ELSEVIER

Food Chemistry 64 (1999) 257–261

Food Chemistry

Analytical, Nutritional and Clinical Methods Section

Quantitative analysis of flavour precursors and pyruvate levels in different tissues and cultivars of onion (*Allium cepa*)

James R. Bacon [a,*], Graham K. Moates [b], Annie Ng [b], Michael J.C. Rhodes [a], Andrew C. Smith [b], Keith W. Waldron [b]

[a] *Biochemistry Department, Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, UK*
[b] *Food Biophysics Department, Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, UK*

Received 23 January 1998; received in revised form and accepted 13 July 1998

Abstract

Onions of three different cultivars were dissected as in commercial peeling operations to give four tissue types: the dry, brown skin, the top/bottom sections, the outer fleshy leaf bases and the inner fleshy leaf bases. All tissue types were assayed for alliinase-produced pyruvate and for flavour precursors (alk(en)yl cysteine sulphoxides) and a positive correlation was observed between the levels of these classes of compounds. In all cultivars, the top and bottom onion sections have the highest levels of both pyruvate and flavour precursors with levels in the dry, brown skin being low or absent. Significant increases in levels of both pyruvate and flavour precursors were observed in inner tissues and top/bottom sections after storage under commercial conditions.

Keywords: Onion; Flavour; Pyruvate; Alk(en)yl cysteine sulphoxides; Storage

1. Introduction

Substantial quantities (4.8 million tonnes, 1987) of onions are produced within the European Community. A substantial proportion of this production is processed (peeled, chopped and packed) to provide ingredients for food products. During this commercial production, significant amounts of waste are produced which consist of the top and bottom sections of the onion, the outer brown scales and the outer fleshy leaf bases which are removed using high pressure water sprays (Rutledge, 1991). Recently, however, the introduction of landfill taxation in the UK and other environmental concerns has encouraged research into the potential exploitation of this onion waste as a source of food ingredients.

One potential by-product of this onion waste is an onion oil, extracted by steam distillation, which could be used as a flavour additive. Onion flavour results only after tissue disruption by the rapid catabolism of endogenous S-alk(en)yl-L-cysteine sulphoxide flavour precursors by the enzyme, alliinase, to produce pyruvate, ammonia and a range of both volatile and non-volatile sulphur compounds, giving the characteristic odour and flavour of onions. Of the flavour precursors present in onions, *trans*-(+)-S-(1-propenyl)-L-cysteine sulphoxide (1-PECSO) is normally found in the highest concentration, together with smaller amounts of (+)-S-methyl-L-cysteine sulphoxide (MCSO) and (+)-S-propyl-L-cysteine sulphoxide (Lancaster & Boland, 1990).

Whilst a large proportion of the onion crop is sold during the harvest period, onions are often stored overwinter to ensure year-round supplies for customers. Freeman and Whenham (1976) have studied the effect of overwinter storage on pyruvate levels in onion bulbs as a measure of flavour intensity. Indeed, Randle (1997) infers from this that flavour precursors must therefore follow the same trend. The effect of storage on levels of flavour precursors has previously only been reported in one publication (Lancaster & Shaw, 1991).

Our objective is to report the differences in flavour precursor levels of onions due to variety, tissue type and storage and to compare these with changes in enzymatically-produced pyruvate levels. Although the changes in pyruvate levels upon storage have been reported previously, little is known about the changes in flavour precursor levels.

* Corresponding author. Tel.: +44-1603-255000; Fax: +44-1603-507723; E-mail: jim.bacon@bbsrc.ac.uk

0308-8146/98/$—see front matter
PII: S0308-8146(98)00156-3

2. Materials and methods

2.1. Materials

Onions (*Allium cepa* L.) of three different cultivars (Hysam, Durco and Grano de Oro) were supplied by the British Onion Producers Association, Louth, UK. The Hysam and Durco varieties were grown in Lincolnshire, UK and the Grano de Oro variety was grown in Spain. The onions (at least 20 bulbs of each variety) were cut and separated into different tissue types, to give (i) top and bottom (approximately 5–10 mm sliced off the top and bottom ends of the onions), (ii) brown dry outer skin, (iii) outer two fleshy layers and (iv) the remaining inner fleshy leaf bases. They were frozen immediately in liquid nitrogen after cutting, and stored at −40°C. The frozen onion tissue was ground under liquid nitrogen in a pestle and mortar before analysis.

For the storage experiment, whole onions from the same source were placed in a commercial store for six months at 0–0.5°C in the dark. These onions were then sectioned as described above for the fresh onions.

All solvents were of AnalaR grade and HPLC grade where applicable. Methanol, chloroform, acetonitrile and hydrochloric acid were obtained from Fisher Chemicals, Loughborough, UK; water was purified via an ELGASTAT Maxima system, ELGA, High Wycombe, UK; hydroxylamine and methyl cysteine were purchased from Sigma Chemical Co., Poole, UK; S-2-propenyl-L-cysteine sulphoxide (2-PECSO) and propyl cysteine were synthesised according to the methods of Iberl, Winkler, Müller, and Knobloch (1990) and Lancaster and Kelly (1983) respectively.

2.2. Methods

2.2.1. Extraction of flavour precursors

Two grams of frozen powdered onion tissue were extracted in 25 ml of methanol:chloroform:water (12:5:3). The solvent contained 0.7 mg ml^{-1} of hydroxylamine as alliinase inhibitor in accordance with the method of Edwards, Musker, Collin, and Britton (1994). Extraction was carried out using a Ystral homogeniser (Ystral GmbH, Dottingen, Germany) before filtration through a glass sinter funnel under vacuum. The residue was then re-extracted with a further 25 ml of solvent. The filtrate was transferred to a separating funnel to which 22.5 ml of chloroform and 27.5 ml of water were added, shaken and allowed to separate. Propyl cysteine was added as an internal standard to assist with quantification. The lower (chloroform) phase was discarded while the aqueous phase was evaporated on a rotary evaporator to a low volume, diluted to 10 ml with water and frozen. All extracts were diluted by a factor of 5 with 0.6 mM HCl and filtered through a 0.22 μm filter (Millipore, Watford, UK) prior to HPLC and LC/MS analysis. All extractions were performed in duplicate.

2.2.2. HPLC analysis

Chromatographic analysis was carried out using an Hewlett–Packard Series HP 1050 with diode array detector and Chemstation software. Samples (20 μl) were injected in duplicate for each extract onto a 250 × 4.6 mm i.d. Spherisorb S5P column (Phase Separation, Deeside Industrial Estate, Queensferry, UK), which was maintained at 30°C, and eluted using 0.6 mM HCl at a flow rate of 0.9 ml min^{-1}. The eluate was monitored at 215 and 280 nm. The results are expressed in equivalent units of the related compound, S-2-propenyl-L-cysteine sulphoxide (2-PECSO).

2.2.3. LC/MS

LC/MS was conducted on a Micromass Platform Electrospray/APCII Mass Spectrometer. Analyzation mode was negative ion electrospray. Samples (20 μl) were injected onto a Spherisorb S5P column, which was maintained at ambient temperature, and eluted using 0.6 mM HCl at a flow rate of 1.0 ml min^{-1}. Acetonitrile (0.5 ml min^{-1}) was added post column and the flow was then split with 50 μl min^{-1} going to the mass spectrometer and the remainder going to a Hewlett–Packard UV detector Model 1090. The ion source temperature of the mass spectrometer was set to 100°C and spectra were scanned at the rate of 3 s over the mass range 70–1000 with an inter-scan delay of 0.1 s.

2.2.4. Pyruvate analysis

The pyruvate levels in the different tissues were analysed according to the method of Wall and Corgan (1992) with the modifications described below.

As a control to measure basal levels of pyruvate, 10 g of frozen powdered onion tissue were mixed with 30 ml of 5% trichloroacetic acid to inactivate the alliinase. After 1 h, the mixture was blended using a Ystral homogeniser for 3 min before making up to 50 ml total volume with distilled water. The suspension was filtered through GF/C filter paper (Whatman International, Maidstone, UK) and the filtrate was analysed for pyruvate after dilution with distilled water by a factor of 5.

To measure total (basal and alliinase-produced) pyruvate, 10 g of frozen powdered onion tissue was blended for 3 min in 30 ml of distilled water. The sample was then left at room temperature for 15 min before making up to 50 ml with distilled water, filtering and diluting an aliquot by a factor of 10.

The prepared samples were then assayed according to the following procedure. Each reaction tube contained 1 ml of diluted filtrate, 1 ml of water, and 1 ml of 2,4-dinitrophenylhydrazine solution (0.0125% DNPH in 2M HCl). A blank was prepared with 2 ml of water and 1 ml of DNPH solution. All reaction tubes were

vortexed and placed in a water bath at 37°C for 10 minutes. After the incubation period, 5 ml of 0.6 M NaOH were added to the tubes before vortexing and allowing to stand for 5 minutes. The DNP hydrazine derivative of pyruvate was measured using a Perkin–Elmer spectrophotometer at 420 nm for both control and sample filtrates. Standards were prepared with sodium pyruvate. Enzymatically (alliinase)-produced pyruvate in each sample was calculated from the difference of the two determinations.

2.2.5. Gravimetric analysis

The dry weight of all tissue types was determined by freeze-drying aliquots in duplicate for 48 h. The freeze-dried samples were weighed and then heated to 60°C overnight to check for residual moisture.

3. Results and discussion

3.1. Effect of variety and tissue type on flavour precursor and pyruvate levels

The distribution of flavour precursors within Hysam, Durco and Grano de Oro onions was investigated. As described previously, the material was sectioned into four tissue types: (1) the outer two fleshy layers, (2) the remaining inner fleshy layers, (3) top and bottom, and (4) the dry, brown skin. The results are reported in Table 1.

1-PECSO was the most abundant flavour precursor with small amounts of MCSO (confirmation of molecular weights of the eluted species was obtained by LC-MS). (+)-S-propyl-L-cysteine sulphoxide was not found in these extracts. This is in agreement with other studies (e.g. Thomas & Parkin, 1994) which report that onions contain MCSO and 1-PECSO (14:86 w/w) with a total precursor content of 100–200 mg/100 g fresh weight. On a fresh weight basis, all three onion varieties exhibited the same distribution with the top and bottom sections having the highest content of flavour precursors (presumably in the fleshy parts rather than the skin or roots). The lowest levels of precursors were observed in the dry, brown skin suggesting that this material is of limited value as a source of flavour compounds. It is not clear whether the low level of flavour precursors observed in the dry, brown skin is due to contamination of this fraction with small amounts of tissue of intermediate scales consisting of both brown and white material, or due to low levels of alliinase activity in this tissue, hence giving low pyruvate values.

If the results are recalculated on a dry weight basis (data not shown), all three varieties exhibit a similar trend in levels of flavour precursors. The outer fleshy layers, however, having a higher water content, have the highest concentration of flavour precursors per gram dry weight, although the top and bottom sections remain a potentially valuable source of flavour components. This assumes, however, that the quality of the onion oil extracted from the top and bottom sections is comparable with that from the whole onion.

In this study, Grano de Oro has higher levels of flavour precursors than either Hysam or Durco suggesting that Grano de Oro has higher flavour potential than the other two varieties. This observation should be treated with caution since it is possible to grow Grano onions as a mild flavoured onion by modification of the growing conditions (Randle, 1997).

Table 1
Levels of flavour precursors and pyruvate in fresh onions

	Dry wt	1-PECSO	MCSO	Pyruvate
	(%)	(μmoles/g fresh weight)		(μmoles/g fresh weight)
Hysam				
Outer fleshy layers	12.1 ± 0.1	5.6 ± 0.7	0.48 ± 0.02	4.4 ± 0.3
Inner fleshy layers	13.3 ± 0.1	5.3 ± 0.2	0.74 ± 0.01	4.0 ± 0.3
Top and bottom	20.7 ± 0.6	7.1 ± 0.4	0.51 ± 0.01	5.3 ± 0.3
Dry brown skin	36.5 ± 1.1	2.8 ± 0.1	n.d.	0
Durco				
Outer fleshy layers	10.4 ± 0	5.7 ± 0.3	0.54 ± 0.01	4.7 ± 0.1
Inner fleshy layers	11.9 ± 0.5	4.9 ± 0.1	0.74 ± 0.01	4.0 ± 0.1
Top and bottom	16.1 ± 0.1	8.7 ± 0.9	n.d	7.2 ± 0.3
Dry brown skin	26.8 ± 1.0	3.3 ± 0.1	n.d.	2.7 ± 0.7
Grano de Oro				
Outer fleshy layers	8.7 ± 0.1	7.4 ± 0.4	0.42 ± 0.05	7.3 ± 0.4
Inner fleshy layers	9.2 ± 0.4	5.5 ± 0.1	0.46 ± 0.06	5.3 ± 0.5
Top and bottom	14.9 ± 0.2	10.6 ± 0.6	0.64 ± 0.01	11.3 ± 0.6
Dry brown skin	55.7 ± 2.1	4.1 ± 0.2	n.d.	1.1 ± 1.5

Values are given as mean ± standard deviation; n.d. = not determined.

Table 1 shows the same trends for the levels of alliinase-produced pyruvate between tissue types as those from the precursor analysis i.e. on a fresh weight basis, the top and bottom sections have the highest pyruvate levels with the dry, brown skin having much lower levels. Results are also in agreement with a more detailed study (Freeman, 1975) using pyruvate to estimate the flavour potential of onion tissues in which the stem tissue was found to contain the most pyruvate on a fresh weight basis.

The results also show differences between the individual onion varieties, with the Spanish variety, Grano de Oro, having the highest pyruvate levels. It is generally accepted that there is a high correlation between levels of enzymatically-produced pyruvate and perception of pungency (Schwimmer & Weston, 1961). However, it should be noted that the varieties were grown at different locations and may be influenced by levels of soil sulphur. The sulphur fertility level has a significant influence on these varietal differences (Randle, Lancaster, Shaw, Sutton, Hay, & Bussard, 1995).

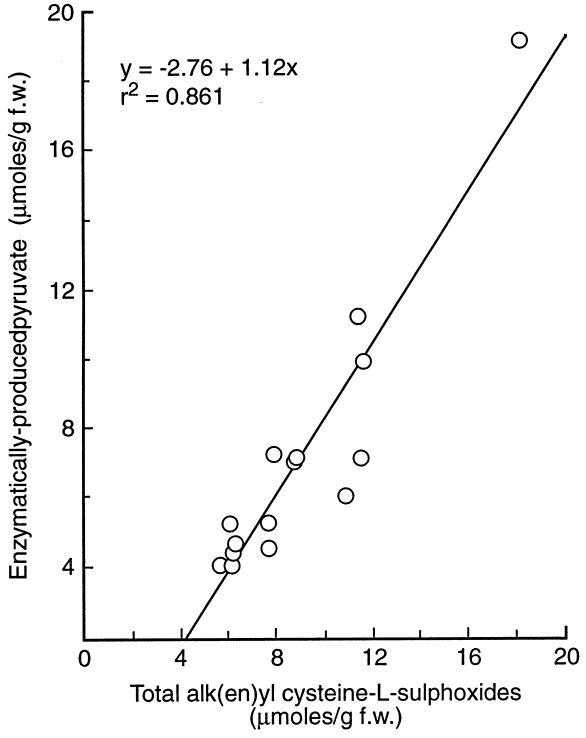

Fig. 1. Correlation between alliinase-produced pyruvate and total levels of alk(en)yl cysteine sulphoxides in onion tissues.

Fig. 1 shows a comparison of the values obtained for levels of enzymatically-produced pyruvate as determined spectrophotometrically and the total flavour precursors present as determined by HPLC in all the white fleshy material and top/bottom sections. It can be seen that a positive correlation ($y = -2.76 + 1.12x$) exists between the sets of data with a regression coefficient of 0.861. Given that a theoretical 1:1 molar ratio exists between pyruvate and its alliinase substrate, flavour precursors (alk(en)yl cysteine sulphoxides), this data demonstrates a significant relationship between these parameters and suggests that both assay techniques may represent a useful estimation of onion pungency.

3.2. *Effect of storage on flavour precursor and pyruvate levels*

After storage for 6 months at 0°C, both varieties show significant increases in levels of 1-PECSO in the inner tissues and the top/bottom sections (Table 2). Levels of 1-PECSO in the outer tissues and the brown skin show no significant changes. Similarly, significant increases in levels of enzymatically-produced pyruvate in the inner tissues and the top/bottom sections were observed. No significant water loss was observed during the storage period (Tables 1 and 2). This would seem to suggest that since the level of sulphur flavour volatiles is dependent on the level of flavour precursors (alk(en)yl cysteine sulphoxides) and the level of pyruvate detected is related to the catabolism of these flavour precursors, these tissues develop a more intense flavour upon storage. It should also be noted that the basal (control) levels of

Table 2
Levels of flavour precursors and pyruvate in stored onions

	Dry wt	1-PECSO	MCSO	Pyruvate
	(%)	(µmoles/g fresh weight)		(µmoles/g fresh weight)
Hysam				
Outer fleshy layers	11.4 ± 0.4	7.1 ± 0.7	0.50 ± 0.05	4.6 ± 0.3
Inner fleshy layers	13.0 ± 0.1	10.2 ± 0.2	0.59 ± 0.04	6.1 ± 0.2
Top and bottom	25.1 ± 2.1	11.5 ± 1.0	n.d.	10.0 ± 0.4
Dry brown skin	55.1 ± 0.4	2.3 ± 0.05	n.d.	0
Grano de Oro				
Outer fleshy layers	7.8 ± 0.1	8.2 ± 0.3	0.47 ± 0.05	7.1 ± 0.2
Inner fleshy layers	8.3 ± 0.1	10.9 ± 0.3	0.56 ± 0.03	7.2 ± 0.9
Top and bottom	18.6 ± 0.6	16.3 ± 1.4	1.59 ± 0.34	19.2 ± 1.4
Dry brown skin	58.5 ± 1.7	3.8 ± 0.2	n.d.	0.6 ± 0.5

Values are given as mean ± standard deviation; n.d. = not determined.

pyruvate in samples taken before and after storage show no significant differences (data not shown).

In alliums, most of the sulphur is stored in the form of non-protein amino acid derivatives (Lancaster & Boland, 1990). This sulphur is taken up from the soil by the roots as sulphate (SO_4^{2-}) and thus, after harvest, no further increase can occur. The observed increase in the levels of the sulphur-containing flavour precursors during storage must therefore be a result of a rearrangement of the total sulphur within the onion bulb to form the alk(en)yl cysteine sulphoxides. Previous studies (Freeman & Whenham, 1976) have shown that enzymatically-produced pyruvate increases up to 190 days' storage and decreases thereafter to 240 days' due to changes in rates of respiration and incidence of sprouting.

4. Conclusions

It is clear from the data presented here that waste from commercial onion peeling operations, i.e. outer fleshy leaf bases and top/bottom sections, can represent a valuable source of flavour ingredients. The levels of flavour precursors and pyruvate observed also suggest that flavour potential of onions can be enhanced by storage under commercial conditions. A correlation was noted between the levels of flavour precursors (S-alk(en)yl cysteine sulphoxides) and enzymatically-produced pyruvate, the standard marker for pungency in onions.

Acknowledgements

The authors acknowledge the financial assistance of the UK Biotechnology and Biological Sciences Research Council and the European Union (contract no. FAIR-CT96-1184), and also the assistance of Dr Fred Mellon with the LC-MS.

References

Edwards, S. J., Musker, D., Collin, H. A., & Britton, G. (1994). The analysis of S-alk(en)yl-L-cysteine sulfoxides (flavour precursors) from species of *Allium* by high performance liquid chromatography. *Phytochemical Analysis, 5*, 4–9.

Freeman, G. G. (1975). Distribution of flavour components in onion (*Allium cepa* L.), leek (*Allium porrum*) and garlic (*Allium sativum*). *Journal of the Science of Food and Agriculture, 26*, 471–481.

Freeman, G. G., & Whenham, R. J. (1976). Effect of overwinter storage at three temperatures on the flavour intensity of dry bulb onions. *Journal of the Science of Food and Agriculture, 27*, 37–42.

Iberl, B., Winkler, G., Müller, B., & Knobloch, K. (1990). Quantitative determination of allicin and alliin from garlic by HPLC. *Planta Medica, 56*, 320–326.

Lancaster, J. E., & Boland, M. J. (1990). Flavour biochemistry. In: H. D. Rabinowitch & J. L. Brewster (Eds.), *Onions and allied crops* (Vol. 3, pp. 33–72). Boca Raton, FL: CRC Press.

Lancaster, J. E., & Kelly, K. E. (1983). Quantitative analysis of the S-alk(en)yl-L-cysteine sulphoxides in onion (*Allium cepa* L.). *Journal of the Science of Food and Agriculture, 34*, 1229–1235.

Lancaster, J. E., & Shaw, M. L. (1991). Metabolism of γ-glutamyl peptides during development, storage and sprouting of onion bulbs. *Phytochemistry*, *30*, 2857–2859.

Randle, W. M. (1997). Onion flavor chemistry and factors influencing flavor intensity. In: *ACS Symposium Series* (Vol. 660, pp. 41–52). Washington, DC: American Chemical Society.

Randle, W. M., Lancaster, J. E., Shaw, M. L., Sutton, K. H., Hay, R. L., & Bussard, M. L. (1995). Quantifying onion flavor compounds responding to sulfur fertility—sulfur increases levels of alk(en)yl cysteine sulfoxides and biosynthetic intermediates. *Journal of the American Society for Horticultural Science, 120*, 1075–1081.

Rutledge, P. (1991). Preparation procedures. In: D. Arthey & C. Dennis (Eds.), *Vegetable processing* (pp. 42–68). Glasgow: Blackie and Sons.

Schwimmer, S., & Weston, W. J. (1961). Enzymatic development of pyruvic acid in onion as a measure of pungency. *Journal of Agricultural and Food Chemistry, 9*, 301–304.

Thomas, D. J., & Parkin, K. L. (1994). Quantification of alk(en)yl-L-cysteine sulfoxides and related amino acids in alliums by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry, 42*, 1632–1638.

Wall, M. M., & Corgan, J. N. (1992). Relationship between pyruvate analysis and flavour perception for onion pungency determination. *Horticultural Science, 27*, 1029–1030.