Journal of Chromatography, 363 (1986) 361–371
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 689

SIMULTANEOUS LIQUID CHROMATOGRAPHIC DETERMINATION OF RIBONUCLEOSIDE-5'-MONOPHOSPHATES AND THEIR ISOMERS IN POTATO TUBERS

T. T. NGUYEN*, P. SPORNS and D. HADZIYEV
Department of Food Science, University of Alberta, Edmonton, Alberta T6G 2P5 (Canada)
(Received March 25th, 1986)

SUMMARY

A liquid chromatographic method has been developed for simultaneous determination of flavor-enhancing nucleotides (guanosine-5'-monophosphate and inosine-5'-monophosphate) along with other RNA breakdown products from raw and processed potatoes. The method consists of potato tuber homogenization in extracting solution of 0.5 *M* perchloric acid, removal of starch by addition of methanol and centrifugation. Prior to injection to a liquid chromatography column (Whatman Partisil SAX), the perchloric acid is removed from the extract by treatment with tri-*n*-octylamine–Freon 113 solution. With isocratic conditions using a solvent of 3% methanol and 0.008 *M* phosphate buffer (pH 4.15) the nucleotides present in potato tubers could be accurately quantitated.

INTRODUCTION

Ribonucleoside-5'-monophosphates are found in most foods of animal and plant origin[1]. Among the nucleotides degraded from ribonucleic acid (RNA), guanosine-5'-monophosphate (5'-GMP) is found to be a powerful flavor enhancer, while adenosine-5'-monophosphate (5'-AMP) with its 6-amino group, is a lesser flavor potentiator[2]. However, it is well known that, in the presence of a deaminase, 5'-AMP is readily converted to inosine-5'-monophosphate (5'-IMP), which is a strong flavor enhancer. In vegetables 5'-GMP and 5'-IMP are present in lesser quantity than other nucleotides such as cytidine-5'-monophosphate (5'-CMP), uridine-5'-monophosphate (5'-UMP) or 5'-AMP[1]. Solms and Wyler[3] reported that the ribonucleoside-5'-monophosphates present in cooked potatoes are due to degradation of RNA during tuber cooking. The methods so far used for detection and quantitation of nucleotides in food are prone to interference and are time consuming[4–7].

With the advent of liquid chromatography (LC) excellent new methods were developed for nucleotide determination. Several methods have been described for separation of a mixture of nucleotides using various LC systems[8–12], but most of these separations were accomplished on "model systems" consisting mostly of a mixture of pure compounds. Several attempts have been made to develop a LC method for

0021-9673/86/$03.50

nucleotide determination in food[13–16] and for biological research[9,10,17–19]. Recently, a review of the use of LC in nucleic acid research was given by Brown[20].

Separation by gradient elution is the commonly used LC method. A high concentration of salt buffer is often needed in the gradient elution method, which can shorten the analytical column life and increase the analysis time because of the need to reequilibrate the column to the initial conditions.

The extraction of nucleotides prior to LC analysis has been discussed by several authors[8,13,19]. Perchloric acid (PCA) or trichloroacetic acid are usually applied to precipitate proteins. Subsequently, the acids are removed by neutralization with potassium hydroxide or by an amine.

This paper describes a modified extraction method using PCA to extract nucleotides from raw and steam-cooked potatoes, followed by removal of soluble starch, protein, and PCA by applying methanol and an amine–Freon solution. In addition an isocratic LC method for the simultaneous analysis of ribonucleoside-5′-monophosphates and 2′- and 3′-isomers of AMP and GMP in potatoes is presented.

MATERIALS AND METHODS

Apparatus and chemicals

LC system. The LC system consisted of a Beckman Model 110 A pump; a 50-μl Rheodyne loop injector; a Data Control Spectromonitor III (Model 1204A, with the UV detector set to 254 nm); a Hewlett-Packard Model 3388A integrator; a Whatman 25 cm × 4.6 mm I.D. Partisil SAX column protected by a 7 cm × 2.1 mm I.D. guard column containing pellicular anion exchanger and by an additional 25 cm × 4.6 mm I.D. pre-injector column (Solvecon) containing silica gel.

LC-grade water was prepared by reverse osmosis (Milli-RO) and purified additionally by Milli-Q-system (Millipore, Bedford, MA, U.S.A.). Methanol and potassium dihydrogen phosphate were LC-grade. Tri-*n*-octylamine (98%) was supplied by Aldrich (Milwaukee, WI, U.S.A.) and Freon 113 by Terochem (Edmonton, Canada). A solution of 0.5 *M* tri-*n*-octylamine in Freon 113 was freshly prepared prior to the extraction step. All other common solvents or chemicals used were of analytical grade.

Standards. All nucleotides used as standards were obtained from Sigma (St. Louis, MO, U.S.A.). Standard compounds were dissolved in water as a stock solution (1 mg/ml). Lower concentrations were freshly prepared by dilution using methanol–water (1:2, v/v) as solvent.

LC separation conditions. The mobile phase used was 3% methanol in 0.008 *M* phosphate buffer (pH 4.15). The buffer was prepared fresh daily and, after degassing, this solvent was used for LC analysis at ambient temperature with a flow-rate of 1.5 ml/min.

Sample preparation

Potatoes. Russet Burbank, Norgold Russet, Norland, Pontiac, Kennebec and Shepody cultivars grown in Southern and Central Alberta were used.

The tubers were stored at 4°C and prior to analysis were reconditioned at 20°C for one week. They were kindly provided by Dr. W. Andrew (Department of Plant

Science, University of Alberta, Edmonton, Canada), the Edmonton Potato Growers Association and I&S Produce Ltd., a local food processor.

Nucleotide extraction. Peeled potato tubers were diced (0.2 × 0.2 cm) and mixed thoroughly to provide uniform sample. About 10 g of sample was weighed and to raw potato sample 10 ml of pre-cooled (4°C) 0.5 *M* PCA were added in order to prevent browning and other enzymatic reactions. Steam-cooked potatoes were prepared under pressure at 100°C for 30 min. The heating was stopped abruptly by immersing the glass beaker with sample in an ice bath. All samples were prepared at least in duplicate.

The raw or cooked potatoes were transferred to a 50-ml container of a Sorvall-Omni mixer. Pre-cooled 0.5 *M* PCA solution of about five times the volume of the sample was then added, followed by homogenization for 30–60 s with the container cooled in an ice bath. The total weight of the slurry obtained was recorded. An aliquot of close to 20 g was transferred to a 50-ml polypropylene centrifuge tube which was then cooled in ice and 10 ml of pre-cooled methanol was added. The resulting PCA–potato–methanol slurry was mixed vigorously and centrifuged at 12 000 *g* for 10 min at 4°C. The supernatant was collected. The residue was re-extracted with 10 ml 0.5 *M* PCA and 5 ml methanol, again centrifuged, and the supernatants combined. The volume was then made up to 50 ml at room temperature.

Treatment of the acid–methanol extract. The procedure adopted to remove PCA from the aqueous nucleotide samples was that developed by Khym[21] as later applied by Chen *et al.*[8] and Riss *et al.*[19]. In our laboratory it was applied by Currie *et al.*[13] for nucleotide extraction from meat samples. A 0.5 *M* tri-*n*-octylamine–Freon 113 solution (solution A) was used to remove PCA from the aqueous nucleotide extract.

Supernatant and solution A in equal volumes were thoroughly mixed in a screw cap test tube for 2 min using a Vortex mixer. The content was then centrifuged at 800 *g* to promote layer separation. Three layers were clearly separated, with the top water layer having a pH of 5.0 and containing all the nucleotides. An aliquot of this layer was then injected into the LC column.

Calculation. The concentration of potato nucleotides was calculated using either peak area or peak height of the 254 nm absorbing effluent. The calculation is based on both wet and dry potato bases as follows:

$$A = \frac{B}{C} \cdot D \cdot \frac{(50 \cdot G)}{(F \cdot H)}$$

where, A is the potato nucleotide content in μg/g; B the peak area or height of sample; C the peak area or height of the standard; D the nucleotide concentration in μg/ml in the standard solution; F the weight in gram of the PCA–potato slurry aliquot; G the total weight in gram of PCA–potato slurry and H the weight of potato sample on fresh or dry basis.

In the above calculation no volume correction is required for the PCA extraction with tri-*n*-octylamine–Freon 113, since the aqueous volume containing the nucleotides did not change during extraction. This was confirmed experimentally with standards and is also shown in the recovery data of Table VI.

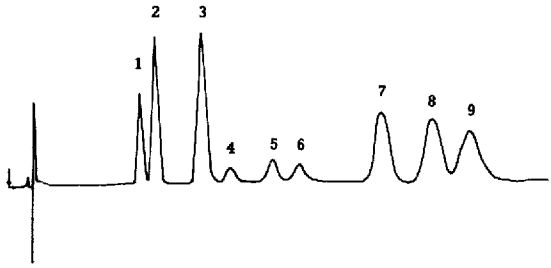

Fig. 1. Liquid chromatogram of nucleotides standard solution. Peak identity: 1 = 5′-CMP (3 μg/ml); 2 = 5′-UMP (3 μg/ml); 3 = 5′-AMP (3 μg/ml); 4 = 5′-IMP (0.6 μg/ml); 5 = 2′-AMP (0.6 μg/ml); 6 = 3′-AMP (0.6 μg/ml); 7 = 5′-GMP (3 μg/ml); 8 = 3′-GMP (3 μg/ml); 9 = 2′-GMP (3 μg/ml). Solvent system: 0.008 *M* phosphate buffer (pH 4.15) containing 3% methanol. Injection volume, 50 μl; flow-rate, 1.5 ml/min; detection at 254 nm.

RESULTS AND DISCUSSION

As shown in Fig. 1 and Table I–III isocratic LC elution separates the four major ribonucleoside-5′-monophosphates of RNA in addition to 5′-IMP and 2′- and 3′-isomers of AMP and GMP. The complete procedure, which also involves the extraction step, lasts only 75 min. The possible interfering compounds, such as deoxyribonucleoside-5′- and 3′-monophosphates, cyclic phosphate esters (2′:3′- and 3′:5′-ribonucleoside esters) were injected into the LC and found to have different retention times (Tables I–III). The corresponding nucleosides and free bases were eluted right after the solvent peak. However, as can be seen in Tables I–III 2′- and 3′-CMP had very similar retention times to 2′-AMP. Also, the retention times of these three compounds were so close to that of 3′-UMP, that an accurate quantitation of 2′-AMP could not be done. Attempts were made to separate these compounds, including changes in buffer molarity, pH, and the methanol to buffer ratio, but without success.

TABLE I

RETENTION TIMES OF RIBONUCLEOTIDES RECORDED BY THE OUTLINED LIQUID CHROMATOGRAPHY PROCEDURE

In this and following Tables, separation conditions are as in Fig. 1.

Nucleotide	*Retention time (min)*
5′-CMP	11.4
5′-UMP	14.3
5′-AMP	17.8
5′-IMP	21.5
2′-UMP	22.1
3′-UMP	23.3
2′-AMP	23.9
3′-CMP	24.0
2′-CMP	24.4
3′-AMP	26.5
5′-GMP	35.8
3′-GMP	39.8
2′-GMP	43.2

TABLE II

RETENTION TIMES OF DEOXYNUCLEOTIDES RECORDED BY THE OUTLINED LIQUID CHROMATOGRAPHY PROCEDURE

Deoxynucleotide	*Retention time (min)*
5′-dCMP	10.1
5′-TMP	13.1
3′-dCMP	15.5
3′-TMP	16.7
5′-dAMP	19.6
3′-dAMP	19.9
3′-dGMP	26.0
5′-dGMP	35.9

The use of 0.01 *M* phosphate buffer (pH 4.0), containing 10% methanol, brought about an overlap of 3′- and 5′-GMP isomers. In the same buffer system but without methanol, 5′-CMP and 5′-UMP remained unresolved. A number of buffer molarities ranging from 0.007 *M* to 0.02 *M*, ratios of methanol to buffer from 1.5 to 15% and also a pH range of 3.60 to 4.50 were systematically examined for their effects on resolution of the compounds assayed. The optimum phosphate buffer molarity was found to be 0.008 *M* at pH 4.15. A change in buffer pH resulted in several unresolved peaks (see Figs. 2 and 3). The optimum ratio of methanol to buffer was 3% (v/v). It was found that the nucleotides could not be well resolved if methanol was omitted.

Further confirmation of the assignment of nucleotide isomers found in potatoes was achieved by periodate oxidation. Addition of an excess of sodium periodate to the sample extract, followed by injection into the LC column, removes ribonucleoside-5′-phosphates whose *cis*-diol sugars are susceptible to oxidation, while 2′- and 3′-ribonucleotide peaks are retained (Fig. 4).

A linear response of peak areas or peak heights at 254 nm was obtained for all nucleotides (Table IV). The detection limits given are for peaks for which the height was at least twice that of the noise level. Table V presents the data for potato

TABLE III

RETENTION TIMES OF CYCLIC NUCLEOTIDES RECORDED BY THE OUTLINED LIQUID CHROMATOGRAPHY PROCEDURE

Nucleotide	*Retention time (min)*
2′:3′-UMP	8.3
2′:3′-CMP	8.4
3′:5′-UMP	8.5
3′:5′-CMP	8.5
2′:3′-AMP	8.9
3′:5′-AMP	9.2
2′:3′-GMP	10.5
3′:5′-GMP	11.0

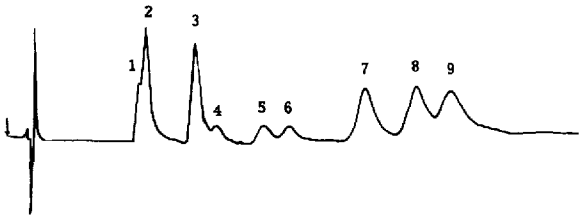

Fig. 2. Liquid chromatogram of nucleotides standard solution. Peak identity: 1 = 5′-CMP; 2 = 5′-UMP; 3 = 5′-AMP; 4 = 5′-IMP; 5 = 2′-AMP; 6 = 3′-AMP; 7 = 5′-GMP; 8 = 3′-GMP; 9 = 2′-GMP. Solvent system: 0.008 *M* phosphate buffer (pH 4.40) containing 3% methanol. Flow-rate, 1.5 ml/min.

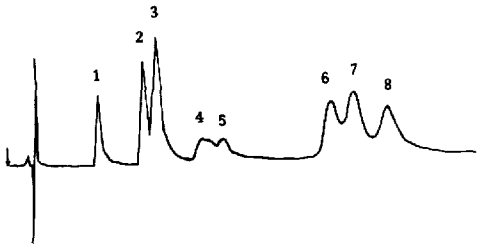

Fig. 3. Liquid chromatography of nucleotides standard solution. Peak identity: 1 = 5′-CMP; 2 = 5′-UMP; 3 = 5′-AMP; 4 = 5′-IMP + 2′-AMP; 5 = 3′-AMP; 6 = 5′-GMP; 7 = 3′-GMP; 8 = 2′-GMP. Solvent system: 0.008 *M* phosphate buffer (pH 3.85) containing 3% methanol. Flow-rate 1.5 ml/min.

nucleotides when measured at three wavelengths. The absorbance ratios at these wavelengths and particularly those of 250/260 and 280/260 were equal to those obtained for pure nucleotides except for 2′-AMP. As already stated this peak was partly co-eluted with 2′- and 3′-CMP and 3′-UMP.

TABLE IV

NUCLEOTIDES: LINEARITY OF PEAK AREA AND HEIGHT MEASUREMENTS (AT A CONCENTRATION RANGE OF 0.5 TO 5.0 ppm)

Detection limits are 100 ppb, except for 2′-, 3′- and 5′-GMPs where detection limits are at 200 ppb. [The American billion (10^9) is meant].

Nucleotide	*Correlation coefficient*	
	Peak area	*Peak height*
2′-AMP	0.9999	0.9996
3′-AMP	0.9999	0.9997
5′-AMP	0.9999	0.9996
5′-CMP	0.9998	0.9997
2′-GMP	0.9981	0.9989
3′-GMP	0.9985	0.9995
5′-GMP	0.9998	0.9986
5′-IMP	0.9999	0.9996
5′-UMP	0.9999	0.9997

TABLE V

NUCLEOTIDES IN ppm PRESENT IN STEAM-COOKED POTATO cv. SHEPODY MEASURED AT VARIOUS WAVELENGTHS

Nucleotide	*Wavelength (nm)*		
	254	*264*	*274*
2'-AMP	7.9	8.4	12.6
3'-AMP	6.5	6.5	6.5
5'-AMP	16.6	17.0	16.8
5'-CMP	19.9	19.6	20.3
2'-GMP	7.8	8.0	7.8
3'-GMP	13.5	13.7	13.9
5'-GMP	20.1	20.6	20.4
5'-UMP	20.6	20.1	20.6

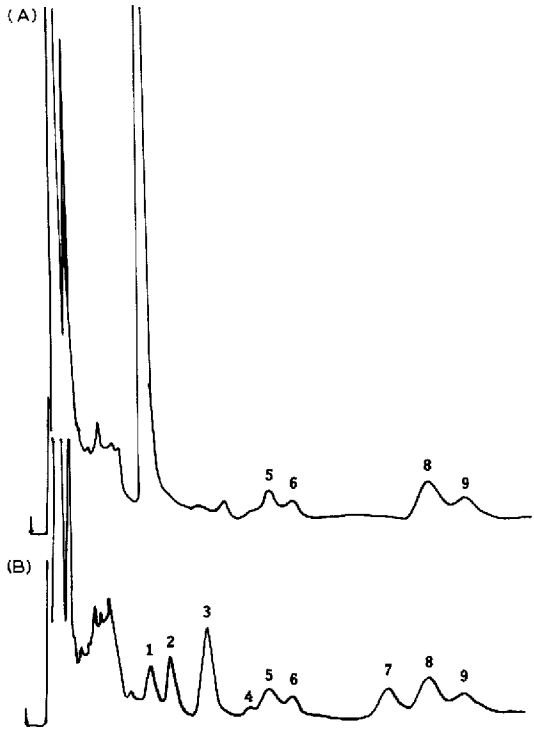

Fig. 4. Liquid chromatogram of nucleotides extracted from steam-cooked potato cv. Pontiac. (B) potato extract prior to and (A) after oxidation with periodate. Peak identity: 1 = 5'-CMP; 2 = 5'-UMP; 3 = 5'-AMP; 4 = unidentified; 5 = 2'-AMP; 6 = 3'-AMP; 7 = 5'-GMP; 8 = 3'-GMP; 9 = 2'-GMP. Separation conditions as in Fig. 1.

The high content of starch in potato parenchyma tissue is gelatinized during steam-cooking. Such gelatinized, and partly leached-out and solubilized starch interferes in the extraction procedure. Several extraction methods were examined to maximize the efficiency of nucleotide recovery. A modification of the extraction procedure developed by Khym[21] proved to be suitable. Khym's procedure applied an amine–Freon solution to remove PCA from the sample extract. However, with cooked potato samples, removal of starch was necessary prior to PCA removal. Thus the modified extraction method developed involves addition of methanol to the homogenized potato–PCA slurry. Methanol addition removes the solubilized starch from the aqueous nucleotide phase and, after centrifugation, provides a clear and transparent supernatant. If methanol is omitted, the supernatant is opaque, as is the nucleotide extract obtained after amine–Freon treatment. Such extracts containing starch impurities are unsuitable for injection into the LC column.

The possibility of interferences by potato tuber constituents other than starch were also investigated.

The contents of reducing sugars, glucose and fructose, and of sucrose in tubers were found at the harvest time to average 0.80 ± 0.14 and 3.35 ± 0.14 g/100 g dry matter respectively. The tubers stored for three months had an increased content of reducing sugars, an average of 1.28 ± 0.17, while the sucrose content decreased to 0.60 ± 0.10 g/100 g dry matter. All these sugars were extracted along with the nucleotides and were washed out from the column appearing with the solvent front. In addition their absorbance above 220 nm were negligible or non-existent.

The major organic acids of potato were oxalic, citric, malic, fumaric and pyroglutamic acid. Citric acid was the predominant one and its content was three to four times higher than malic acid, the next major acid in the tuber. These acids were mostly washed out with the solvent front and the absorbance above 220 nm was low, while at 254 nm it was non-existent. Pyroglutamic acid was eluted at a retention time (t_R) of 9.7 min, and was detected at levels well above those found in tubers.

Major phenolic acids present in potatoes were chlorogenic and caffeic acid, while other phenolic acids were present in traces. The high absorbing chlorogenic acid was eluted at t_R = 13.3 and did not interfere with ribonucleotides; however in deoxynucleotide separation it shouldered the peak of 5'-TMP.

Major free amino acids of potato, extracted along with the nucleotides, were aspartic and glutamic acids. These acidic amino acids were present in raw tubers at a level of 28.5 and 20.3 mg/g dry matter, while in steam-cooked samples these amounts were lowered to 23.0 and 18.5 mg/g dry matter, respectively. These acids were eluted at t_R = 9.3 min (aspartic) and 6.5 min (glutamic acid), and their detection limit at 254 nm was 10 mg/ml. This limit is much higher than the average 0.4 mg/ml of acids found in potato extract.

The neutral and polar aromatic amino acids such as tyrosine, phenylalanine and tryptophan (average content in raw tuber 4.0 mg/g dry matter), exhibited high absorbance but were readily washed out from the column, appearing with the solvent front. Other neutral and basic amino acids were also readily washed out, facilitated by the nature of the strong anion-exchange resin in the column.

Recovery studies and the precision of analysis results using steam-cooked potatoes, are illustrated by some results given in Tables VI and VII. Both analyses were performed on portions of a single tuber carried through the entire extraction pro-

TABLE VI

NUCLEOTIDE RECOVERIES FROM STEAM-COOKED POTATOES cv. PONTIAC

Nucleotide	*Added (ppm)*	*Content originally present in potatoes (ppm)*	*Total found (ppm)*	*Recovery* (%)*
2′-GMP	100	59.5	157.3	98
	150	58.7	211.1	101
	200	59.0	258.3	100
3′-GMP	100	91.9	192.0	100
	150	90.8	245.2	103
	200	91.2	294.8	102
5′-GMP	100	74.6	172.7	98
	150	73.6	229.9	104
	200	74.0	280.5	103
5′-UMP	100	68.1	171.5	103
	150	67.2	223.6	104
	200	67.6	276.0	104

* Recovery (%) = (total found − content originally present)/(amount added) × 100.

cedure for each analysis. They provided a proof that no nucleotide losses occur by starch adsorption and/or interaction with potato proteins.

Since heat treatment at acidic conditions might bring about isomerization of 2′- and 3′-isomers into an equimolar mixture of these isomers for both purine and pyrimidine ribonucleotides[22,23] and thus provide a source of error, pure 2′-AMP and 2′-GMP, and 3′-AMP and 3′-GMP were subjected to the same heat treatment as potatoes. Their standard solutions were boiled for 30 min in 0.05 *M* phosphate buffer

TABLE VII

PRECISION ANALYSIS IN A STEAM-COOKED POTATO cv. KENNEBEC

Nucleotide	*Content in ppm/potato fresh weight*					
	Extraction					
	1	*2*	*3*	*4*	*Mean*	*S.D.*
3′-AMP	7.0	7.0	7.1	6.9	7.0	0.07
5′-AMP	7.1	7.4	7.0	7.4	7.2	0.18
5′-CMP	9.2	9.0	9.2	9.4	9.2	0.13
2′-GMP	14.9	14.6	14.6	14.8	14.7	0.17
3′-GMP	27.2	26.9	26.8	27.1	27.0	0.17
5′-GMP	5.0	5.0	5.1	5.1	5.0	0.05
2′-UMP	2.0	2.1	2.0	2.1	2.1	0.07
5′-UMP	10.5	10.3	10.8	10.6	10.6	0.20

TABLE VIII

NUCLEOTIDES CONTENT (ppm/DRY MATTER) FOUND IN SOME RAW POTATO CULTIVARS, GROWN IN SOUTHERN AND CENTRAL ALBERTA

Nucleotide	*Potato cultivar*				
	*Kennebec**	*Norgold Russet*	*Norland*	*Pontiac*	*Russet Burbank*
5'-AMP	85.0	97.2	76.2	15.0	12.0
5'-GMP	17.2	14.3	16.0	N.D.**	N.D.
5'-UMP	68.6	193.6	31.2	45.6	150.6

* Moisture contents are as follows: Kennebec, 80; Norgold Russet, 80; Norland, 81; Pontiac, 81 and Russet Burbank, 78.

** Below the detection limit.

TABLE IX

NUCLEOTIDES CONTENT (ppm/DRY MATTER) FOUND IN SOME STEAM-COOKED POTATO CULTIVARS, GROWN IN SOUTHERN AND CENTRAL ALBERTA

Nucleotide	*Potato cultivar*				
	Kennebec	*Norgold Russet*	*Norland*	*Pontiac*	*Russet Burbank*
3'-AMP	32.5	18.9	8.5	27.0	13.4
5'-AMP	48.0	74.5	80.5	94.0	86.1
5'-CMP	39.0	115.9	74.9	67.8	86.5
2'-GMP	87.3	46.7	37.7	52.3	26.6
3'-GMP	112.5	97.4	71.1	103.0	110.9
5'-GMP	51.1	65.5	67.6	92.5	82.3
5'-UMP	94.4	141.5	98.8	92.8	140.6

(pH 6.0, close to the pH of potatoes). After this treatment the usual extraction procedure followed and the standards were examined by LC. These data revealed that no isomer interconversion occurred.

Results for five commercially-grown potato cultivars are presented in Tables VIII and IX. After steam-cooking the amount of ribonucleoside-5'-monophosphates in all cultivars was much higher than the amount found in raw tubers. In all cultivars 5'-IMP was absent. The 2'- and 3'-isomers are present in cooked rather than in raw potatoes, indicating thermal and/or an enzymatic breakdown of the tuber RNA. Further research into this breakdown is in progress.

CONCLUSION

The procedure outlined provides a rapid and accurate analysis for nucleotides in raw and cooked potatoes. A rapid isocratic LC method was developed for food

analysis which involves simultaneous determination of ribonucleoside-5′-monophosphates and the 2′- and 3′-isomers of AMP and GMP. The method is also suitable for following the thermal or enzymatic degradation of nucleic acid during food processing.

REFERENCES

1 M. D. Considine (Editor), *Foods and Food Production Encyclopedia*, Van Nostrand Reinhold, New York, 1982, p. 705.
2 A. Kuninaka, in R. Teranishi, A. Flath and H. Sugisawa (Editors), *Flavor Research, Recent Advances*, Marcel Dekker, New York, 1981, p. 305.
3 J. Solms and R. Wyler, *ACS Symp. Ser.*, 115 (1979) 175.
4 E. Dumelin and J. Solms, *Potato Res.*, 19 (1976) 215.
5 W. Hashida, T. Mouri and I. Shiga, *Food Technol.*, 20 (1966) 95.
6 H. G. Lento, J. A. Ford and A. E. Denton, *J. Food Sci.*, 29 (1963) 435.
7 N. Nakajima, K. Ichimawa, I. Yoshimura, C. Kuriyama, M. Kamada and E. Fujita, *J. Agr. Chem. Soc. Jpn.*, 37 (1963) 558.
8 S. C. Chen, P. R. Brown and D. M. Rosie, *J. Chromatogr. Sci.*, 15 (1977) 218.
9 R. A. De Abreu, J. M. VanBaal, C. H. M. M. de Bruyn, J. A. J. M. Bakkeren and E. D. A. M. Schretlen, *J. Chromatogr.*, 229 (1982) 67.
10 A. Floridi, C. A. Palmerini and C. Fini, *J. Chromatogr.*, 138 (1977) 203.
11 R. A. Hartwick and P. R. Brown, *J. Chromatogr.*, 112 (1975) 651.
12 S. Rokushika, Z. Y. Qui and H. Hatano, *J. Chromatogr.*, 320 (1985) 335.
13 R. W. Currie, P. Sporns and F. H. Wolfe, *J. Food Sci.*, 47 (1982) 1226.
14 Y. Kitada, M. Inone, K. Tamase, M. Imou, A. Hasuike, M. Sasaki and K. Tanigawa, *J. Assoc. Off. Anal. Chem.*, 66 (1983) 632.
15 T. T. Nguyen and P. Sporns, *J. Assoc. Off. Anal. Chem.*, 67 (1984) 747.
16 N. Parris, S. A. Palumbo and T. J. Montville, *J. Food Protect.*, 26 (1983) 614.
17 F. S. Anderson and R. C. Murphy, *J. Chromatogr.*, 121 (1976) 251.
18 L. S. Folley, S. D. Power and R. O. Poyton, *J. Chromatogr.*, 281 (1983) 199.
19 T. L. Riss, N. L. Zorich, M. D. Williams and A. Richardson, *J. Liq. Chromatogr.*, 3 (1980) 133.
20 R. P. Brown, *HPLC in Nucleic Acid Research, Methods and Applications*, Marcel Dekker, New York, 1984.
21 J. X. Khym, *Clin. Chem.*, 21 (1975) 1245.
22 D. M. Brown and A. R. Todd, *J. Chem. Soc.*, (1952) 44.
23 W. E. Cohn, *J. Am. Chem. Soc.*, 72 (1950) 2811.