +44 1382 562731; fax +44 1382 562426).

10.1021/jf0717900 CCC: $37.00
Published on Web 10/19/2007

cooked potato taste (*16–18*). Thus, the presence of salt, sugars, or alkaloids does not enhance potato flavor, although their presence at high levels may decrease palatability.

Limited data on the levels of several umami compounds in potato tubers have been published (*17*). Although raw potatoes contain only very small amounts of 5′-nucleotides, cooked potatoes contain appreciable levels, higher than most other plant-derived foods examined. Several studies have addressed the levels of free amino acids (including glutamic acid) in potato tubers and also examined changes in amino acid levels during storage (*19, 20*). It is clear that the soluble protein and amino acid contents of potato tubers are modified substantially during storage, with larger effects observed after 3 months of storage at 10 °C than at 4 °C (*20*). At harvest, the major free amino acids are asparagine, glutamine, glutamic acid, arginine, and aspartic acid, with some cultivar-dependent variations in the contribution of these amino acids (*20, 21*). Additionally, the soil nitrogen fertilization regimen affects amino acid content, with a high level of nitrogen fertilization associated with increased glutamine content (*22*). Potassium fertilization can enhance tuber potassium content (*23*), and as potassium may enhance umami taste intensity (*14*), this could also be a factor in potato flavor.

Only sparse taste panel data are available to support the importance of umami compounds in defining potato flavor. The effects of supplementing boiled potato with glutamic acid and glutamic acid plus nucleotides both resulted in a "stronger" potato taste, as assessed by a trained panel consisting of 18 judges. Additionally, an aqueous mixture of amino acids and nucleotides that reproduces the levels found in boiled potatoes "had practically no odor, but an agreeable basic potato-like taste" (*16*). Furthermore, a mixture with components replicating the amino acid and nucleotide concentrations of a preferred boiled potato was judged to taste better than that replicating a less preferred boiled potato (*24*). More recently, it was shown that supplementation of mashed potato with different amounts of monosodium glutamate and disodium inosate and guanylate can enhance the sensory evaluation scores for mashed potato, although no information on endogenous levels was included in this analysis (*25*). In general, correlation of sensory evaluation scores for cooked potato flavor from different cultivars with umami compound measurements is lacking. Recent work has demonstrated that boiled tubers from *Solanum phureja* score better in sensory evaluations than those from *Solanum tuberosum* (*26*). The *S. phureja* tubers have a distinctive (preferred) mouthfeel and a higher intensity of flavor attributes than *S. tuberosum*. The aim of the current paper is to compare the levels of the amino acids and ribonucleotides with umami taste in *S. phureja* and *S. tuberosum* tubers at a range of tuber developmental stages and during several processing methodologies. By comparing the levels of the these umami compounds with sensory evaluation data, the aim was to determine whether or not these compounds are likely to be important components of potato flavor.

MATERIALS AND METHODS

Plant Material. Potato-breeding clones and cultivars examined in this study were grown in field trials during 2006 at Gourdie Farm (Dundee, U.K., 56°, 28′, 27″ north; 3°, 4′, 11″ west) using normal agronomic practices. Tubers were planted on April 26, 2006, and were analyzed at three harvest periods: H1, harvested early in development (harvested on July 12, 2006) when average tuber weight was 20–40 g; H2, harvested at maturity when average tuber weight was 150–200 g (harvested on October 9, 2006, following acid burndown of foliage); H3, mature tubers that had been stored at 4 °C for 6 weeks. Approximately 1 kg of average-sized mature tubers was selected and pooled, and each tuber was manually cut into eighths. These pools were subdivided into nine with each constituting a single representative sample. Triplicate samples were used for three cooking time points (either steaming or boiling as indicated in the text). All raw and cooked tuber samples were immediately frozen in liquid nitrogen and freeze-dried. The freeze-dried samples were ground in a laboratory mill fitted with a 0.5 mm sieve and stored at −20 °C prior to analysis. The clones used in this study were *S. phureja* 333-16, Mayan Gold, Inca Sun, and DB257-28 and *S. tuberosum* cultivars Montrose, Pentland Dell, Maris Piper, and Record.

Analysis of 5′-Nucleotides. Triplicate freeze-dried potato samples (approximately 100 mg) were extracted into 5 mL of 5% perchloric acid (VWR Ltd., Lutterworth, U.K.) by vortexing for 5 s, followed by end over end mixing on a blood rotator for 60 min at 4 °C. Following centrifugation at 4 °C at 3000*g* for 5 min, 2.5 mL of supernatant was transferred to a fresh 15 mL centrifuge tube containing 50 μL of BDH 4080 indicator dye (VWR Ltd.). The pH range of the sample was adjusted to approximately 6.5 by the dropwise addition of a solution of 5 mol/L K_2CO_3 (VWR Ltd.). After removal of potentially explosive insoluble $KClO_3$ salts, by centrifugation at 3000*g* for 10 min, the supernatant was applied to a strong anion exchange–solid phase extraction (SAX SPE) column (100 mg, acetate counter ion, Alltech, Carnforth, U.K.). The column was washed with 4 mL of deionized H_2O prior to elution of 5′-nucleotides with 4 mL of 2 mol/L formic acid (VWR Ltd.). Extracts were lyophilized and resuspended in 200 μL of deionized water prior to analysis by high-performance anion-exchange chromatography (HPAEC), using a CarboPac PA-1 column [Dionex (UK) Ltd., Camberley, U.K.], as detailed in ref *27*. Nucleoside monophosphate reference standards 5′-AMP and 5′-GMP (Sigma-Aldrich, Gillingham, U.K.) were used for peak identification and quantification using standard curves. 5′-Nucleotide recovery percentages were consistently 55–65% as determined by comparing spiked (with standards) and unspiked samples that had undergone the extraction procedure. The results presented are not corrected for recovery percentage.

Analysis of Amino Acids. Triplicate freeze-dried potato samples (approximately 100 mg) were extracted into 5 mL of buffer consisting of methanol/water/acetic acid [49:49:2 v/v/v (*28*)]. Samples were vortexed for 5 s followed by end over end mixing on a blood rotator for 60 min at 4 °C. The extracts were centrifuged at 3000*g* for 5 min, and the supernatant was passed through a 0.45 μm filter (VWR Ltd.) prior to analysis. Amino acids were derivatized with *o*-phthaldialdehyde prior to separation by high-performance liquid chromatography (HPLC) (*29*). Separation was performed using a Zorbax Eclipse AAA column (4.6 × 150 mm, 5 μm) on an Agilent 1100 HPLC equipped with a G1313A autosampler, a G1312A binary pump, and a G1315A fluorescence detector (Agilent Technologies, Wokingham, U.K.). A binary solvent gradient of 0–14% B (0–6 min), 14% B (6–11 min), 14–50% B (11–16 min), 50% B (16–20 min), 50–100% B (20–30 min), 100% B (30–32 min), and 100–0% B (32–36 min) at a flow rate of 0.8 mL min^{-1} was used [solvent A, 83 mmol/L sodium acetate/methanol (4:1) with tetrahydrofuran added at 1% v/v; solvent B, 83 mmol/L sodium acetate/methanol (1:4)], and the column temperature was kept at 20 °C. Fluorescence detection was set at excitation = 360 nm, emission = 455 nm, and PMT gain = 10. Amino acids were quantified by comparison with the AA-S-18 (Sigma-Aldrich, Gillingham, U.K.) reference amino acid mixture supplemented with asparagine, glutamine, tryptophan, and γ-aminobutyric acid. Amino acid recovery percentages were consistently 80–90% as determined by comparing spiked (with standards) and unspiked samples that had undergone the extraction procedure. Data shown are not corrected for recovery percentage.

Equivalent Umami Calculation. The equivalent umami concentration (EUC, grams of MSG per 100 g) is the concentration of MSG equivalent to the umami intensity given by a mixture of MSG and the 5′-nucleotide and is represented by the addition equation (*13*)

$$Y = \sum a_i b_i + 1218\left(\sum a_i b_i\right)\left(\sum a_j b_j\right) \quad (1)$$

where Y is the EUC of the mixture, a_i is the concentration of each umami amino acid (Glu or Asp), a_j is the concentration of each umami

5′-nucleotide (5′-GMP or 5′-AMP), b_i is the relative umami concentration (RUC) for each umami amino acid to MSG (Glu = 1, Asp = 0.077), b_j is the RUC for each umami 5′-nucleotide to 5′-IMP (5′-GMP = 2.3, 5′-AMP = 0.18), and 1218 is a synergistic constant. All concentrations must be in grams per 100 g.

Sensory Evaluation. Potatoes were stored in a cold store at 2 °C from harvest until being assessed for sensory characters. Potatoes were peeled and cut into cubes of approximately 30 g weight. Samples (500 g) were boiled with 1000 mL of boiling distilled water and 1% cooking salt for a predetermined length of time predicted by initial penetrometer tests (between 6 and 10 min). Samples were drained of excess water, squeezed through a potato ricer, then transferred to prewarmed bowls, covered with foil, and kept warm in an oven at 70 °C prior to serving to a trained sensory panel for assessment. The panel members were trained following standard guidelines (*30*) ensuring the use of a standardized vocabulary, and all panel members demonstrated good sensory acuity. The sensory assessors rated the potatoes according to a series of sensory attributes, including a "catch all" trait, acceptability, which measures an assessor's subjective opinion of the degree to which he or she likes or dislikes the flavor of the cooked potatoes. The flavor attributes intensity, creaminess, and sweetness were also assessed. All samples were coded and presented in a defined order to allow assessment of sample, assessor, order of tasting, carry-over, and session effects. All attributes were scored on a scale from 0 (poor rating for the character) to 100 (good rating for the character). Five clones per session were tested, and each clone was evaluated in three separate sessions. For each session there were between 8 and 12 assessors. Data were collected using a computer-assisted interface. All assessments were carried out in triplicate in isolated, purpose-built booths with controlled airflow and lighting. Assessors were invited to rinse their palates between samples. The experimental results were collated and analyzed using a proprietary package (KwikSense, Hannah InterActions Ltd., Ayr, U.K.) as well as Genstat version 9 (Lawes Agricultural Trust). The mean values for each attribute (three replicates) were computed. Data for five genotypes for the flavor attributes, flavor intensity, flavor creaminess, flavor sweetness, and the overall acceptability attribute are presented here.

In-Gel RNase Assay. Substrate-based sodium dodecyl sulfate–polyacrylamide gel electrophoresis of potato protein extracts was performed as described in ref *31*. Lyophilized tuber powder (0.2 g) was extracted in 2 mL of citrate protein extraction buffer [150 mmol/L citric acid–Na_2HPO_4, pH 3, 0.1 mmol/L phenylmethanesulfonyl fluoride (PMSF)]. In addition, proteins were extracted using an acetate buffer [0.1 mol/L sodium acetate, pH 5.2; (*32*)] with the addition of PMSF at a final concentration of 0.1 mmol/L. All protein extractions were performed at 4 °C. Samples were clarified by centrifugation (10 000*g* for 10 min), and the protein was quantified using the DC Protein Assay (Bio-Rad, Hertfordshire, U.K.). Proteins (40 μg) were separated by electrophoresis prior to visualization of RNase activity as described previously (*31*).

RNase Activity Assay. RNase enzyme activity assay was performed spectrophotometrically as described previously (*32*) with the addition of PMSF at a final concentration of 0.1 mmol/L. Absorbances at 260 and 280 nm were recorded, and one enzyme unit is defined as a change of 1 OD at 260 nm per milligram of protein per hour.

Phosphohydrolytic Enzyme Activity Assays. Freeze-dried powder (0.5 g) was extracted into 5 mL of sodium citrate extraction buffer [0.05 mol/L, pH 6.0 containing 2 mmol/L cysteine and 0.1 mmol/L PMSF; (*33*)] by grinding in a mortar and pestle for 10 s. Samples were clarified by centrifugation (10000*g* for 5 min); 2.5 mL of extract was desalted using a Sephadex G25 gel filtration column (NAP10 column, GE Healthcare UK Ltd., Buckinghamshire, U.K.) pre-equilibrated with sodium citrate extraction buffer. Specific phosphohydrolytic enzyme activities were determined (*34*) using a variety of synthetic substrates: *p*-nitrophenyl phosphate for the phosphomonoesterase activities, bis-*p*-nitrophenyl phosphate as substrate for nonspecific phosphodiesterases, and thymidine 5′-monophospho-*p*-nitrophenyl ester substrate for phosphodiesterases that can specifically hydrolyze the 5′-phosphodiester bonds. Each substrate (Sigma-Aldrich, Gillingham, U.K.) was used for assays performed under alkaline (33 mmol/L Tris-HCl buffer, pH 8.7 (*35*)) and acidic (33 mmol/L ammonium acetate buffer, pH 5.7) conditions. All assays were performed at 50 °C; the liberated *p*-nitrophenol was monitored at 405 nm, and all analyses were performed in triplicate.

Statistical Analysis. Student's *t* test method was used (paired, two-tailed distribution) to test the statistical relationship between cultivars for amino acid and 5′-nucleotide levels and nuclease activities.

RESULTS

Formation of 5′-Nucleotides during Cooking. To investigate the formation of 5′-nucleotides during cooking, tubers (cultivars *S. phureja* Mayan Gold and *S. tuberosum* Pentland Dell) were analyzed at time points during the boiling or steaming process. Nucleotides were analyzed using HPLC and quantified by comparison to authentic reference standards. In accordance with published literature (*16*) 5′-nucleotide levels were very low in raw tubers. However, 5′-GMP and 5′-AMP accumulated in tubers during cooking (**Figure 1**). 5′-GMP and 5′-AMP levels rose sharply during the first 5 min of cooking time before reaching a plateau. The levels of 5′-nucleotides were slightly higher (although not significant using Student's *t* test) in steamed tubers compared with boiled tubers, possibly indicating that steaming is the better cooking method. Interestingly, levels of the most potent umami 5′-nucleotide, 5′-GMP, were ca. 2–3-fold higher in the Mayan Gold compared with Pentland Dell tubers for both cooking methods at the plateau level (**Figure 1A**,**B**). Additionally, the same trend was observed for 5′-AMP, where the levels were ca. 2- and 4-fold higher in Mayan Gold than in Pentland Dell for boiling and steaming, respectively (**Figure 1C**,**D**).

Once it was established that 5–10 min of cooking time was optimal for the release of 5′-nucleotides, levels were compared in *S. tuberosum* (cultivars Montrose and Pentland Dell) and *S. phureja* tubers (cultivars Inca Sun and Mayan Gold) throughout development and storage. Levels of 5′-nucleotides were determined in raw and steamed samples at the three different harvests, defined under Materials and Methods (**Figure 2**). The formation of 5′-nucleotides upon cooking is again evident for all three harvest time points. At harvest stage 1 the levels of 5′-GMP and 5′-AMP are not significantly different (at $P = 0.05$ level) between the *S. phureja* and *S. tuberosum* cultivars (**Figure 2**). However, at harvest stage 2 the 5′-GMP levels were significantly higher ($P \leq 0.015$) in *S. phureja* compared with *S. tuberosum* (e.g., Inca Sun 2.6-fold higher than Montrose). The levels of 5′-GMP were maintained in the *S. phureja* Inca Sun tubers at harvest stage 3, whereas the levels in *S. tuberosum* cultivars and Mayan Gold declined slightly. A similar pattern was observed for levels of 5′-AMP during development and storage (**Figure 2B**). For example, Inca Sun 5′-AMP levels were 2.0- and 2.6-fold higher than those in Montrose at harvest stages 2 and 3, respectively.

Tuber Free Amino Acid Content. Amino acids were analyzed using HPLC and quantified by comparison to authentic reference standards. The levels of amino acids determined were in the same range as previously published values (*20*, *21*). Tubers were analyzed at the same three harvest points as used for the 5′-nucleotides. In both *S. phureja* and *S. tuberosum* cultivars the amides asparagine and glutamine predominated and comprised between 20 and 50% (w/w) and between 15 and 45% (w/w), respectively, of the total free amino acid pool (see Supporting Information Table 1). The profiles of individual amino acids showed variation between cultivars, and the total amino acid levels were generally around 2-fold lower in tubers harvested early in development (H1) compared with harvest stages 2 and 3. Of particular interest were the levels of the umami amino acids glutamate and aspartate (**Figure 3**). The

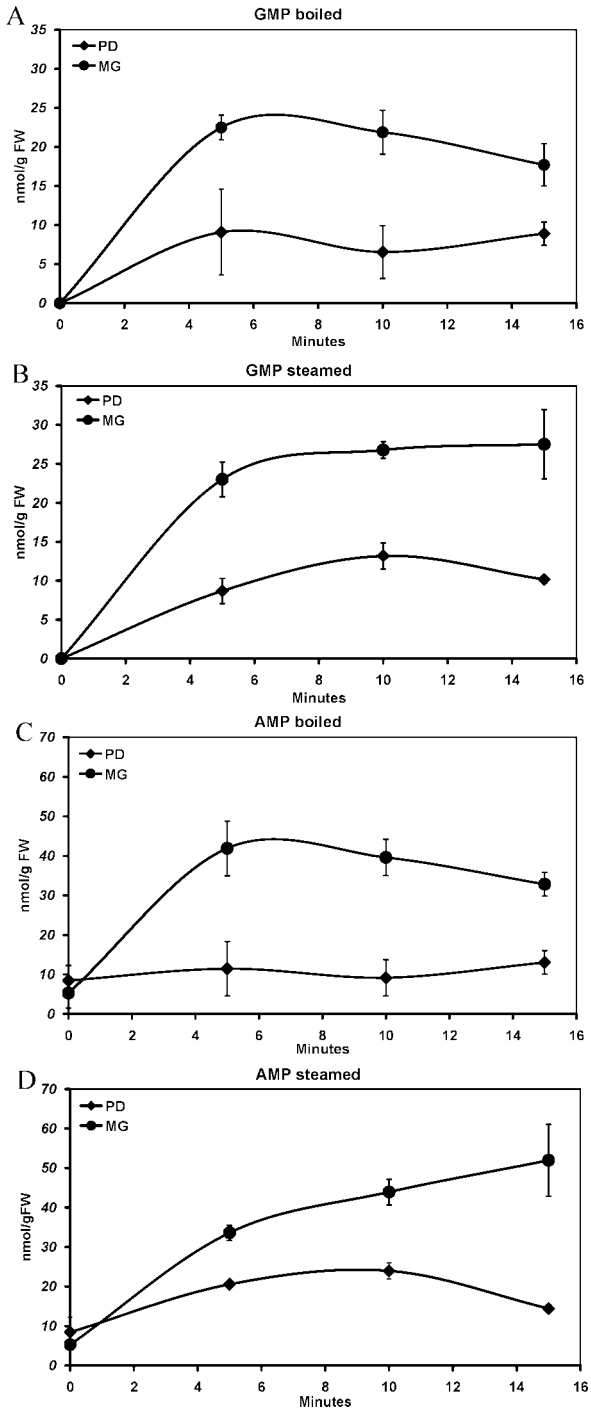

Figure 1. Comparison of the kinetics of 5′-nucleotide formation during cooking for potato cultivars Pentland Dell (PD) and Mayan Gold (MG): (**A**) GMP boiled; (**B**) GMP steamed; (**C**) AMP boiled; (**D**) AMP steamed. AMP, adenosine 5′-monophosphate; GMP, guanosine 5′-monophosphate; FW, fresh weight. Error bars represent standard error of the mean (SEM), $n = 3$.

most striking difference observed was the higher levels of glutamate in the *S. phureja* cultivars compared with *S. tuberosum* cultivars. The levels of glutamate at tuber maturity were significantly ($P \leq 0.08$) higher in Mayan Gold and Inca Sun compared with Montrose and Pentland Dell.

Equivalent Umami Concentration. The intensity of umami flavor depends on the synergistic interaction between 5′-nucleotides and certain flavor amino acids. Equivalent umami concentrations (EUC) were calculated as described above using the levels of aspartate, glutamate, 5′-GMP, and 5′- AMP and

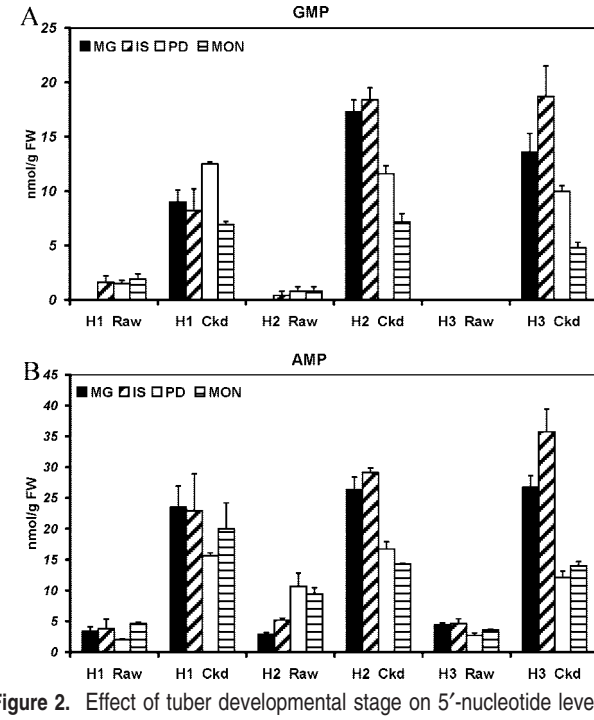

Figure 2. Effect of tuber developmental stage on 5′-nucleotide levels in raw and cooked (Ckd) potato cultivars Mayan Gold (MG), Inca Sun (IS), Pentland Dell (PD), and Montrose (MON). H1, harvest 1; H2, harvest 2; H3, harvest 3 (see text for details). Error bars represent the SEM, $n = 3$.

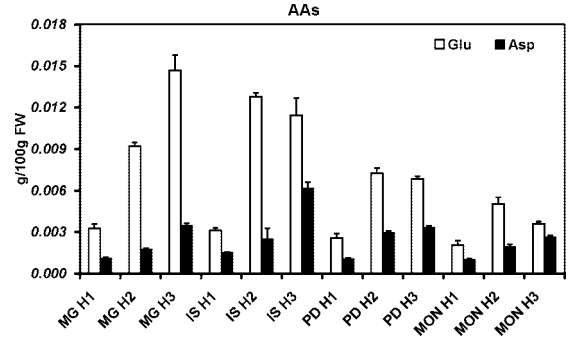

Figure 3. Effect of tuber developmental stage on flavor amino acid levels in cooked potato cultivars Mayan Gold (MG), Inca Sun (IS), Pentland Dell (PD), and Montrose (MON). Glu, glutamic acid; Asp, aspartic acid; H1, harvest 1; H2, harvest 2; H3, harvest 3 (see text for details). Error bars represent the SEM, $n = 3$.

the equation given in ref *13* (**Figure 4**). The EUC values calculated for *S. phureja* cultivars Mayan Gold and Inca Sun were not significantly different (at $P = 0.1$ level) from those of *S. tuberosum* cultivars Montrose and Pentland Dell at the earliest harvest stage (H1). However, at later harvest stages the EUC values were significantly higher in *S. phureja* compared with *S. tuberosum* with differences ranging from between 2.6-fold for Mayan Gold versus Pentland Dell harvest stage 3 ($P = 0.046$) and 6.5-fold for Inca Sun versus Montrose harvest stage 3 ($P = 0.0062$).

5′-Nucleotide-Liberating Enzyme Assays. Previously it has been suggested that the presence of 5′-nucleotides in potato tubers may be due to the enzymatic breakdown of RNA during cooking (*17*). To investigate this hypothesis, nuclease enzyme activities (RNase and phosphomono- and diesterases) were compared in *S. tuberosum* (Maris Piper and Pentland Dell) and *S. phureja* (Mayan Gold and 257-28) mature tubers. Substrate-based in-gel RNase assays showed no differences in RNase

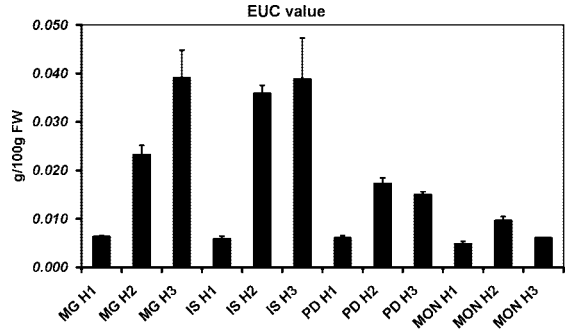

Figure 4. Effect of tuber developmental stage on equivalent umami concentrations (EUC) in cooked potato cultivars Mayan Gold (MG), Inca Sun (IS), Pentland Dell (PD), and Montrose (MON). H1, harvest 1; H2, harvest 2; H3, harvest 3 (see text for details). Error bars represent the SEM, $n = 3$.

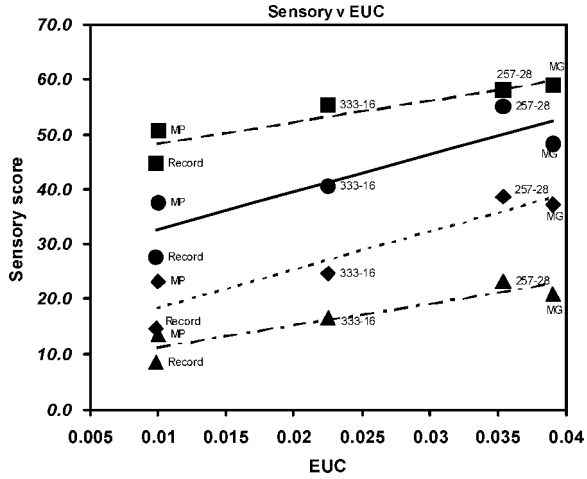

Figure 5. Correlation of sensory evaluation scores with equivalent umami concentration of potato: (squares) flavor intensity; (circles) acceptability; (diamonds) sweet flavor; (triangles) creamy flavor. *S. tuberosum* cultivars Maris Piper (MP) and Record were compared with *S. phureja* clones DB333-16 and DB257-28 and cultivar Mayan Gold (MG).

banding patterns between the *S. tuberosum* and *S. phureja* cultivars (Supporting Information Figure 1). Additionally, no significant differences were observed between the *S. tuberosum* and *S. phureja* cultivars for total RNase activity assays (Supporting Information Table 2). As the differences in the levels of 5′-nucleotides could not be attributable to variation in RNase activity, the activities of phosphohydrolytic enzymes were investigated. Both phosphomonoesterase and phosphodiesterases (specific and nonspecific) were examined in *S. tuberosum* and *S. phureja* cultivars (Supporting Information Table 2). No significant differences were observed in phosphohydrolytic enzyme activity.

Sensory Evaluation and Umami Level Correlation. Sensory evaluations of boiled mature tubers from both *S. tuberosum* (cultivars Maris Piper and Record) and *S. phureja* (clones DB333-16, DB257-28 and the variety Mayan Gold) were carried out by a trained panel. Overall acceptability and the flavor attributes flavor intensity, flavor creaminess, and flavor sweetness scores were plotted against calculated EUC values to determine the level of correlation (**Figure 5**). Clear and positive correlations were observed between all sensory scores and EUC values (for flavor intensity, $R^2 = 0.83$; flavor creaminess, $R^2 = 0.85$; flavor sweetness, $R^2 = 0.86$; and acceptability, $R^2 = 0.79$).

DISCUSSION

Previously it has been hypothesized that potato flavor is largely due to the levels of umami compounds that develop in cooked tubers (*16*, *17*). Evidence in support of this hypothesis is presented in this paper. It is clearly demonstrated that there are significantly higher levels of glutamate and 5′-GMP in cooked mature tubers of *S. phureja* cultivars than in those of *S. tuberosum* cultivars. Glutamate is the most potent commonly occurring amino acid in terms of its umami flavor, and 5′-GMP is the most potent 5′-nucleotide. Calculation of the equivalent umami concentration, taking into account the levels of the major umami amino acids (glutamate and aspartate) and 5′-nucleotides (5′-GMP and 5′-AMP), shows levels that are up to 2.3-fold higher in *S. phureja* tubers compared with the highest levels measured in mature *S. tuberosum* cultivars. Sensory evaluations of boiled tubers from five cultivars were carried out using a trained panel. There are good correlations between the overall acceptability score and flavor attributes and the EUC value. The three *phurejas* that were analyzed are related in that 333-16 and Mayan Gold share a common parent with 257-28. Mayan Gold and 257-28 were both considered to have commercially acceptable flavors, and the former is now available in supermarkets. In contrast, 333-16 was rejected for commercialization because of unacceptable taste and texture. The level of detection of glutamate in solution varies between individuals, but a mean value of 1.5 mmol/L or ca. 250 mg/L has been published (*36*). It may be significant that the EUC for *S. tuberosum* cultivars is close to this threshold, whereas for *S. phureja*, the level will clearly exceed the threshold and perhaps accentuate the difference in taste. Interestingly, both glutamate and 5′-nucleotide levels were found to be elevated in the *S. phureja* tubers; the main factor affecting the increased EUC was the elevated level of 5′-GMP.

The formation of 5′-nucleotides was examined during the time course of cooking using several different boiling or steaming methods. In raw tubers the levels of 5′-nucleotides were very low, indicating that these compounds are formed during processing. More consistent and higher levels (ca. 10%) of 5′-nucleotides were measured on steaming than by boiling in water. 5′-GMP levels were maximal after 5–10 min of steaming for both *S. phureja* and *S. tuberosum* cultivars. Previously it has been suggested that 5′-nucleotides accumulate due to the action of nucleases during cooking processes, particularly due to RNA degradation (*37*). Ribonucleases are active under the pH and temperature conditions that occur during heating, particularly at around 50 °C (*38*). As the temperature of potato tissues increases slowly from 40 to 60 °C during some cooking processes (for example, boiling), nuclease activity may be significant (*17*). Despite the proposed involvement of RNases, no differences in RNase, or phosphohydrolytic enzyme, activities could be detected in extracts from *S. tuberosum* and *S. phureja* under our assay conditions. It may be that more subtle differences in these activities, which escape detection in our extraction and assay methods, account for the difference. For example, the RNase activity maxima may be different in *S. phureja* and *S. tuberosum*.

There was a large effect of developmental stage on the level of cooked 5′-nucleotides. Interestingly, as tuber development progressed, the effect on 5′-nucleotide levels was different for the *S. phureja* and *S. tuberosum* cultivars. In small developing tubers of both cultivars 5′-nucleotide levels were generally lower and levels in the *S. tuberosum* cultivars were not significantly different from those in the *S. phureja* cultivars. Conversely, at tuber maturity, whereas low levels of 5′-GMP could be detected

in the *S. tuberosum* tubers, significantly higher levels are present in cooked *S. phureja* tubers. In addition, levels of 5′-GMP remained higher in the *S. phureja* tubers, compared with *S. tuberosum*, after 6 weeks of storage at 4 °C. The implication of this finding in view of the demonstrated association of umami level and taste is that these compounds contribute to taste differences of tubers at different developmental stages. The changes that occur during tuber development that account for the differences in 5′-nucleotide levels are not yet clear. It is possible that changes in starch structure or content and/or textural differences may underpin the effect. It is known, for example, that *S. phureja* tubers cook significantly more quickly than *S. tuberosum* tubers (*26*). This may affect the accessibility of RNases with their substrate, assuming this is the mode of 5′-nucleotide formation.

A consistently higher glutamate level was measured in the mature *S. phureja* tubers than in those from *S. tuberosum*. The amino acid biosynthetic networks are complex and heavily regulated, although most of the biosynthetic genes have been cloned. Additionally, the contribution of amino acids synthesized in the tuber and amino acids imported from leaves remains to be fully resolved (*39*), but clearly amino acid transporters could have a key role (*40*). In view of the importance of amino acids in quality traits, understanding the mechanisms that control storage organ levels should be a priority.

As there are multiple factors that may be affecting the levels of the key umami metabolites, it is a challenging problem to dissect the molecular mechanisms involved. A factor not considered in this study is the potential for inorganic ions such a potassium and magnesium to modulate the umami taste. The levels of these ions should be included in further work. It is of significance that in crosses of *S. phureja* and *S. tuberosum* there is considerable variation in the levels of these compounds (data not shown). This may imply that a genetic approach might be applicable, for example, comparing quantitative trait loci (QTLs) for sensory traits with the map location of candidate genes. Other recently developed tools include a nearly whole transcriptome microarray for potato, which could be used to identify gene expression differences correlated with the trait of interest.

ABBREVIATIONS USED

AMP, adenosine 5′-monophosphate; Asp, aspartic acid; EUC, equivalent umami concentration; Glu, glutamic acid; GMP, guanosine 5′-monophosphate; HPAEC, high-performance anion exchange chromatography; HPLC, high-performance liquid chromatography; IMP, inosine 5′-monophosphate; IS, Inca Sun; MG, Mayan Gold; MON, Montrose; MSG, monosodium glutamate; OD, optical density; PD, Pentland Dell; PDE, phosphodiesterase; PME, phosphomonoesterase; PMSF, phenylmethanesulfonyl fluoride; PMT, photomultiplier tube; QTL, quantitative trait loci; SAX-SPE, strong anion exchange–solid phase extraction.

Supporting Information Available: Free amino acid contents of potato cultivars, 5′-nucleotide-liberating enzyme activity assays, and in-gel RNase assay. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Wang, Y.; Kays, S. J. Analytically directed flavour selection in breeding food crops. *J. Am. Soc. Hortic. Sci.* **2003**, *128*, 711–720.

(2) Mutti, B.; Grosch, W. Potato odorants of boiled potatoes. *Nahrung* **1999**, *43*, 302–306.

(3) Maga, J. A. Potato flavor. *Food Rev. Int.* **1994**, *10*, 1–48.

(4) Taylor, M. A.; McDougall, G. J.; Stewart, D. Potato flavour and texture. In *Potato Biology and Biotechnology*; Vreugdenhil, D., Ed.; Elsevier: Amsterdam, The Netherlands, 2007.

(5) Oruna-Concha, M. J.; Duckham, S. C.; Ames, J. M. Comparison of volatile compounds isolated from the skin and flesh of four potato cultivars after baking. *J. Agric. Food Chem.* **2001**, *49*, 2414–2421.

(6) Oruna-Concha, M. J.; Bakker, J.; Ames, J. M. Comparison of the volatile components of two cultivars of potato cooked by boiling, conventional baking and microwave baking. *J. Sci. Food Agric.* **2002**, *82*, 1080–1087.

(7) Duckham, S. C.; Dodson, A. T.; Bakker, J.; Ames, J. M. Volatile flavour components of baked potato flesh: a comparison of eleven potato cultivars. *Nahrung* **2001**, *45*, 317–323.

(8) Duckham, S. C.; Dodson, A. T.; Bakker, J.; Ames, J. M. Effect of cultivar and storage time on the volatile flavor components of baked potato. *J. Agric. Food Chem.* **2002**, *50*, 5640–5648.

(9) Dobson, G.; Griffiths, D. W.; Davies, H. V.; McNicol, J. W. Comparison of fatty acid and polar lipid contents of tubers from two potato species, *Solanum tuberosum* and *Solanum phureja*. *J. Agric. Food Chem.* **2004**, *52*, 6306–6314.

(10) Halpern, B. P. Glutamate and the flavour of foods. *J. Nutr.* **2000**, *130*, 910–914.

(11) Bellisle, F. Nutritional effects of umami in the human diet. *Food Rev. Int.* **1998**, *14*, 309–319.

(12) Beksan, E.; Schieberle, P.; Robert, F.; Blank, I.; Fay, L. B.; Schlichtherle-Cerny, H.; Hofmann, T. Synthesis and sensory characterization of novel umami-tasting glutamate glycoconjugates. *J. Agric. Food Chem.* **2003**, *51*, 5428–5436.

(13) Yamaguchi, S.; Yoshikawa, T.; Ikeda, S.; Ninomiya, T. Measurement of the relative taste intensity of some L-α-amino acids and 5′-nucleotides. *J. Food Sci.* **1971**, *36*, 846–849.

(14) Ugawa, T.; Kurihara, K. Enhancement of canine taste responses to umami substances by salts. *Am. J. Physiol.* **1994**, *266*, 944–949.

(15) Rotzoll, N.; Dunkel, A.; Hofmann, T. Quantitative studies, taste reconstitution, and omission experiments on the key taste compounds in Morel mushrooms (*Morchella deliciosa* fr.). *J. Agric. Food Chem.* **2006**, *54*, 2705–2711.

(16) Solms, J. Nonvolatile compounds and flavour. In *Gustation and Olfaction*; Olhloff, G., Thomas, A. F., Eds.; Academic Press: London, U.K., 1971; pp 92–110.

(17) Solms, J.; Wyler, R. Taste components of potatoes. In *Food Taste Chemistry*; Boudreau, J. C., Ed.; ACS Symposium Series 115; American Chemical Society: Washington, DC, 1979; pp 175–184.

(18) Maga, J. A.; McNeill, J. G. Nucleotide composition of incubated potatoes from five potato cultivars. *Lebensm.-Wiss. -Technol.* **1986**, *19*, 31–33.

(19) Brierley, E. R.; Bonner, P. L. R.; Cobb, A. H. Factors influencing the free amino acid content of potato (*Solanum tuberosum* L.) tubers during prolonged storage. *J. Sci. Food Agric.* **1996**, *70*, 515–525.

(20) Brierley, E. R.; Bonner, P. L. R.; Cobb, A. H. Aspects of amino acid metabolism in stored potato tubers (cv. Pentland Dell). *Plant Sci.* **1997**, *127*, 17–24.

(21) Davids, S. J.; Yaylayan, V. A.; Turcotte, G. Use of unusual storage temperatures to improve the amino acid profile of potatoes for novel flavouring applications. *Lebensm.-Wiss. -Technol.* **2004**, *37*, 619–626.

(22) Burton, W. G. *The Potato*, 3rd ed.; Longman Scientific and Technical: Essex, U.K., 1989.

(23) Allison, M. F.; Fowler, J. H.; Allen, E. J. Responses of potato (*Solanum tuberosum*) to potassium fertilizers. *J. Agric. Sci.* **2001**, *136*, 407–426.

(24) Buri, R.; Signer, V.; Solms, J. Die Bedeutung von freien aminosauren und nukleotiden fur den flavor von gekochten kartoffeln. *Lebensm.-Wiss. -Technol.* **1970**, *3*, 63–66.

(25) Barylko-Pikielna, N.; Kostyra, E. Sensory interaction of umami substances with model food matrices and its hedonic effect. *Food Qual. Pref.* **2007**, *18*, 751–758.
(26) De Maine, M. J.; Lees, A. K.; Muir, D. D.; Bradshaw, J. E.; Mackay, G. R. Long-day-adapted Phureja as a resource for potato breeding and genetic research. In *Potato, Global Research and Development*; Paul Khurana, S. M., Shekhawat, G. S., Singh, B. P., Pandey, S. K., Eds.; Indian Potato Association: Shimla, India, 2000; Vol. I, pp 134–137.
(27) Tomiya, N.; Ailor, E.; Lawrence, S. M.; Betenbaugh, M. J.; Lee, Y. C. Determination of nucleotides and sugar nucleotides involved in protein glycosylation by high-performance anion-exchange chromatography: sugar nucleotide contents in cultured insect cells and mammalian cells. *Anal. Biochem.* **2001**, *293*, 129–137.
(28) Matsuda, F.; Yamada, T.; Miyazawa, H.; Miyagawa, H.; Wakasa, K. Characterization of tryptophan-overproducing potato transgenic for a mutant rice anthranilate synthase α-subunit gene (*OASA1D*). *Planta* **2005**, *222*, 535–545.
(29) Jones, B. N.; Pääbo, S.; Stein, S. Amino acid analysis and enzymatic sequence determination of peptides by an improved *o*-phthaldialdehyde precolumn labelling procedure. *J. Liq. Chromatogr.* **1981**, *4*, 565–586.
(30) Stone, H.; Sidel, J. *Sensory Evaluation Practices*, 3rd ed.; Academic Press: London, U.K., 2004.
(31) Yen, Y.; Green, P. J. Identification and properties of the major ribonucleases of *Arabidopsis thaliana*. *Plant Physiol.* **1991**, *97*, 1487–1493.
(32) Shivakumar, P. D.; Vasanthi, N. S.; Shetty, H. S.; Smedegaard-Petersen, V. Ribonucleases in the seedlings of pearl millet and their involvement in resistance against downy mildew disease. *Eur. J. Plant Pathol.* **2000**, *106*, 825–836.
(33) Dumelin, E.; Solms, J. The formation of 5′-nucleotides during cooking of potatoes. *Potato Res.* **1976**, *19*, 215–228.
(34) Dolapchiev, L. B.; Bakalova, A. T.; Vassileva, R. A.; Ilieva, M. P.; Mihneva, M. D. Tobacco plant cell cultures with high content of extracellular phosphohydrolases. *Enzyme Microb. Technol.* **1996**, *19*, 384–388.
(35) Mamillapalli, R.; Haimovitz, R.; Ohad, M.; Shinitzky, M. Enhancement and inhibition of snake venom phosphodiesterase activity by phospholipids. *FEBS Lett.* **1998**, *436*, 256–258.
(36) Soldo, T.; Blank, I.; Hofmann, T. (+)-(*S*)-Alapyridaine-a general taste enhancer. *Chem. Senses* **2003**, *28*, 371–379.
(37) Buri, R.; Solms, J. Ribonucleic acid–a flavor precursor in potatoes. *Naturwissenschaften* **1971**, *58*, 56.
(38) Singh, A.; Ai, Y.; Kao, T.-h. Characterization of ribonuclease activity of three S-allele-associated proteins of *Petunia inflata*. *Plant Physiol.* **1991**, *96*, 61–68.
(39) Fischer, W. N.; André, B.; Rentsch, D.; Krolkiewicz, S.; Tegeder, M.; Breitkreuz, K.; Frommer, W. B. Amino acid transport in plants. *Trends Plant Sci.* **1998**, *3*, 188–195.
(40) Koch, W.; Kwart, M.; Laubner, M.; Heineke, D.; Stransky, H.; Frommer, W. B.; Tegeder, M. Reduced amino acid content in transgenic potato tubers due to antisense inhibition of the leaf H^+/amino acid symporter StAAP1. *Plant J.* **2003**, *33*, 211–220.

Received for review June 18, 2007. Revised manuscript received September 6, 2007. Accepted September 9, 2007. This work was supported by the Scottish Executive Environment and Rural Affairs Department.

JF0717900