Onion Pungency Testing and Consumer Calibration

John Golding
NSW Department of Primary Industries

Project Number: VN04016

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This report is published by Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the onion industry.

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of the onion industry.

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ISBN 0 7341 1347 1

Published and distributed by:
Horticultural Australia Ltd
Level 1
50 Carrington Street
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399
E-Mail: horticulture@horticulture.com.au

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FINAL REPORT

Onion Pungency Testing and Consumer Calibration

Dr. John Golding
Gosford Horticultural Institute
NSW Department of Primary Industries
Locked Bag 26 Gosford NSW 2250

NSW DEPARTMENT OF
PRIMARY INDUSTRIES

Horticulture Australia **Project Number VN04016**

Onion Pungency Testing and Consumer Calibration

Organisation: NSW Department of Primary Industries

Research Team:
Principal Researcher:
John Golding — Research Horticulturist. Gosford Horticultural Institute. NSW Department of Primary Industries

Team Members:
Lorraine Spohr — Biometrician. Gosford Horticultural Institute. NSW Department of Primary Industries

Richard Meyer — Analytical Chemist. Wagga Wagga Agricultural Institute. NSW Department of Primary Industries

Dr. Patrick O'Riordan — Food Quality (Measurement & Perception) Food Science Australia. North Ryde (Sydney)

May 2006

This project was funded by the onion levy facilitated by HAL in partnership with Onions Australia. The Australian Government provides matched funding for all HAL's research and development activities.

Table of Contents

Table of Contents - continued

Acknowledgments

We wish to thank and acknowledge the financial contribution and support of the following organisations and individuals:

- New South Wales Department of Primary Industries

- Horticulture Australia Limited.

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['Onion Pungency Testing and Consumer Calibration' (VN 04016)]

We would also like to sincerely thank Professor Bill Randle, students and staff from the Department of Horticulture at the University of Georgia for their time, invaluable expertise, discussions and organisation. We also appreciate the time and discussions with National Onion Labs Inc. (David Burrell and Dr. Davey Kopsell) who took time out of their very busy schedule during harvest to show us around and talk with us about their program and the Georgia Vidalia onion industry.

We would also like to thank Trevor Twigden for sharing his vast knowledge and experience.

Nick Mendham (Enza Zaden Australia) and Alan Duff (Queensland Department of Primary Industries and Fisheries) for supply of onions for the trial.
Dr. Neil Coombes (Wagga Wagga Agricultural Institute) and Steve Morris (Wollongbar Agricultural Institute), NSW Department of Primary Industries for help with experimental design and statistical analysis.
Simon Drum (Horticulture Australia) and the Onion Research and Development Committee for helpful suggestions and guidance.

Media Summary

There is a considerable market in Australia for onions with a mild onion taste for their use in salads. However there was no way to ensure that consumers would consistently receive mild tasting onions, as there was no way to reliably measure onion pungency. Pungency is responsible for the hot onion taste when eating onions. This lack of a reliable cost-effective test for pungency was limiting the development of the Australian mild onion industry.

This project adapted and developed methods for extracting juice from the onion and to measure the chemical associated with the pungent taste in onions. This naturally occurring chemical in onions that is associated with pungency is called pyruvate. This was done at the NSW Department of Primary Industries, Wagga Wagga Agricultural Institute. The NSW DPI laboratory in Wagga is now accepting commercial samples to measure onion pungency.

Having successfully developed a reliable and cost-effective test for onion pungency, the project assessed whether onions could be grouped into categories by consumers based on the pyruvate levels in the onion. The pungency levels in a range of onions were measured and assessed by both specialist tasters and regular consumers. This taste testing was undertaken by Food Science Australia in Sydney and showed that consumers prefer mild onions, and they could reliably distinguish between the different classes of onion pungency. The results will provide industry with a tool to manage the establishment of the mild onion industry in Australia.

Technical Summary

There is large apparent potential for the development of the mild onion in Australia. However the Australian mild onion industry lacked a reliable cost-effective test for pungency. Pungency is responsible for the typical hot flavour of some onions. To guarantee that mild onions are not pungent, the development of a rapid and cost effective method for the assessment of onion pungency (pyruvate) was critical for industry.

This project adapted and developed the standard onion juice extraction and pungency testing procedure at NSW Department of Primary Industries, Wagga Wagga Agricultural Institute. An onion press was constructed was used in measuring onion pyruvate levels using the modified 'Schwimmer and Weston' method. The onion press pneumatically crushes the onion samples to extract the juice without the production of heat which can affect the pungency measurement. The pyruvate concentration in the juice was measured the modified 'Schwimmer and Weston' method at Wagga. This laboratory is accredited with the National Association of Testing Authorities (NATA) which means all systems and results are quality assured from the NATA ensuring the reproducibility, quality and rigour of the results. This assurance and external auditing is essential for the commercial testing service.

Having established a reliable and cost effective method to measure onion pungency, the project calibrated pyruvate levels in a range of onions to the Australian palate utilising comprehensive taste panel comparisons. These trained and consumer panel assessments of raw onions were conducted at Food Science Australia in Sydney. The results show that trained panel could reliably and accurately perceive differences in pyruvate levels (pungency) between different classes of onions based on their pyruvate concentration. Similarly the 100 untrained consumers could not detect differences in pungency between onions with the lower levels of pyruvate, but were able to reliably tell these onions from the higher levels of pyruvate. Conversely, the degree of consumer *liking* of the different onions classes varied with perceived pungency. As expected, onions with the lower levels of pyruvate (less than 6 $\mu M.mL^{-1}$ pyruvate) were equally *likable*, with the more pungent onions equally *un-likable*. Additional relationships between onion *flavour intensity* or *liking* and the background of the consumer (gender, age etc) were analysed. A further questionnaire of consumer attitudes and beliefs towards onions was also collected and analysed. The results will provide industry with a tool to manage the establishment of the mild onion industry in Australia.

1. Introduction

The Australian Onion Industry believes there is a significant potential for a mild onion industry in Australia. The mild onion (or Vidalia onion) industry in the state of Georgia (USA) generates over A$120 million each year. Mild onions are not pungent (hot) and are generally eaten raw in salads and sandwiches. However to guarantee that mild onions are not pungent, the development of a rapid and cost effective method for the assessment of onion pungency was critical for the Australian Onion Industry.

Onions Australia and Horticulture Australia Ltd. funded a research and development project commencing in April 2005 for the development of a rapid and cost effective method for the assessment of onion pungency using the modified 'Schwimmer and Weston' method. The project also calibrated this pungency assessment method to the Australian palate utilising comprehensive taste panel comparisons. The rational and scope of this project is outlined in the original Horticulture Australia *Expression of Interest* (Appendix 1) and summarised:

Project Title
'Onion Pungency Testing and Consumer Calibration' (VN 04016)

Project Objectives

- Develop a reliable and reproducible pungency test utilising the modified 'Schwimmer and Weston' method
- Calibrate the 'Schwimmer and Weston' method against the Australian palate utilising extensive taste panel comparisons
- Construct an onion juice press, establishing a recognised testing facility that will enable rapid and cost effective sampling of onion pungency

This was a collaborative project with NSW Department of Primary Industries (DPI) at Gosford Horticultural Institute with the pungency testing being conducted through NSW DPI Diagnostic and Analytical Services (DAS) at the Wagga Wagga Agricultural Institute. Richard Meyer is the chief chemist at DAS Wagga running the pungency testing. This project also utilised the extensive practical expertise of Food Science Australia (Dr. Patrick O'Riordan) at North Ryde in Sydney to calibrate the pungency assessment to the Australian palate utilising comprehensive taste panel comparisons.

Pungency Background

The characteristic flavour of onions develops when the tissue is cut or damaged. The enzyme alliinase which is localised in the vacuole, is released to hydrolyse the flavour precursors, collectively known as the *S*-alk(en)yl cysteine sulfoxides (ACSOs), which are localised in the cytoplasm. This gives rise to pyruvate, ammonia and the many volatile sulphur compounds associated with flavour and odour (Figure 1). The reaction of the enzyme and substrate to produce sulphur volatiles is the central point of onion flavour biochemistry.

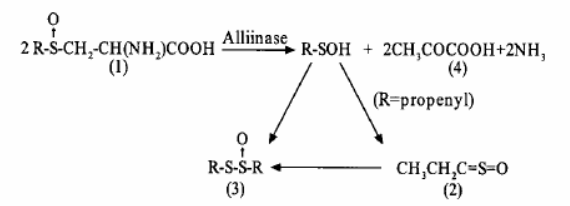


Figure 1 Flavour reaction in onion

(1) alkenyl cysteine sulfoxides:
R= CH_3 (methyl); **R=** CH_3-CH_2-CH_2 (propyl); **R=** CH_3 CH_3-CH=CH (propenyl) ;
(2) thiopropanal *S*-oxide ;(3) thio- CH_3-CH=CH sulphinates ; and (4) pyruvic acid
(from Lancaster *et al*., 1998)

In onions there are three ACSOs ((*1) above*), (+)-*S*-methyl-L-cystine sulphoxide (MCSO), (+)-*S*-propyl-L-cystine sulphoxide (PCSO) and *trans* (+)-*S*-(1-propenyl)-L-cystine sulphoxide (1-PRENCSO), however PRENCSO generally predominates (Randle *et al*., 1995). The unstable sulphenic acids re-arrange over time to produce disulfides and other sulphur compounds.
(*Z, E*) Propanethial *S*-oxide or the lachrymatory factor (LF) arises from the hydrolysis of 1-propyl cysteine sulphoxide (1-PRENCSO) and is responsible for the tear producing, mouth burn and heat associated with eating onions. Sensory attributes from the LF can be overwhelming and can dominate the experience of eating onions with high levels of 1-PRENCSO.

The current testing of pungency in Georgia is based on measuring pyruvate (a breakdown product of the ACSOs) by spectrophotometry (see Section 4). This method is relatively rapid, although sample preparation can restrict sample through-put.

In Georgia, if Vidalia onions are measured to have a pungency less than 3.5 $\mu M.mL^{-1}$ pyruvate, then they can marketed as Certified Extra Sweet^{TM}. If the pungency is less than 5.0 $\mu M.mL^{-1}$ pyruvate, then the onions can be marketed as Certified Sweet^{TM}. If the onions are above 5.0 $\mu M.mL^{-1}$ pyruvate, then the onions can not be marketed as Vidalia onions as they are considered too pungent to classify as 'Vidalia' onions.

2. Study visit to Georgia (USA) for Discussions on Mild Onion Pungency Testing

It was recommended in February 2005 by the Onion Research and Development Committee that John Golding (Project leader) and Trevor Twigden (Chair Onion R & D Committee) meet with Professor Bill Randle at the University of Georgia for detailed discussions about the plans and scope of the project.

In May 2005, Trevor Twigden and John Golding spent five days with Dr. Randle's laboratory and associated visits (30 April – 8 May 2005). These discussions and visits with industry were crucial in the development, planning of the project and future directions for the Australian onion industry. A full travel report to the University of Georgia for discussions on mild onion pungency testing is included as Appendix 2.

2.1 Discussions and meetings with Dr. Bill Randle and staff at the University of Georgia, Athens

These discussions and visits were invaluable for the directions and scope of the project and the development of the Australian mild onion industry. Outcomes of the visit with Bill Randle and others at the University of Georgia include:

- Construction of the onion press (see Section 3 – Onion Press)

- Pyruvate testing procedure
  Precise details of onion pungency testing procedure were examined. This included onion crushing, juice collection, sample handling, measurement of pyruvate using spectrophotometer, preparation and handling of solvents and standards, safe use of chemicals, data handling and analysis. In order to resolve any confusion between testing facilities, the Georgia State Department of Agriculture published actual sampling and testing procedure (see full NSW DPI Travel Report – Appendix 2). This was adapted to develop the pyruvate testing by Richard Meyer at NSW Department of Primary Industries Wagga Wagga Agricultural Institute.

- Sampling schedule for meaningful analysis of pungency from an onion population
  The most important factor for pyruvate testing is sampling of the onion bulbs from the entire field population. There is huge field variation in pungency which determines the level of sampling required to ensure the onion population can be classified as 'mild'. Research over many years at the University of Georgia has concluded that two (10 onion bulb) samples per acre are required to assess the pungency of the crop under Georgia's specific climatic and agronomic conditions.

- Lachrymatory Factor (LF)
  The lachrymatory factor (LF) is a chemical compound that is responsible for the mouth burn and tears production when eating some onions. The validity and ability to routinely quantify LF is not obvious in the current scientific literature and was not considered in this initial Horticulture Australia proposal (February 2005). However developments at the University of Georgia and the National Onion Labs Inc. have increased the importance of LF in onion pungency testing. The lachrymatory factor was measured in each onion used in the sensory trials for this project.

- Sensory analysis

  Another important outcome of this visit was the potential to use the same onion for both the pungency testing and the sensory analysis. We were initially planning to sample different onions from the same onion population for the chemical and sensory analysis. This was because the literature stated that the same onion could not be used due to chemical changes in the cut onion. However re-slicing the cut onion-half before presentation to the taste panel overcomes this issue. The potential to use the same onion for both tests increases the robustness and reliability of the data.

Under-cut Vidalia onions near Lyons, GA
Onions are allowed to air 'cure' before hand harvesting.

Trevor Twigden (Onions Australia), Dr Davey Kopsell (National Onion Labs Inc) and Dr Bill Randle (University of Georgia)

2.2 Discussions and meetings with National Onion Labs Inc

National Onion Labs Inc. is a private pungency testing laboratory based in Collins Georgia. It was formed in 1998 to ensure that Vidalia sweet onions did have low levels of pyruvate. National Onion Labs Inc. now provides a wide range of customised services to sweet onion producers in North America (eg. Washington State, Texas etc) and many Central and South American (e.g. Peru etc) locations. Since 1998 the company has used GPS (global positioning systems) field sampling and laboratory based testing of onion flavour. Using precision farming techniques, the National Onion Labs Inc. take two 10 bulb sample per acre and a single soil sample per acre for complete soil and nutrient analysis. The matching the of onion yield, size and pungency data of each sample to the field soil nutrient analysis in each paddock is a very powerful agronomic technique and has been successful for the growers in the scheme, not only with reducing pungency and field variation, increasing size and yields but with premiums being paid by US supermarkets. The agronomic database collected over the years by National Onion Labs Inc. is a valuable asset and will continue to assist the company manage onion yield and quality for its clients.

Vidalia onion pungency testing preparation Onion press in operation

National Onions Labs, Inc. Collins GA, USA

2.3 Recommendations from visit

This visit to the University of Georgia was invaluable to this project and the Australian industry. The outcomes of the visit fast-tracked the development and reliability of the pungency test and helped in designing appropriate and reliable sensory analysis.

Project recommendations

From discussions with Professor Bill Randle and others, some fundamental changes to the initial proposal were included to improve the confidence and reliability of the results. Some of these main issues included:

- Removing the necessity to measure background pyruvate (Yoo and Pike, 2001). From the initial scientific literature the levels of background pyruvate were an issue in determining onion pungency. However discussions with industry and experienced chemists removed the need to measure background pyruvate.
- In addition to pyruvate and soluble solids content (SSC(%)), lachrymatory factor (LF) using GC/FID was also measured
- The ability to use the same onion for both the chemical and sensory analysis (with precautions)

Other minor changes and developments from discussions and demonstrations at the University of Georgia of the current pyruvate method were implemented in the project.

Industry recommendations

The Australian onion industry must carefully consider the management of the crucial issues of field sampling for pungency and mild onion certification.

3. Onion Press

The extraction of juice from the onion is an important limiting step in the cost-effective and reliable pyruvate determination. The construction of an onion juice press was a key outcome of the project:

Project Outcomes

- Develop a reliable and reproducible pungency test utilising the modified 'Schwimmer and Weston' method
- Calibrate the 'Schwimmer and Weston' method against the Australian palate utilising extensive taste panel comparisons
- Import or construct an onion juice press, establishing a recognised testing facility that will enable rapid and cost effective sampling of onion pungency

(EOI, December 2005 – Appendix 1)

3.1 Onion Press Construction

The onion press pneumatically crushes the onion (under constant pressure) to extract the juice which is used for pungency testing. The pneumatic pressing of the onion immediately releases the juice from the flesh under normal room temperatures. In contrast other juice extraction procedures, such as blending, which can take significant time and also generate heat. This excess heat and time can denature the alliinase enzyme (protein) and bias the estimation of pyruvate in the juice. Hence pneumatically crushing the flesh to obtain the juice is preferred.

An onion press was constructed from plans adapted from the University of Georgia (Bill Randle). This was done by John Zoutendyk (Senior Laboratory Craftsman at NSW DPI, Wagga), Richard Meyer (Analytical Chemist at NSW DPI, Wagga) and Alan Palmer (Engineer at NSW DPI, Trangie). Plans for the onion press were developed and adapted. The press was locally constructed and commissioned at NSW DPI Wagga Wagga. This was supervised and is currently run by Richard Meyer.

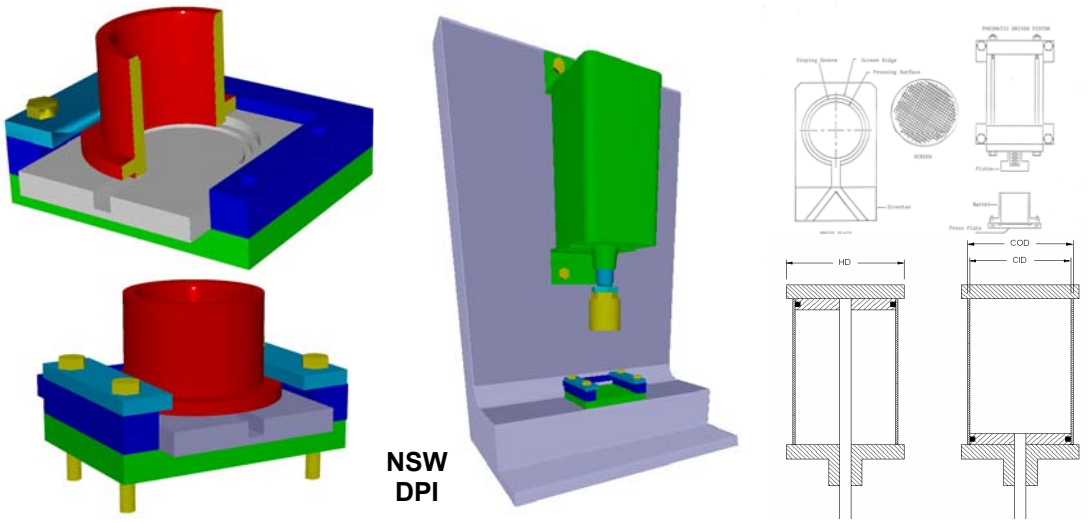


Figure 2 Onion press designs and operating press at NSW Department of Primary Industries, Wagga Wagga Agricultural Institute

3.2 Press Verification

The efficacy and reproducibility of the pneumatic onion press was assessed by comparing the pyruvate concentrations of both onion halves using the juice extracted by the onion press (Section 4). This was compared to the reproducibility of pyruvate concentrations of onion halves prepared using a blender extraction, where the onion half was weighed, and blended with an equal weight of water for 30 seconds in a commercial blending unit. The macerate was then coarsely filtered and the resulting juice analysed at the same time as the juice obtained from the press.

The results are shown in Table 1. Statistical analysis using a t-test on the relative errors showed there were no differences in the reproducibility between the two preparation techniques (p = 0.05 significance level). However the blender extraction method is not recommended due to potential large errors.

Prep	Date analysed	Analysis	ref #	Tube No.	raw	Pyruvate (mmoles/mL)	Relative Error	Difference
NT	11.11.05	RM	blender 1	PT 140 wedge 011	0.108	3.92		
NT	11.11.05	RM	blender 1	PT 140 wedge 012	0.114	4.16	-3.0	-0.2
NT	11.11.05	RM	blender 2	PT 140 wedge 013	0.0449	1.40		
NT	11.11.05	RM	blender 2	PT 140 wedge 014	0.0412	1.25	5.6	0.1
NT	11.11.05	RM	blender 3	PT 140 wedge 015	0.0414	1.26		
NT	11.11.05	RM	blender 3	PT 140 wedge 016	0.0411	1.24	0.5	0.0
NT	11.11.05	RM	blender 4	PT 140 wedge 017	0.0464	1.46		
NT	11.11.05	RM	blender 4	PT 140 wedge 018	0.0448	1.39	2.2	0.1
NT	11.11.05	RM	blender 5	PT 140 wedge 019	0.0513	1.65		
NT	11.11.05	RM	blender 5	PT 140 wedge 020	0.0392	1.17	17.2	0.5
NT	11.11.05	RM	blender 6	PT 140 wedge 021	0.0442	1.37		
NT	11.11.05	RM	blender 6	PT 140 wedge 022	0.0515	1.66	-9.6	-0.3
NT	11.11.05	RM	Press 1	PT 140 wedge 023	0.125	4.60		
NT	11.11.05	RM	Press 1	PT 140 wedge 024	0.134	4.96	-3.8	-0.4
NT	11.11.05	RM	Press 2	PT 140 wedge 025	0.117	4.28		
NT	11.11.05	RM	Press 2	PT 140 wedge 026	0.138	5.12	-8.9	-0.8
NT	11.11.05	RM	Press 3	PT 140 wedge 027	0.115	4.20		
NT	11.11.05	RM	Press 3	PT 140 wedge 028	0.112	4.08	1.4	0.1
NT	11.11.05	RM	Press 4	PT 140 wedge 029	0.107	3.88		
NT	11.11.05	RM	Press 4	PT 140 wedge 030	0.114	4.16	-3.5	-0.3
NT	11.11.05	RM	Press 5	PT 140 wedge 031	0.131	4.84		
NT	11.11.05	RM	Press 5	PT 140 wedge 032	0.134	4.96	-1.2	-0.1

Table 1 Onion press verification. Onion juice samples were obtained using the onion press or the blender extraction procedure. Samples of both halves were extracted separately and analysed as per Section 4.

4. Pungency Testing

A key outcome of this project was the development of a reliable pungency test:

Project Outcomes

- Develop a reliable and reproducible pungency test utilising the modified 'Schwimmer and Weston' method
- Calibrate the 'Schwimmer and Weston' method against the Australian palate utilising extensive taste panel comparisons
- Import or construct an onion juice press, establishing a recognised testing facility that will enable rapid and cost effective sampling of onion pungency

(EOI, December 2005 – Appendix 1)

4.1 Pyruvate Testing Procedure

The testing procedures and regulations adopted by the State of Georgia that were developed by Dr. Bill Randle using the modified 'Schwimmer and Weston' method were followed at NSW DPI Wagga Wagga.

A flowchart of the onion preparation and analysis procedure is outlined in Figure 3. This was based on the standard pungency analysis testing procedure (as prescribed the Georgia State Agriculture Department and conducted at the University of Georgia) and summarised as:

- A core sample or wedge is cut from the onion
- The sample is squeezed in the onion press (Section 3)
- 0.5 ml of the juice is put into a 40 ml test tube
- The slurry is allowed to sit for 10 minutes
- 1.5 ml of 5% trichloroacetic acid is added to each test tube and vortexed
- 18 ml of deionized water is added to each test tube, which is vortexed and capped
- Approximately 5mL of sample used for pyruvate determination using the automated Flow Injection Analyser (FIA):
    - 1ml of 2,4-dinitrophenylhydrazine and 1ml of deionized water is added and mixed
    - Samples are incubated at 60ºC for 2½ minutes
    - 5 ml of 0.6 N sodium hydroxide and mixed
    - Samples are measured on a spectrophotometer at 520nm. Standards are made and run at the same conditions to create a standard curve

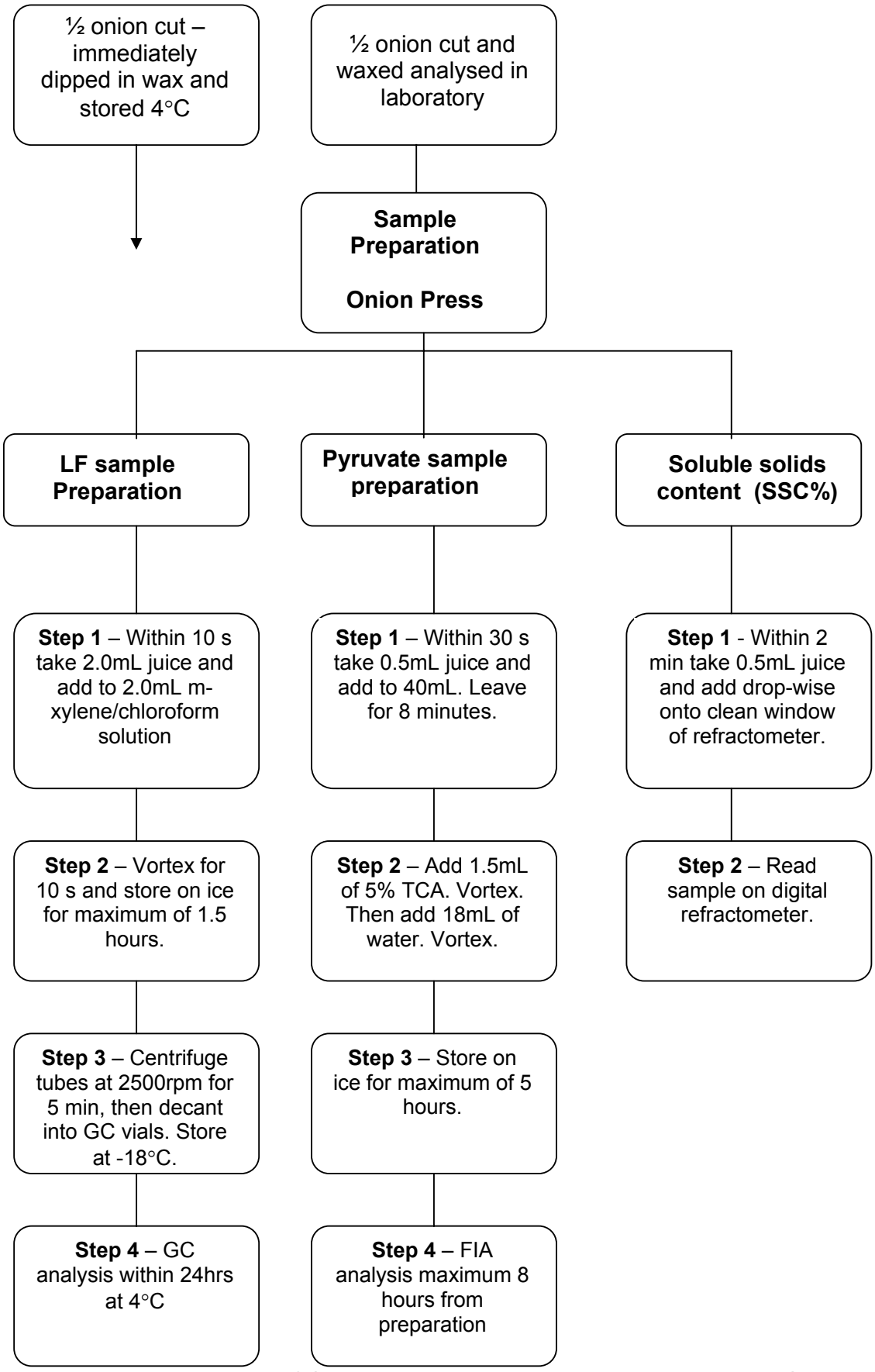


Figure 3 Flow chart of Onion Analysis Preparation Procedure at NSW DPI

4.1.1 Development and Validation of Flow Injection Analyser (FIA) Technology for Pyruvate Determination

A significant improvement to the University of Georgia pyruvate method was the development of Flow Injection Analyser (FIA) technology into the method (Figure 4). This analysis was chosen as the desired method as it had distinct advantages over the traditional manual spectrophotometric (University of Georgia) method including:

1. Minimising operator error giving better accuracy and reproducibility. Error is reduced as the FIA automatically dispenses chemical reagents at a continuous rate, rather than an operator dispensing volumes from calibrated pipettes. During the incubation period, the samples analysed by the FIA are incubated at elevated temperatures for exactly the same time and temperature, avoiding any potential errors due to density change from temperature, and inconsistent incubation times.

2. Faster throughput of samples. The FIA method developed analyses the samples in approximately 2.5 minutes (once initially prepared), eliminating the 10 minute incubation time of the manual method. Samples are presented in racks of 60, with a total capacity of 300 samples.

3. Greater efficiency. Once the instrument is set up, it is essentially operator free, eliminating the need for trained personnel to process the samples manually. The calibrations are done automatically, and there is a completely electronic traceable data.

Figure 4 Flow Injection Analyser (FIA) used for pyruvate measurement at NSW DPI Wagga Wagga Agricultural Institute

Validation of FIA Pyruvate Method and Method Performance

- **FIA Performance Validation**

The FIA method was developed following extensive method development and various performance validations. These details are presented in Appendix 3. Figure 5 illustrates the data collection form the FIA software platform. Concentrations are calibrated and calculated from peak areas.

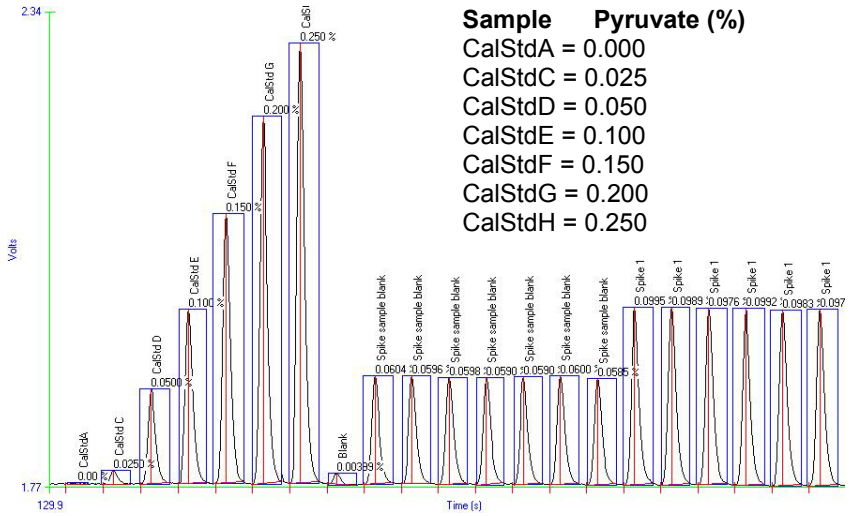
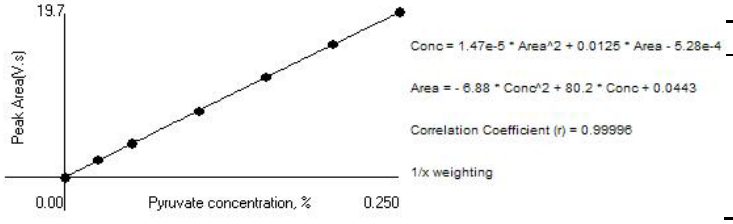


Figure 5 Calibration and sample data collected from Flow Injection Analyser (note consistency of response in spike data)

- **Method Linearity, Sensitivity and Range**

The method linearity was checked according to NATA guidelines (NATA, 2004). Six standards were prepared to cover a calibration range of 1 - 11 μM.mL^{-1} pyruvate at equally spaced intervals.
Least squares regression is applied to the data set (peak areas) to establish the relationship between the instrument response (y) and the pyruvate concentration (x) for which the linear model is: $y = a + bx$

The results are shown in Figure 6. The regression statistics show that there is no indication of non-linear behaviour across the pyruvate concentration range studied.



Regression Statistics	
Multiple R	0.999974
R Square	0.999948
Adjusted R Square	0.999938
Standard Error	0.05 μM.mL^{-1}
Observations	7

Figure 6 Linearity of FIA response across the range of expected pyruvate concentrations (1 - 11 μM.mL^{-1} pyruvate)

Sensitivity of the method is given by the slope or gradient of the calibration graph. The greater the sensitivity the better the method is able to distinguish small changes in analyte concentration. For the data presented in Figure 6, the sensitivity is 0.76 (peak area units.mL.μM^{-1}.
The working range for the method was not determined; however the calibration set covers a practical level of 1 - 12 $\mu M.mL^{-1}$ pyruvate. In this project over 1,249 onions were tested within the panel set across a range of varieties. The largest pyruvate concentration was found to be 11.8 $\mu M.mL^{-1}$ pyruvate and the lowest was found to be 1.2 $\mu M.mL^{-1}$ pyruvate. This is described in more detail in Section 4.2.

- **Matrix Effects**

The only matrix effects reported in the scientific literature appear to be from interference associated with the presence of endogenous absorbing material (Anthon and Barrett, 2003). This particulate material would be expected to be at lower concentrations in pressed juice than in blended onion tissue / water extractions. Anthon and Barrett (2003) report that the interference is reduced greatly by measuring the absorbance at 515nm rather than 420nm. This has been the approach that we have adopted.
Matrix effects were investigated by analysing onion juice spiked with various levels of pyruvate and measuring the recovery compared with theoretical recoveries. The results are shown in Appendix 3. Recoveries varied from 101 -104 % with the average recovery 102% of the theoretical value. This indicates that matrix effects in the onion juice studied was minimal. There appears to be a very small positive bias associated with this measurement, although this could easily be explained by a small amount of laboratory error and would have to be verified by further studies.

- **Method Selectivity**

2,4-Dinitrophenylhydrazine (DNPH) reacts with carbonyl groups in dilute acid to produce products with chromophores with a peak around 450nm (Sokol, 1976). Other compounds which can produce competing chromophores are glyoxylic acid and acetaldehyde. Glyoxylic acid has a molar absorptivity of 10,500 at 450nm (equivalent to that of pyruvate). The chromophore of acetaldehyde is much more instable and has a molar absorptivity 90% less than that of pyruvate. Glyoxylic acid has not been reported as significantly interfering in onions.

- **Other Method Performance Parameters**

Accuracy and precision data are presented in Appendix 3.6 (Test Verification Record). The repeatability was tested by repeating seven measurements of prepared samples under the same conditions and determining the coefficient of variation (COV) for the data set. It was found that the coefficient of variation was in the order of 1% for a reading of 1.8 $\mu M.mL^{-1}$ pyruvate. The accuracy was determined by analysing spiked samples in a similar way and was found to be also in the order of 2%. This COV is very low and is considered acceptable.

The Limit of Detection (LOD) of a method is the smallest concentration of an analyte that can be readily distinguished from zero and is determined by repeat analysis of a sample which is in the lowest practical level for detection. Using this method, the LOD was determined to be in the order of 0.08 $\mu M.mL^{-1}$ pyruvate. The Limit of Quantitation (LOQ) of a method is defined as the lowest concentration of analyte which can be reported with an acceptable level of uncertainty, and is normally calculated by multiplying the LOD by a factor (usually 3). The LOD for this FIA method at NSW DPI has been determined as being 0.39 $\mu M.mL^{-1}$ pyruvate. In practice mild onion varieties will be unlikely to get as low as the LOD.

- **Collaborative data**

It is important to validate the results of any new method with other methods available. A comparison of the manual method (University of Georgia) with the automated FIA (NSW DPI) is shown in Figure 7. This shows a very strong correlation between the two methods of measuring pyruvate in onion juice ($r^2 = 0.98$).

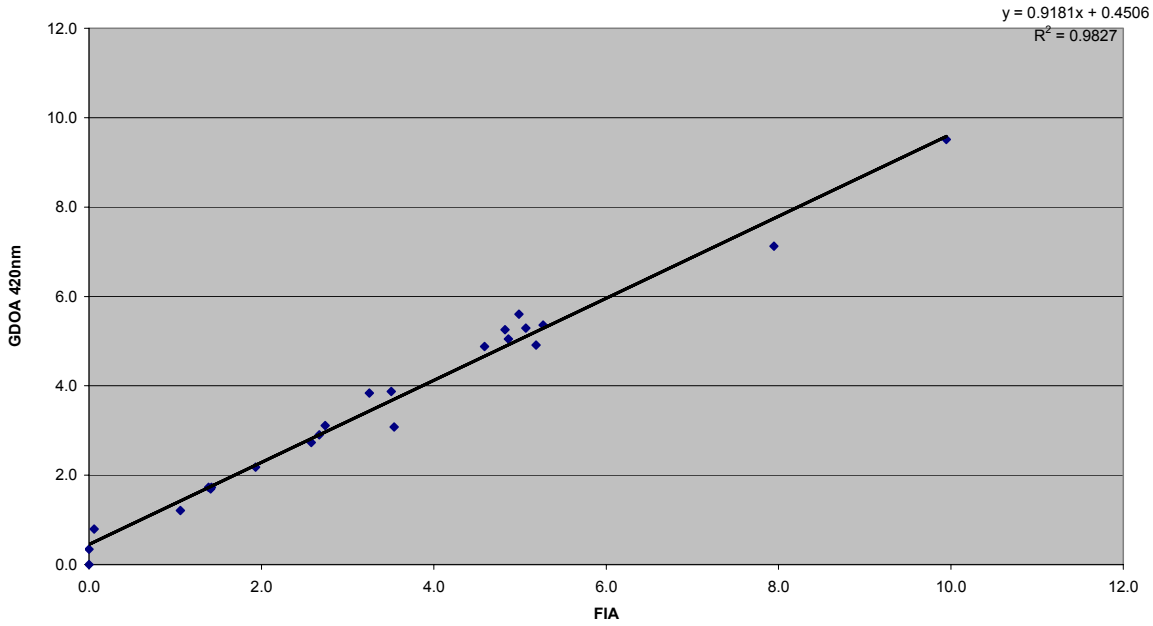


Figure 7 Correlation between the Flow Injection Analyser (FIA) method and the standard manual procedure for measuring pyruvate in onion juice ($r^2 = 0.98$).

- **Estimation of Measurement Uncertainty (MU)**

Measurement Uncertainty (MU) is defined as 'a parameter associated with the result of a measurement, which characterises the dispersion of the values that could reasonably be attributed to the measurand' (ISO, 1993). The estimation of uncertainty was determined according to NATA guidelines. Appendix 3.7 presents the results of calculations for the MU for this method. In summary, over the range of 1 - 12 $\mu M.mL^{-1}$ pyruvate concentration, the expanded uncertainty (Uc) was found to be 0.5 $\mu M.mL^{-1}$ pyruvate. This is considered adequate for this level of determination.

- **NSW DPI Onion Pyruvate Testing Service**

The pyruvate testing service is currently available through NSW DPI Diagnostic and Analytical Services (DAS) at the Wagga Wagga Agricultural Institute. This laboratory is accredited with the National Associations of Testing Authorities (NATA) which means all systems and results are quality assured from the national laboratory testing authority ensuring the reproducibility, quality and rigour of the results. This assurance and external auditing is essential for any commercial analytical testing service. The NSW DPI Diagnostic and Analytical Service (DAS) is committed to providing reliable, accurate and cost-effective analytical services to the Australian onion industry.

4.2 Preliminary Survey of Pyruvate Levels

In order to obtain sufficient numbers and range of onions with the correct pungency levels for the sensory analysis, a preliminary survey of a range of onions was conducted. It was planned to use this information, to select enough onions of each variety to cover each pungency class used for the sensory work. This would minimise excess sampling and provide more confidence in selecting and categorising the correct number and range of onions required for the main sensory assessments.

Aim

Survey a range of onion varieties for pungency to use in the main sensory assessments.

Materials and Methods

Nine varieties of onions were sourced from Queensland and New South Wales in October 2005 (Amada, Aurion / Aquarius, Early Locker Brown, Cavalier, Early Red, EX 450, M5345, N5410, Rio Red Rocks). Onions were assessed for pyruvate as outlined in Section 4.1.

Results

Sample pyruvate concentrations ($\mu M.mL^{-1}$) of each onion variety used in the preliminary survey is shown in Table 2.

	Variety	Mean ($\mu M.mL^{-1}$)	Standard Dev
1	Amada	2.77	0.74
2	Aurion / Aquarius	5.17	1.32
3	Early Locker Brown	2.74	0.95
4	Cavalier	5.57	1.28
5	Early Red	2.74	1.03
6	EX 450	2.61	1.41
7	M5345	5.72	1.64
8	N5410	4.75	1.35
9	Rio Red Rocks	8.86	1.57

Table 2 Average pyruvate concentration (μmoles pyruvate.mL^{-1}) of each onion variety (and standard deviation)

The values of individual onions are graphically demonstrated in Figure 8, where the variety number corresponds to the variety in Table 2. Note the large degree of bulb to bulb variability. For example within this batch of 20 Cavalier onions, the lowest pyruvate concentration was 3.4 $\mu M.mL^{-1}$ pyruvate and maximum was 8.9 $\mu M.mL^{-1}$ pyruvate.

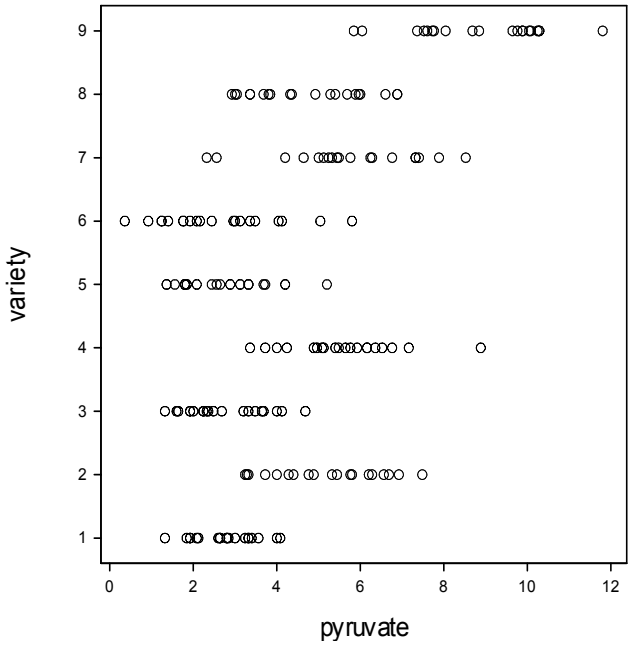


Figure 8 Pyruvate (μM.mL^{-1} pyruvate) distribution of nine onion varieties used in the preliminary survey (n = 20 onions for each variety)

Examining the data set as a whole, it is apparent that Rio Red Rocks was excluded from further consideration for the main sensory trial as this batch of onions were too pungent for the sensory analysis (pyruvate 8.8 μM.mL^{-1}). Assuming the pyruvate levels of each onion variety conform to the Gaussian distribution, then the sampled onion populations is represented in Figure 9.

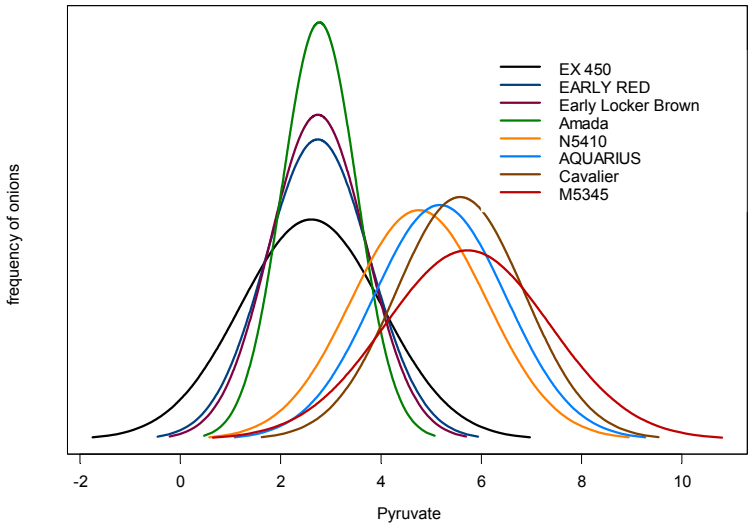


Figure 9 Simulated frequency distribution of onions used in the preliminary survey

The object of this preliminary trial was to obtain a representative sample of pyruvate classes for the main sensory trial. However if we sampled equal numbers of onions from each variety the resultant distribution would be the summation of these curves and would have a dominance of onions in the 2 - 4 $\mu M.mL^{-1}$ pyruvate classes (Figure 10).

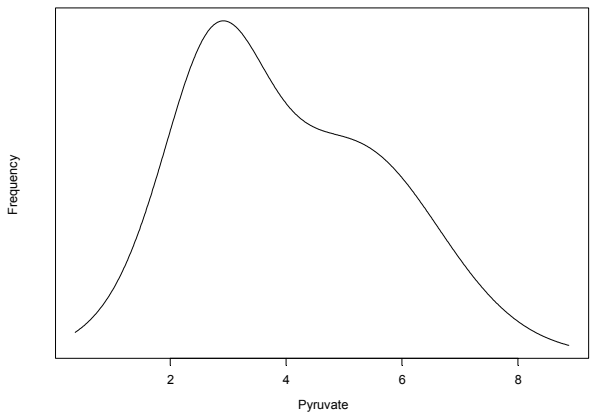


Figure 10 Predicted frequency distribution of onions if similar numbers of onions were equally sampled.

After selecting different combinations of onions, the best solution was to obtain equal numbers of EX450 and Aquarius onions to result in a distribution of pyruvate pungencies similar to this distribution (Figure 11).

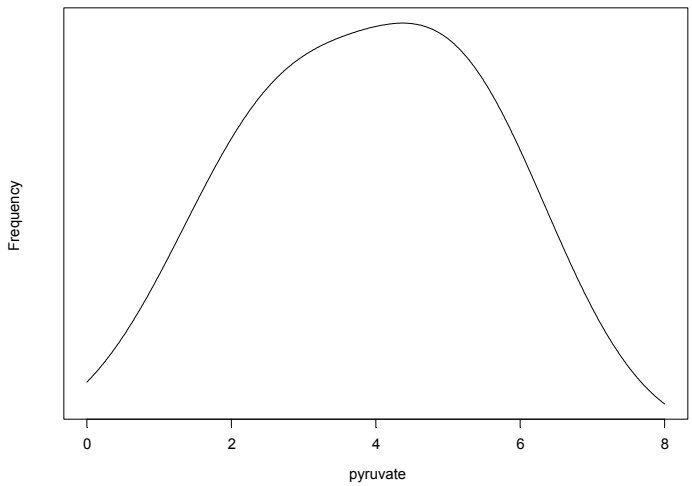


Figure 11 Predicted frequency distribution of onions selected from equal numbers of EX450 and Aquarius onions.

This was the ideal solution based on the preliminary trial, however there were not enough onions to fulfil this solution, and other onions were requested.

Unfortunately other batches of onions, of even the same variety, contained significantly different pungency levels. For example a new batch of EX450 onions (n = 107 onions) averaged 7.0 $\mu M.mL^{-1}$ pyruvate, compared to the initial batch used (2.6 $\mu M.mL^{-1}$ pyruvate). Similarly with Cavalier onions (n = 16) averaged 4.1 $\mu M.mL^{-1}$ pyruvate (compared to 5.6 $\mu M.mL^{-1}$ pyruvate in initial analysis), and K5161 onions (n = 149 onions) averaged 4.0

μM.mL^{-1} pyruvate (compared to 5.7 μM.mL^{-1} pyruvate in the initial analysis). This lack of continuity between sample batches of the same variety meant the onions used in the preliminary trial could not be relied upon to give similar pungency levels for the sensory trial. This is a salient lesson for both researchers and industry, in not assuming variety will give similar levels of pungency between batches. This reinforces the dominant role that climatic and geographic factors play in determining onion pyruvate content.
This inconsistency of varietal pungency levels between batches resulted in proceeding into the sensory study with no guidelines for selection of varieties or numbers of onion bulbs required for analysis. Fortunately with a lot of hard and late night work at the onion testing laboratory at Wagga, the adequate numbers of samples were prepared from other batches and sent to Food Science Australia for sensory analysis.

4.3 Lachrymatory Factor (LF)

During discussions with both Dr. Randle and the National Onion Labs Inc. it became clear that the lachrymatory factor (LF) is an important aspect of onion pungency. LF ((*Z, E*) propanethial *S*-oxide) arises from the hydrolysis of 1-propyl cysteine sulfoxide (1-PRENCSO) and is responsible for the mouth burn and heat associated with eating onions. Sensory attributes from the LF can be overwhelming and can dominate the experience of eating onions with high levels of 1-PRENCSO. This is in addition to the pyruvate levels in the onion.

The validity and ability to routinely quantify LF is not obvious in the scientific literature and was not considered in the initial HA pungency proposal (February 2005). However recent developments at the University of Georgia and the National Onion Labs Inc. have increased the importance of LF in onion pungency testing.

Traditionally LF has not been quantified in any quality or sensory assessment, but it is now strongly believed that the LF is a significant contributor to pungency, especially in 'borderline' pungent onions (around 4-5 μM.mL^{-1} pyruvate) and in onions that are not from the Granex type. This would be the case in with some of mild onions grown in Australia where it is believed that these onions would have high concentrations of 1-PRENCSO (LF precursor). It was thought that both the pungency (pyruvate) and LF of onions grown in the southern areas of Australia may be a significant factor in consumer acceptability.

It was noted in numerous discussions in Georgia that two onions both with similar pyruvate concentrations (eg. 4 μM.mL^{-1} pyruvate) can have significantly different perceived pungencies, due to differences in LF. One onion which may have low LF will taste mild and sweet, whilst the high LF onion (although having similar pyruvate concentrations) can taste extremely pungent. High LF will cause extreme mouth burn and tear production. It is now thought that the sometimes poor correlations in the scientific literature between perceived pungency and pyruvate concentration may be improved with the inclusion of the LF into the equation. The relationship between pyruvate, LF and TSS and sensory analysis has not been explored in any study. Although previous studies have made good correlations between pyruvate and sensory perceived pungency (eg Wall and Corgan 1992), it is expected that the introduction of LF into this study may improve this relationship.
In this study, LF was measured in every onion that were assessed in the sensory panels (trained and consumer panel). However pyruvate was still the predominant factor in this trial. All sensory work was designed with pyruvate being the primary factor of classification, as outlined in the original Horticulture Australia *Expression of Interest* (Appendix 1).

4.3.1 Measurement of the Lachrymatory Factor

The measurement of the Lachrymatory Factor in onion juice was conducted using the University of Georgia method without modification. Quantification was conducted on the same instrument and column using the same instrument parameters (Appendix 3.3).

Initial information from Tim Coolong, PhD student at The University of Georgia (pers comm.), suggested that the LF was stable for at least a couple of hours under refrigeration. LF stability studies were conducted at NSW DPI Wagga Wagga Agricultural Institute. The results showed that samples stored at 4°C showed less than 5% breakdown after 24 hours and there appeared to be no significant breakdown in samples that were stored at -18°C for 24 hours. Samples stored at room temperature had no significant breakdown after 4 hours; but were completely degraded after 24 hours at room temperature.

These studies were significant as they allowed us to properly control the temperature conditions of the extractions to ensure sample integrity was intact. Sample extractions were immediately stored on ice, as suggested in the original method, and then stored at -18°C until analysed (maximum 12 hours). Samples were placed on a refrigerated manifold on the Shimadzu GC 17A at 4°C while they were analysed for LF.

5. Sensory Analysis

A key outcome of this project was the calibration of the pyruvate to the Australian palate:

Project Outcomes

- Develop a reliable and reproducible pungency test utilising the modified 'Schwimmer and Weston' method
- Calibrate the 'Schwimmer and Weston' method against the Australian palate utilising extensive taste panel comparisons
- Import or construct an onion juice press, establishing a recognised testing facility that will enable rapid and cost effective sampling of onion pungency

(EOI, December 2005 – Appendix 1)

5.1 Preparation and sourcing onion samples for sensory analysis

5.1.1 Source of onions

Onions were sourced from Queensland and New South Wales. The onion varieties used for the sensory analysis were: Cavalier, Brown, EX450, K5161, NS410 and Aurion. Each onion was cut in half. One half of the onion was crushed in the onion press, juice collected and pyruvate, LF and SSC(%) measured (as per Section 4).

After cutting the onion in half, the exposed cut surface was immediately sealed in food grade wax with a muslin / cheesecloth foundation for increased strength and enhanced wax integrity (Figure 20). The continuous wax coating prevented any contamination and prevented any water loss from the cut surface. These onions were refrigerated and sent to Food Science Australia's North Ryde Centre in Sydney for sensory analysis. The maximum time between cutting the onion for chemical analysis and sensory assessment was two weeks. To minimise any development of pungency between sampling and sensory analysis onion halves were kept refrigerated (5^{o}C) during transport and storage.

The results of the chemical characteristics (pyruvate, LF and SSC(%)) of onions used in sensory assessments follow.

5.1.2 Pyruvate

A summary of the pyruvate classes used for the sensory analysis is shown in Table 3. Note there is considerable overlap of pyruvate concentrations between varieties within the sample population (Figure 12). Variety was not a factor in classification. Onions were classified according to their pyruvate concentration only. To illustrate this point, the number of onions used in pyruvate class 4-5 $\mu M.mL^{-1}$ pyruvate were; 11 Brown, 25 Cavalier, 10 EX450, 17 K5161, 31 NS410 and 69 Orion onions.

Variety	Pyruvate 1 <4 μM.mL^{-1}	Pyruvate 2 4-5 μM.mL^{-1}	Pyruvate 3 5-6 μM.mL^{-1}	Pyruvate 4 6-7 μM.mL^{-1}	Pyruvate 5 <7 μM.mL^{-1}
Brown	4	11	48	86	66
Cavalier	23	25	17	3	0
EX450	1	10	21	51	93
K5161	43	17	3	1	0
NS410	32	31	17	7	1
Orion	59	69	50	9	2

Table 3 Classification of onions into pyruvate groupings according to their pyruvate concentration (μM.mL^{-1})

Variety	Pyruvate 1 <4 μM.mL^{-1}	Pyruvate 2 4-5 μM.mL^{-1}	Pyruvate 3 5-6 μM.mL^{-1}	Pyruvate 4 6-7 μM.mL^{-1}	Pyruvate 5 <7 μM.mL^{-1}
Brown	3.35	4.56	5.48	6.50	7.63
Cavalier	3.26	4.54	5.40	6.59	
EX450	3.88	4.58	5.52	6.51	7.95
K5161	3.45	4.38	5.36	6.32	
NS410	3.51	4.51	5.41	6.31	7.28
Orion	3.48	4.47	5.39	6.30	7.10
Class Average	**3.47**	**4.49**	**5.44**	**6.49**	**7.81**

Table 4 Pyruvate concentration (μM.mL^{-1}) means for each variety

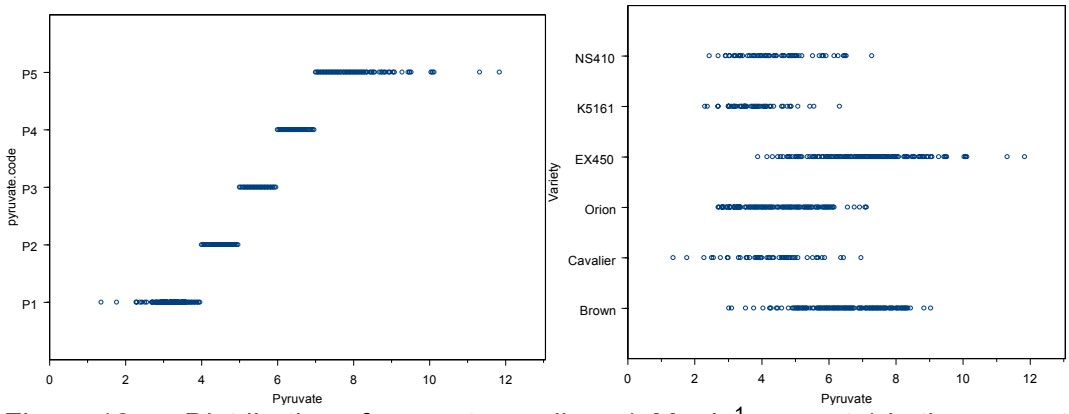


Figure 12 Distribution of pyruvate readings (μM.mL^{-1} pyruvate) in the pyruvate categories and onion varieties tested in both sensory panels

5.1.3 Lachrymatory Factor

The distribution of the lachrymatory factor ($\mu M.mL^{-1}$) within and between the onion varieties tested is shown in Figure 13.

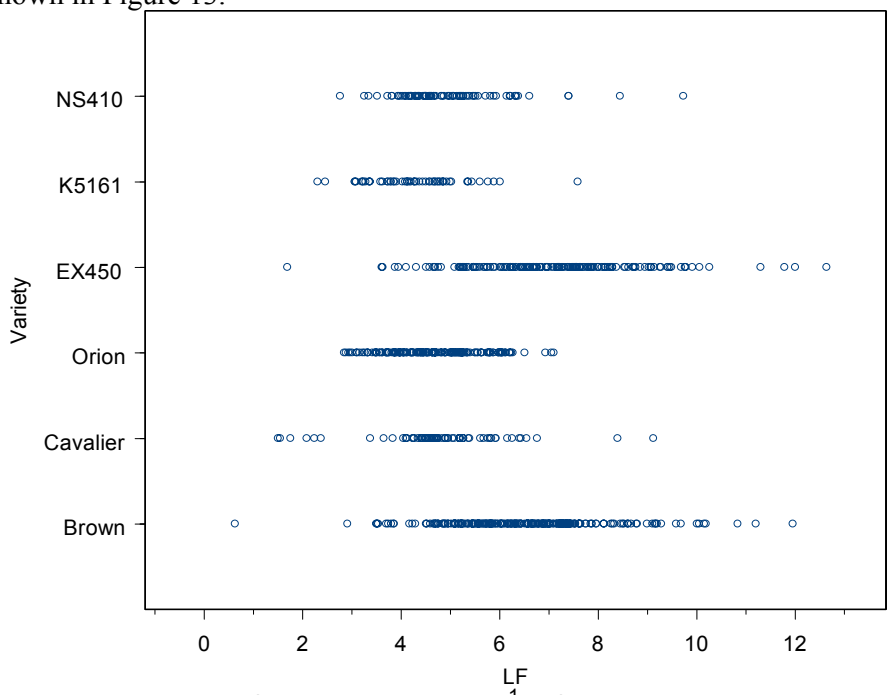


Figure 13 Lachrymatory factor (LF) ($\mu M.mL^{-1}$) of the onion varieties tested in both sensory panels

5.1.4 Soluble Solids Content (SSC(%))

The distribution of the soluble solids content (%) within and between the onion varieties tested is shown in Figure 14.

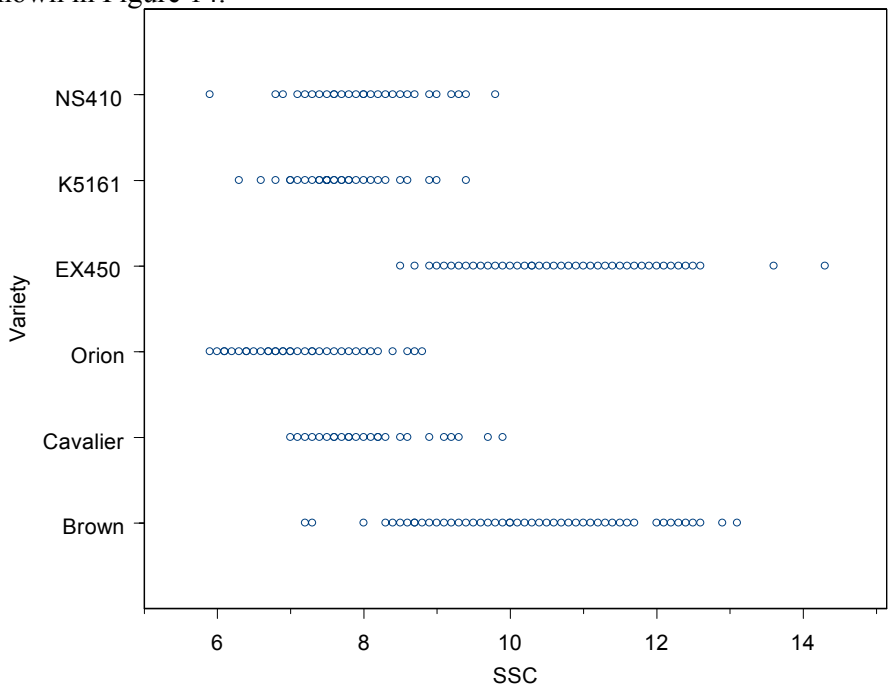


Figure 14 Soluble solids content (SSC(%)) of the onion varieties tested in both sensory panels

5.1.5 Relationships between LF, pyruvate and SSC(%)

The relationship between LF and pyruvate is described in Figures 15 and 16. The linear equation fitted to describe the relationship between pyruvate and LF can be described as:

$$y = 0.86 (\pm 0.02)\ x + 0.95 (\pm 0.13) \qquad (r^2 = 0.66)$$

Where y = LF and, x is the measured pyruvate

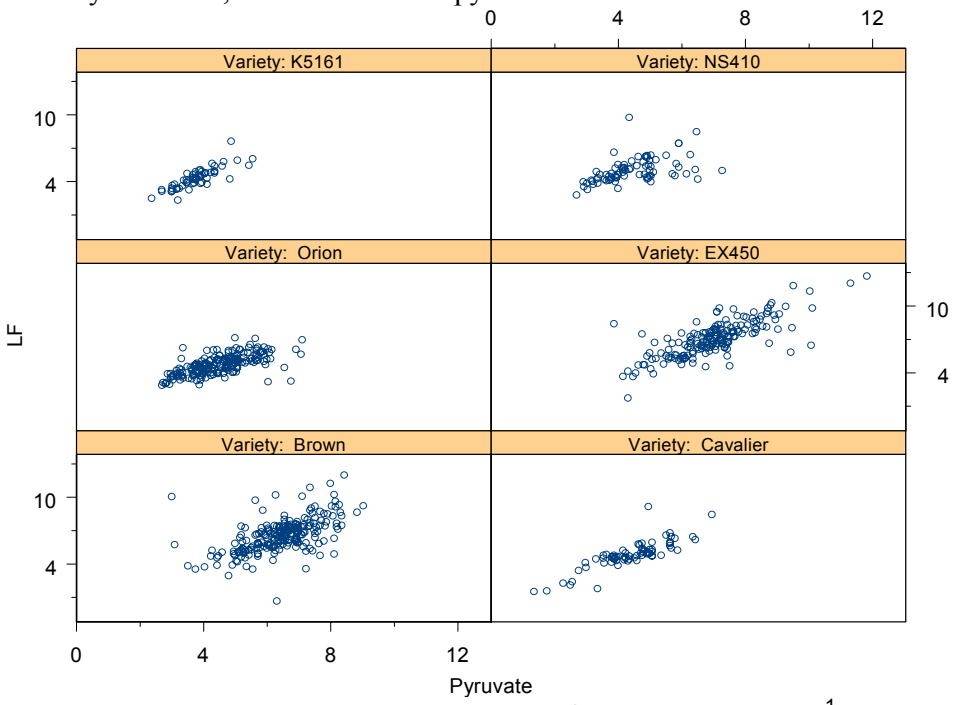


Figure 15 Relationship between lachrymatory factor (LF) ($\mu M.mL^{-1}$) and pyruvate ($\mu M.mL^{-1}$ pyruvate) in each of the onion varieties tested in both sensory panels

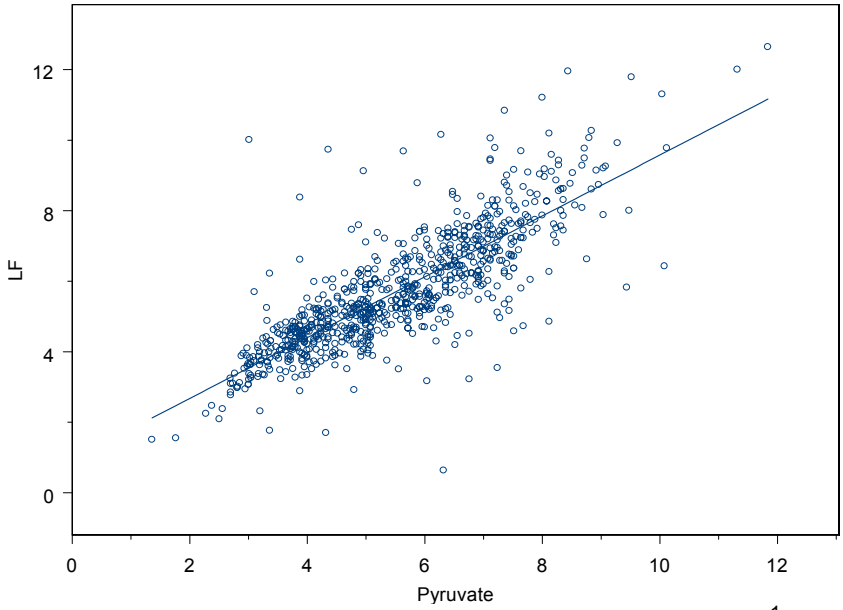


Figure 16 Relationship between lachrymatory factor (LF) ($\mu M.mL^{-1}$) and pyruvate ($\mu M.mL^{-1}$ pyruvate) in all onions tested in both sensory panels

The relationship between SSC(%) and pyruvate is poor and is described in Figure 17. The linear equation fitted to describe the relationship between SSC(%) and pyruvate can be described as:

$$y = 0.78 (\pm 0.03)\ x + 4.68 (\pm 0.16) \qquad (r^2 = 0.50)$$

Where y = SSC(%) and, x is the measured pyruvate

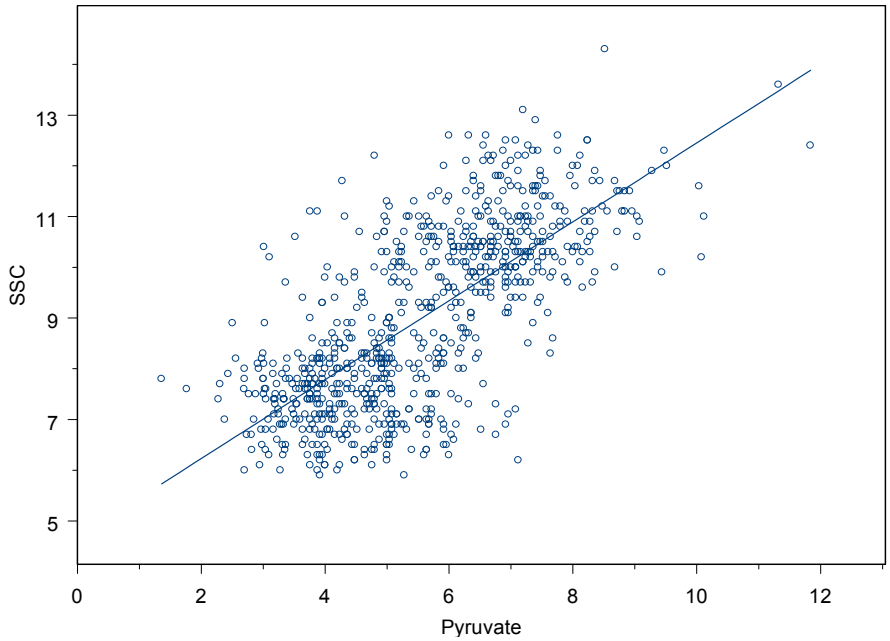


Figure 17 Relationship between of soluble solids content (%) and pyruvate ($\mu M.mL^{-1}$ pyruvate) in all onion tested in both sensory panels

The relationship between LF and SSC(%) is also poor and is shown in Figure 18. The linear equation fitted to describe the relationship between LF and SSC(%) can be described as:

$$y = 0.62\ (\pm\ 0.03)\ x + 0.15\ (\pm\ 0.24) \qquad (r^2 = 0.41)$$

Where y = LF and, x is the SSC(%)

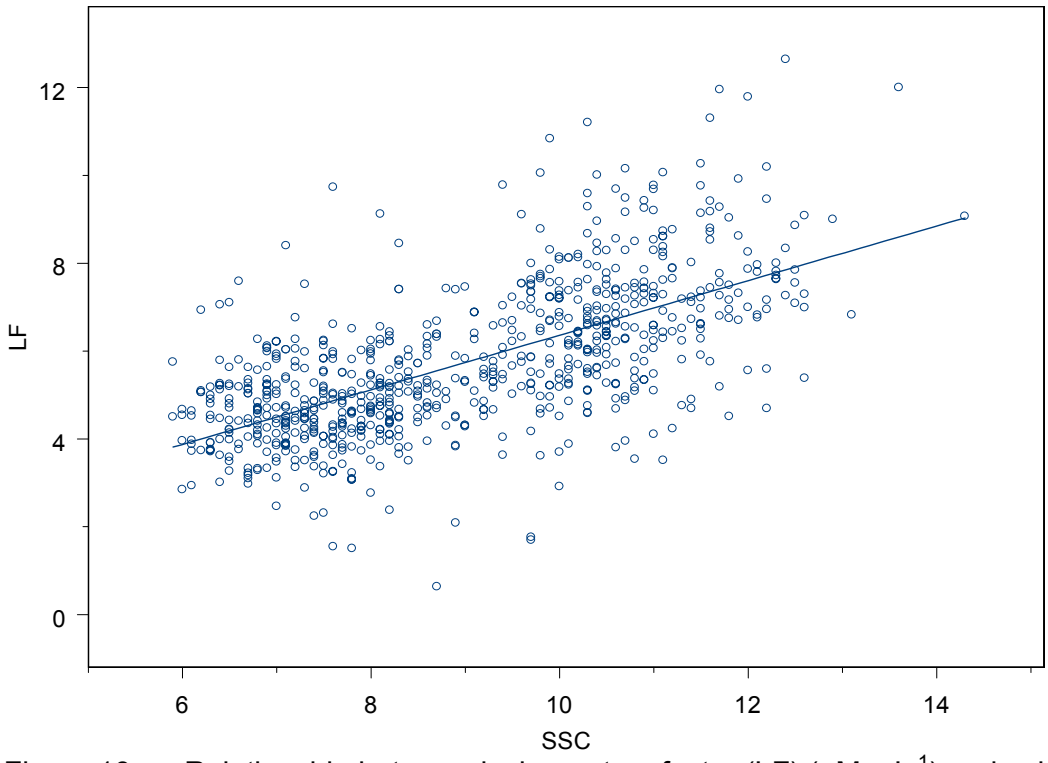


Figure 18 Relationship between lachrymatory factor (LF) ($\mu M.mL^{-1}$) and soluble solids content (%) in all onions tested in both sensory panels

5.2 Trained Panel Assessment

5.2.1 Background

A trained sensory panel was used to provide objective measurements of onion *pungency*. This approach was used to ensure the generation of purely perceptual measurements of *pungency*, without the influence of biasing psychological factors commonly associated with naïve consumer measurement of diagnostic sensory attributes. Such factors include affective weighting to cognitive utilities such as 'taste' and poor alignment for individual sensory attributes, both of which can lead to large residual variation (noise) in sensory attribute data. Therefore, the objective of using trained panel-type assessment was:

- Generate objective perceptual information that could be combined with corresponding instrumental measurements of pyruvate to determine the relationship between a physical stimulus (pyruvate) and the perceived human response (*pungency*).
- Combine objective perceptual information with corresponding subjective-level information (*liking*, opinion of flavour strength and classification as generated by the consumer panel) to determine the relationships between a perceptual stimulus (*pungency*) and the affective response (*like/dislike, mild/strong*).

A hypothetical example of this relationship is depicted graphically in Figure 19.

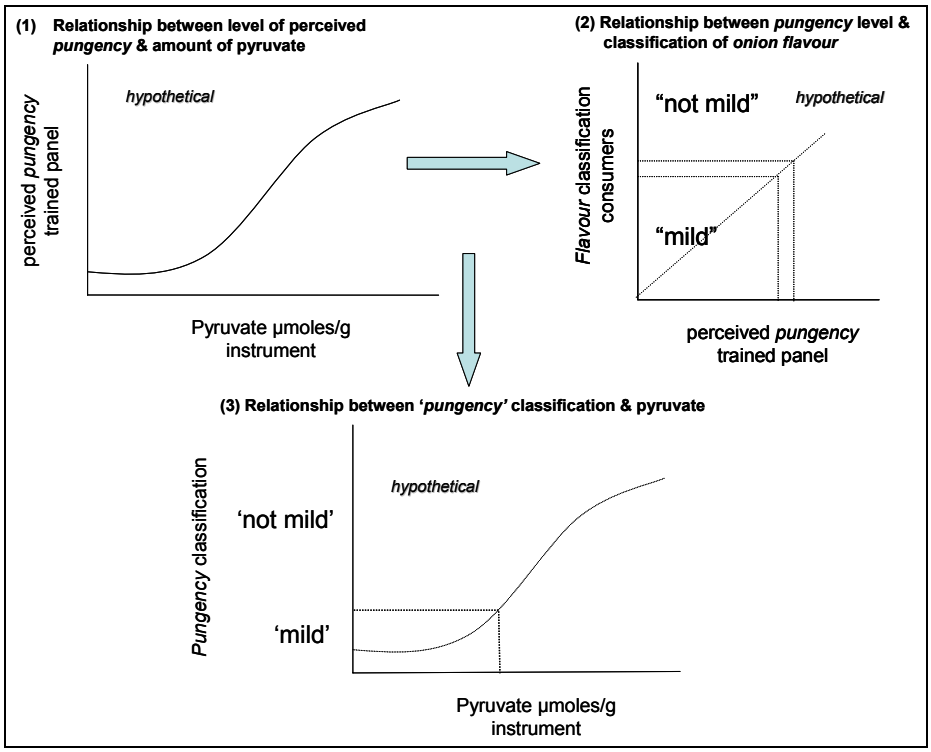


Figure 19 Schematic illustration of a hypothetical relationship between perceived onion *pungency intensity* (trained panel), pyruvate levels (instrument) and classification of *pungency* (consumer)

The trained panel were also used to provide objective measurements of onion sweetness and lachrymatory factor to address any interactions that might occur between physicochemical components (e.g. pyruvate and soluble sugars) at a perceptual level during consumption and not accounted for by isolated instrumental measurements.

5.2.2 Onion Storage and Preparation

All onions were stored in darkness at a temperature of 5°C. Prior to evaluation, onions were removed from cool storage and allowed to equilibrate to room temperature (20 ± 1°C) for at least 30 minutes prior to sampling. To minimise the influence of inter-product variation an appropriate preparation and sampling procedure was developed prior to trained panel and consumer assessment.

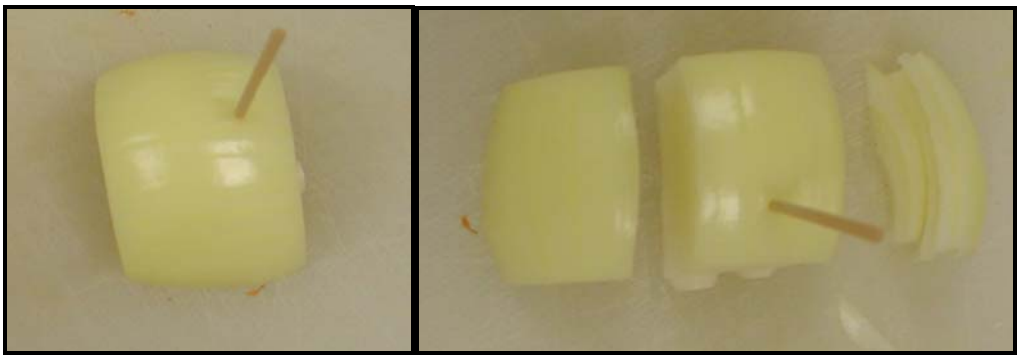
All onions were prepared immediately prior to assessment. Onions were cut with a large sharp knife on a plastic cutting board. A clean knife and cutting board were used for each new sample (Figure 20).

Figure 20 Receipt of half onions at Food Science Australia
Protective food grade wax was removed and samples prepared for sensory analysis

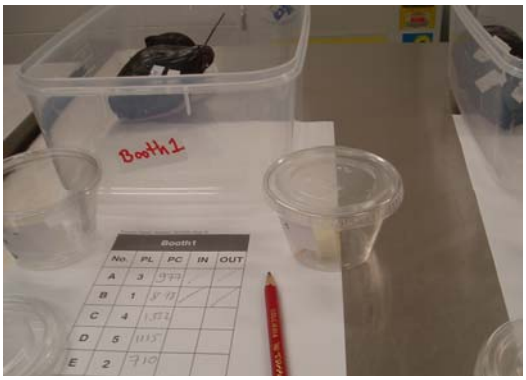
1. A sliver of ~5mm was cut off the base of the onion sample where it had been waxed.
2. The bottom and the top of the onion sample were then removed to keep the equatorial region.
3. The first outer layer (skin) was removed.

4. A tooth pick was inserted in the middle part of the onion to maintain layer integrity.

5. The two sides were then removed to obtain an equatorial region with a longitudinal width of approx 4cm.



6. The sample was placed in a plastic container, blind labelled with an appropriate 3 digit code. The lid was replaced and the sample was presented to the assessor for evaluation.



7. Assessors (trained assessors and consumers) were instructed to consume the onions in a manner similar to that outlined. At least one bite of the onion was consumed during assessment and all assessors were instructed to consume the onion as normal (i.e. assessors were encouraged to swallow the sample).

All sensory testing took place in the sensory laboratory at Food Science Australia's North Ryde Centre in Sydney according to International Standards on Sensory Analysis (ISO 6658:1985). A panel of ten trained assessors participated in the study. These assessors were previously screened for sensory acuity and were trained to profile the sensory characteristics of food products.

5.2.3 Panel Training

Prior to onion evaluation, the panel participated in five two-hour discussion group training sessions to describe the perceived sensations associated with onion *pungency*. These in turn were devolved into a descriptive vocabulary of *pungency* attributes. Furthermore, the panel received exposure (limited) to perceived onion sweetness and LF to facilitate inclusion of appropriate attributes to measure these sensations during the pungency evaluation. Those onions used during panel training were chosen to cover the range of pungency, sweetness and LF experiences likely to be encountered in the chosen experimental treatments (pyruvate level 1-5). The session plans for panel training are outlined in Appendix 4.1.

Training the trained panel for pyruvate assessments

Five discussion group training sessions were conducted on five consecutive days with ten onions of each pyruvate classification (x5 classification) presented at each training session:

	Pyruvate Level	Actual range of pyruvate concentrations used
1	< 4 $\mu M.mL^{-1}$ pyruvate	(3.22 – 3.64)
2	4 – 5 $\mu M.mL^{-1}$ pyruvate	(4.36 - 4.64)
3	5 – 6 $\mu M.mL^{-1}$ pyruvate	(5.12 – 5.64)
4	6 – 7 $\mu M.mL^{-1}$ pyruvate	(6.00 – 6.96)
5	> 7 $\mu M.mL^{-1}$ pyruvate	(7.16 – 11.56)

Training the panel for LF and SSC(%) assessments

LF and SSC(%) were of secondary importance to the primary aim of this project (pyruvate). However exposure to LF and SSC(%) was conducted during two training sessions.

Ten onions of each of three classifications of LF were used to train the panel in one session. As there is no sensory information on LF, three arbitrary classifications were chosen to encompass the ranges of LF expected in the main experiment:

Classification of LF groups	
1	2.0 - 2.8 $\mu mol.mL^{-1}$
2	4.8 only
3	7.5 - 8.0

Similarly ten onions of each of three classifications of SSC(%) were used to expose the trained panel to onion sweetness during one training session. Three arbitrary classifications were chosen to encompass the ranges of SSC(%) expected in the main experiment:

Classification of SSC(%) groups	
1	5.6 - 5.9%
2	7.2% only
3	9.0 - 9.9%

During the training sessions the odour, flavour (aroma and taste) and aftertaste of the onions was screened for uni-dimensional (e.g. *throat pungency*, *sweetness*) and integrated (e.g. *overall pungency intensity*) attributes associated with pungency. Once an informal list of attributes was generated, panel consensus (mutual understanding of each descriptive attribute) and performance (consensus ranking of products using each descriptive attribute) was measured throughout the training process (Appendix 4.1). The final descriptive sensory vocabulary is outlined in Appendix 4.2. Definitions of all descriptive terms were generated by panel consensus (Appendix 4.2) and where necessary, reference standards were developed to aid assessors in the evaluation of difficult sensory attributes.

5.2.4 Onion Evaluation

Onion samples were evaluated in individual booths under red lighting in the sensory laboratory at Food Science Australia's Sydney Centre. Red lighting was used to minimise the appearance (predominantly colour) as an associative cue (cross-modal interaction) for product taste. Panellists had free access to tap water (room temperature), unsalted crackers, full cream milk or buttermilk, cranberry juice, cream cheese, banana, parsley and coconut to aid in palate cleansing during the 'wash-out' period between each sample. Furthermore, a ten minute wash-out period was enforced between each sample to allow assessor palates' to return to a baseline level (desensitisation) following onion sensitisation.

Sensory attributes were scored on unstructured 100 mm continuous line scales anchored at both ends (at 5% and 95%) with extremes of each descriptive term (Appendix 4.2). Where necessary, consensus attribute scaling was aided through the use of appropriate reference standards (Appendix 4.2). The complete descriptive sensory vocabulary along with corresponding line scale anchors is outlined in Appendix 4. Data were recorded and stored using the Compusense five sensory data acquisition programme (Guelph, Ont., Canada). Strict controls were in place to ensure that all differences identified were the result of true product differences rather than any competing extraneous factors.

5.2.5 Statistical Methods

- **Experimental Design**

A three dimensional design was used to accommodate a comparison of all pyruvate treatments by each assessor and session. Five samples, each of a designated pyruvate level and a specific instrument measurement of pyruvate, were assigned to assessors according to the design. In total, six consecutive individual evaluation sessions were conducted. Samples were evaluated in a sequential monadic order, according to the balanced design to reduce the effect of positional (order of presentation) bias. The order of presentation of the pyruvate levels was balanced in the 3-dimensional design. Session, panellist and order were the main design elements.

The randomisation of the design was restricted so that each of the five pyruvate levels was tested by each panellist in a session. An efficient experimental design was sought using the DiGGer (Coombes, 2002) search program. Blocking factors used in the search were panellist, panellist.order and session.order where order was the order of tasting the five samples. Autoregressive correlation (parameter 0.5) between samples within a panellist.session was also used.
The design used had each pyruvate level being tested twice across panellists for each session.order block. The blocking structure for the analysis was thus panellist + panellist.session + panellist.order + session.order.

Panellists recorded their responses for each onion in the following order (as defined in Appendix R):

1. *Pungent odour*
2. *Sweetness*
3. *Mouth pungency*
4. *Throat pungency*
5. *Nose pungency*
6. *Eye pungency*
7. *Overall pungency*
8. *Sweet aftertaste*
9. *Mouth pungency aftertaste*
10. *Throat pungency aftertaste*
11. *Nose pungency aftertaste*
12. *Eye pungency aftertaste*
13. *Overall pungency aftertaste*

The data for one of the panellists was omitted from the analysis due to their lack of discrimination between the pyruvate levels. This caused the design to become unbalanced.

Correlations between the 13 sensory attributes and pyruvate reading were calculated.

- **Mixed model analysis of instrument pyruvate reading and the 13 sensory response attributes in the trained panel assessments**

The relationship between each attribute and pyruvate reading was modelled using a mixed linear regression approach (Searle, 1971) in which the variation was separated into fixed and random effects. The analysis was conducted using ASReml (Gilmour *et al*. 2000).The use of random effects allows inference from these results to extend to the wider population of randomly chosen panellists, rather than only to the particular nine panellists used in this study.

The fitted model was:

$$y = \text{pyruvate} + \textit{panellist} + \textit{panellist.order} + \textit{panellist.session} + \textit{session.order} + \textit{error}$$

where y = sensory attributes (e.g. *Overall pungency*) and the *italicised* terms are included in the model as random effects.

The ASReml program was also used to conduct a mixed effects analysis of variance with pyruvate category as the fixed treatment effect and random effects of panellist, panellist.order, panellist.session and session.order for each sensory attribute. This blocking structure reflected the experimental design.

5.2.6 Trained Panel Results

5.2.6.1 Correlation Matrix

The overall correlation matrix that relates all 13 sensory attributes and pyruvate instrument reading from the trained panel is shown in Table 5. The results show there are obvious correlations between sensory attributes such as *mouth pungency* and *mouth pungency aftertaste*. For example to find the correlation between pyruvate reading and *nose pungency*, locate row 14 (pyruvate) and column 5 (*nose pungency*). The value is 0.20. Row 4 column 3 contains the correlation between *mouth pungency* and *mouth pungency aftertaste* (0.73).
Some interesting correlations exist, such as with *overall pungency* and pyruvate. These are further explored when examining the different levels of pyruvate between onions.

1	1.00													
2	0.92	1.00												
3	0.39	0.31	1.00											
4	0.40	0.39	0.73	1.00										
5	0.51	0.50	0.46	0.45	1.00									
6	0.54	0.62	0.31	0.46	0.83	1.00								
7	0.49	0.40	0.86	0.71	0.54	0.35	1.00							
8	0.56	0.57	0.73	0.84	0.56	0.58	0.79	1.00						
9	0.30	0.31	0.26	0.25	0.48	0.36	0.34	0.35	1.00					
10	-0.03	-0.09	0.00	-0.24	-0.38	-0.44	-0.04	-0.22	-0.05	1.00				
11	-0.06	-0.05	-0.11	-0.22	-0.17	-0.22	-0.14	-0.17	-0.03	0.66	1.00			
12	0.48	0.38	0.71	0.48	0.50	0.35	0.77	0.58	0.30	0.04	-0.03	1.00		
13	0.54	0.51	0.59	0.70	0.53	0.56	0.68	0.81	0.29	-0.16	-0.07	0.67	1.00	
14	0.17	0.12	0.27	0.30	0.20	0.14	0.34	0.32	0.12	0.00	-0.04	0.36	0.34	1.00
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**

1	*Eye pungency*
2	*Eye pungency aftertaste*
3	*Mouth pungency*
4	*Mouth pungency aftertaste*
5	*Nose pungency*
6	*Nose pungency aftertaste*
7	*Overall pungency*
8	*Overall pungency aftertaste*
9	*Pungent odour*
10	*Sweet aftertaste*
11	*Sweetness*
12	*Throat pungency*
13	*Throat pungency aftertaste*
14	Pyruvate reading

Table 5 Correlation coefficients for the trained panel for the assessments: *Eye pungency*, *Eye pungency aftertaste*, *Mouth pungency*, *Mouth pungency aftertaste*, *Nose pungency*, *Nose pungency aftertaste*, *Overall pungency*', *Overall pungency aftertaste*, *Pungent odour*, *Sweet aftertaste*, *Sweetness*, *Throat pungency*, *Throat pungency aftertaste* and the instrument pyruvate reading.

5.2.6.2 Regression Analysis

The fitted relationships between the sensory attribute *throat pungency* and pyruvate reading for each of the nine panellists are shown in Figure 20, together with the raw data. These results demonstrate the variation between the trained panellists. For example the *throat pungency* responses for panellist 3 showed a strong linear relationship with the level of pyruvate concentration, whilst panellist 1 was less consistent (i.e. more variable).
The results of the other 12 sensory attributes are shown in Appendix 5.1.

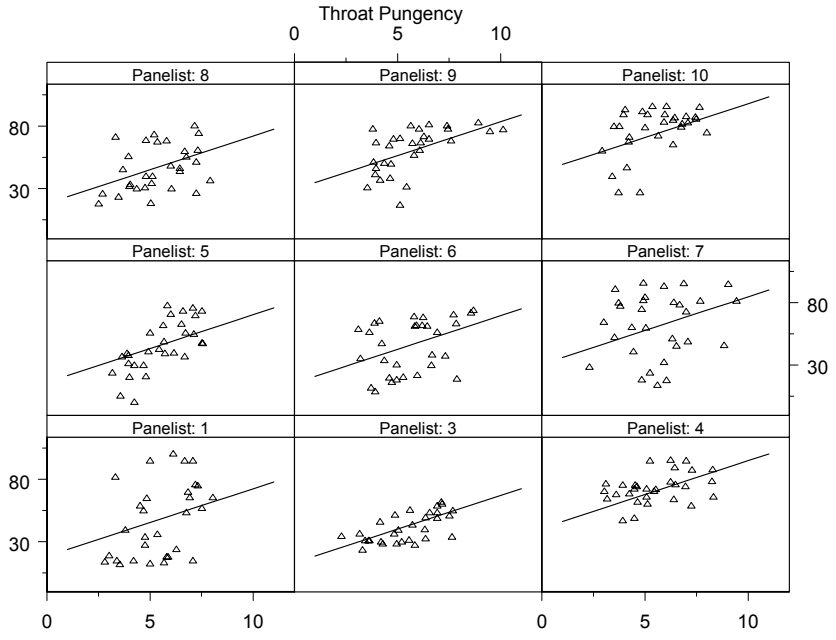


Figure 20 Fitted and observed relation between throat pungency and pyruvate reading for each of the nine panellists.

A summary of the mixed linear regression results is shown in Table 6. The trained panellists' responses for all sensory attributes, apart from *sweetness* and *sweet aftertaste*, were positively related to increasing pyruvate ($p < 0.01$). The results show that the relation between *throat pungency* and pyruvate was the strongest with a slope of 5.41 ± 0.67.
The responses for the sensory attributes *sweetness* and *sweet aftertaste* were not related to pyruvate.

Sensory attribute	F	prob	Slope ± se		Constant ± se	
Pungent odour	8.22	.003	1.53	0.53	35.63	4.99
Sweetness	0.71	0.403	-0.50	060	38.52	4.36
Mouth pungency	44.26	<.001	4.52	0.68	33.56	7.10
Throat pungency	65.44	<.001	5.41	0.67	25.05	5.77
Nose pungency	39.59	<.001	3.72	0.59	17.16	8.48
Eye pungency	32.86	<.001	2.27	0.40	4.90	5.99
Overall pungency	66.46	<.001	4.62	0.57	32.36	5.76
Sweet aftertaste	0.15	.695	-0.13	0.35	20.81	3.66
Mouth pungency aftertaste	65.67	<.001	4.66	0.57	22.73	6.39
Throat pungency aftertaste	58.38	<.001	4.73	0.62	20.49	4.86
Nose pungency aftertaste	23.86	<.001	2.41	0.49	13.78	7.69
Eye pungency aftertaste	22.79	<.001	1.53	0.32	5.85	5.56
Overall pungency aftertaste	71.88	<.001	4.11	0.48	26.84	5.16

Table 6 Summary of the mixed model regression analysis of each of the sensory attributes assessed by the training panel. 'F' is the F-probability statistic, 'prob' is the probability, 'Slope ± se' is the slope of the relationship ± the standard error of the slope, and 'constant ± se' is the constant in the relationship ± the standard error of the constant.

5.2.6.3 Relationship between Pyruvate Category and Trained Panel Assessments

The effects of pyruvate category on each of the 13 sensory attributes were assessed in separate analyses. As would be expected based on the previous regression analysis using pyruvate reading, a significant effect of pyruvate category was observed for all attributes except *sweetness* and *sweet aftertaste*.

Overall pungency is an integrated sensory attribute that relates a range of different *pungency* attributes. The results of *overall pungency* are shown in Table 7 and show that the trained panel could not distinguish between onions in the two lowest categories of pyruvate (less than 4 $\mu M.mL^{-1}$ pyruvate and 4-5 $\mu M.mL^{-1}$ pyruvate). However the trained panel could reliably detect those onions in pyruvate category 3 (5-6 $\mu M.mL^{-1}$ pyruvate) and these were different to those onions greater than 6 $\mu M.mL^{-1}$ pyruvate. The trained panellists could not distinguish between onions that were between 6 – 7 $\mu M.mL^{-1}$ pyruvate and those onions greater than 7 $\mu M.mL^{-1}$ pyruvate. The panellists thought these onions were the most pungent.
The results of the other *pungency* attributes (*eye pungency*, *mouth pungency*, *throat pungency*, *nose pungency*, *pungent odour*, *eye pungency aftertaste*, *mouth pungency aftertaste*, *nose pungency aftertaste*, *throat pungency aftertaste*, *overall pungency aftertaste*) were analysed and produced similar results (Tables 8, 9, 10, 11, 12, 15, 16, 17, 18, 19). However the panellists' distinction between the different pyruvate classes were not as clear cut as the *overall pungency*, where there was sometimes overlap between the pyruvate classes (e.g. *pungent odour* (Table 12)).

The results of the perception of onion *sweetness* between the different pyruvate categories showed that the trained panel could not detect any differences in *sweetness* (or *sweetness aftertaste*) (Tables 13 and 14).

Overall Pungency

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	Predicted Value
1	(< 4 μM)	50.6 a
2	(4 – 5 μM)	50.4 a
3	(5 – 6 μM)	55.7 b
4	(6 – 7 μM)	64.5 c
5	(> 7 μM)	68.2 c

Table 7 *Overall pungency* of onions as assessed by trained panel (n = 9).
Overall pungency was defined as the overall pungency of the onion flavour from 'low' to 'high' as measured on a continuous scale from 0 to 100.
(*Least Significant Difference* = 4.5)

Eye Pungency

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	Predicted Value
1	(< 4 μM)	14.1 ab
2	(4 – 5 μM)	13.1 a
3	(5 – 6 μM)	17.3 bc
4	(6 – 7 μM)	20.1 cd
5	(> 7 μM)	22.3 d

Table 8 *Eye pungency* of onions as assessed by trained panel (n = 9). *Eye pungency* was defined as the effect on the eyes of the onion flavour from 'low' to 'high' as measured on a continuous scale from 0 to 100. (*Least Significant Difference* = 3.2)

Mouth Pungency

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	Predicted Value
1	(< 4 μM)	49.8 a
2	(4 – 5 μM)	53.1 ab
3	(5 – 6 μM)	56.6 ab
4	(6 – 7 μM)	66.2 c
5	(> 7 μM)	67.0 c

Table 9 *Mouth pungency* of onions as assessed by trained panel (n = 9). *Mouth pungency* was defined as the pungency effect in the mouth of the onion flavour from 'low' to 'high' as measured on a continuous scale from 0 to 100. (*Least Significant Difference* = 5.4)

Throat Pungency

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	Predicted Value
1	(< 4 μM)	45.9 a
2	(4 – 5 μM)	46.7 a
3	(5 – 6 μM)	53.2 b
4	(6 – 7 μM)	62.7 c
5	(> 7 μM)	66.0 c

Table 10 *Throat pungency* of onions as assessed by trained panel (n = 9). *Throat pungency* was defined as the *pungency* effect on the throat of the onion flavour from 'low' to 'high' as measured on a continuous scale from 0 to 100. (*Least Significant Difference* = 5.4)

Nose Pungency

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	Predicted Value
1	(< 4 μM)	32.8 a
2	(4 – 5 μM)	31.1 a
3	(5 – 6 μM)	34.6 a
4	(6 – 7 μM)	44.0 b
5	(> 7 μM)	45.9 b

Table 11 *Nose pungency* of onions as assessed by trained panel (n = 9). *Nose pungency* was defined as the pungency effect in the *nose* of the onion flavour from 'low' to 'high' as measured on a continuous scale from 0 to 100. (*Least Significant Difference* = 4.7)

Pungent Odour

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	Predicted Value
1	(< 4 μM)	42.6 ab
2	(4 – 5 μM)	40.4 a
3	(5 – 6 μM)	42.2 ab
4	(6 – 7 μM)	45.7 bc
5	(> 7 μM)	49.4 c

Table 12 *Pungent odour* of onions as assessed by trained panel (n = 9). *Pungent odour* was defined as the pungent odour the onion flavour from 'low' to 'high' as measured on a continuous scale from 0 to 100. (*Least Significant Difference* = 4.2)

Sweetness

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	Predicted Value
1	(< 4 μM)	37.1
2	(4 – 5 μM)	34.5
3	(5 – 6 μM)	37.1
4	(6 – 7 μM)	37.1
5	(> 7 μM)	33.0

Table 13 *Sweetness* of onions as assessed by trained panel (n = 9). *Sweetness* was defined as the sweet taste in the mouth of the onion flavour from 'low' to 'high' as measured on a continuous scale from 0 to 100. There was no significant difference between pyruvate levels

Sweet aftertaste

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	Predicted Value
1	(< 4 μM)	19.7
2	(4 – 5 μM)	20.3
3	(5 – 6 μM)	20.6
4	(6 – 7 μM)	20.7
5	(> 7 μM)	19.1

Table 14 *Aftertaste sweetness* of onions as assessed by trained panel (n = 9). *Sweetness* after taste was defined as the sweet taste in the mouth after eating the onions as measured on a continuous scale from 0 to 100. There was no significant difference between pyruvate levels

Eye Pungency Aftertaste

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	Predicted Value
1	(< 4 μM)	12.2 ab
2	(4 – 5 μM)	10.9 a
3	(5 – 6 μM)	14.4 bc
4	(6 – 7 μM)	17.4 d
5	(> 7 μM)	16.6 cd

Table 15 *Eye pungency aftertaste* of onions as assessed by trained panel (n = 9). *Eye pungency aftertaste* was defined as the level of pungency in the eyes after eating the onions as measured on a continuous scale from 0 to 100. (*Least Significant Difference* = 2.5)

Mouth Pungency Aftertaste

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	Predicted Value
1	(< 4 μM)	39.7 a
2	(4 – 5 μM)	42.7 ab
3	(5 – 6 μM)	46.7 b
4	(6 – 7 μM)	55.7 c
5	(> 7 μM)	55.3 c

Table 16 *Mouth pungency aftertaste* of onions as assessed by trained panel (n = 9). *Mouth pungency aftertaste* was defined as the level of mouth pungency after eating the onions as measured on a continuous scale from 0 to 100. (*Least Significant Difference* = 4.6)

Nose Pungency Aftertaste

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	Predicted Value
1	(< 4 μM)	22.9 a
2	(4 – 5 μM)	22.6 a
3	(5 – 6 μM)	26.8 b
4	(6 – 7 μM)	32.2 c
5	(> 7 μM)	30.9 c

Table 17 *Nose pungency aftertaste* of onions as assessed by trained panel (n = 9). *Nose pungency aftertaste* was defined as the level of nose pungency after eating the onions as measured on a continuous scale from 0 to 100. (*Least Significant Difference* = 3.9)

Throat Pungency Aftertaste

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	Predicted Value
1	(< 4 μM)	37.2 a
2	(4 – 5 μM)	39.6 a
3	(5 – 6 μM)	46.7 b
4	(6 – 7 μM)	53.9 c
5	(> 7 μM)	55.6 c

Table 18 *Throat pungency aftertaste* of onions as assessed by trained panel (n = 9). *Throat pungency aftertaste* was defined as the level of pungency in the *throat* after eating the onions as measured on a continuous scale from 0 to 100. (*Least Significant Difference* = 4.9)

Overall Pungency Aftertaste

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	Predicted Value
1	(< 4 μM)	42.4 a
2	(4 – 5 μM)	42.5 a
3	(5 – 6 μM)	49.0 c
4	(6 – 7 μM)	55.6 d
5	(> 7 μM)	58.0 d

Table 19 *Overall pungency aftertaste* of onions as assessed by trained panel (n = 9). *Overall pungency aftertaste* was defined as the level of pungency in the eyes after eating the onions as measured on a continuous scale from 0 to 100. (*Least Significant Difference* = 3.9)

5.2.6.4 Principal Components Analysis of Perceived Sensory Attributes

To compare similarities and differences between the complex set of sensory attributes and instrument pyruvate, means and variances were calculated for each attribute and principal component analysis (PCA) was conducted on the correlation matrix of the data.

Associations between onions and the direct and *aftertaste* sensory attributes were determined using a bi-plot analysis (Gabriel, 1971) and shown in Figures 21 and 22. The bi-plot presents the first two principal components axes as well as the data for each onion. As expected, the *sweet* and *sweet aftertaste* responses were well separated from the other attributes. *Throat* and *mouth pungency* responses were highly correlated. *Nose* and *eye pungency* were different to *throat* and *mouth pungencie*s, but similar to each other.

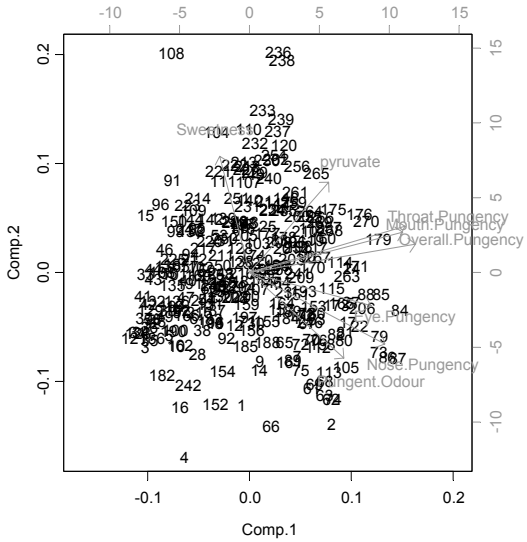


Figure 21 Bi-plot showing principal components analysis for direct sensory attributes and pyruvate for the raw data. The attributes were; *Eye pungency*, *Mouth pungency*, *Nose pungency*, *Overall pungency*, *Pungent odour*, *Sweetness* and *Throat pungency*.

PC1 (read horizontally) accounted for 46% of the variation in the direct sensory attributes, while PC2 (read vertically) accounted for a further 13% of the variation in the direct sensory attributes, not accounted for by PC1.

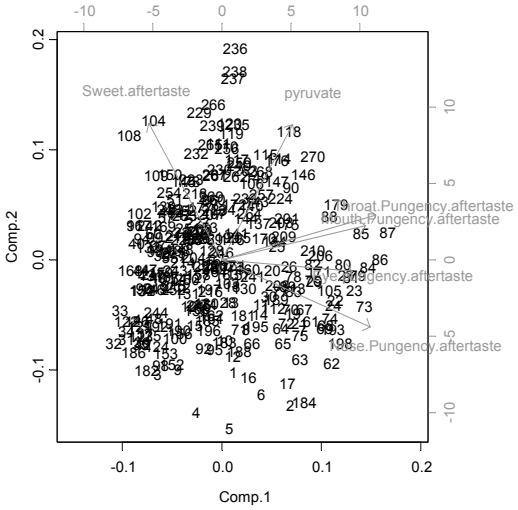


Figure 22 Bi-plot showing principal components analysis for *aftertaste* attributes and pyruvate for the raw data. The *aftertaste* attributes were; *Eye pungency aftertaste*, *Mouth pungency aftertaste*, *Nose pungency aftertaste*, *Overall pungency aftertaste*, *Sweet aftertaste* and *Throat pungency aftertaste*.

PC1 (read horizontally) accounted for 52% of the variation in the *aftertaste* sensory attributes, while PC2 (read vertically) accounted for a further 16% of the variation in the *aftertaste* sensory attributes, not accounted for by PC1.

To simplify the data, PCA was conducted on the means (9 panellists x 6 sessions) for each of the 13 sensory attributes for each pyruvate level as shown in Figure 23.

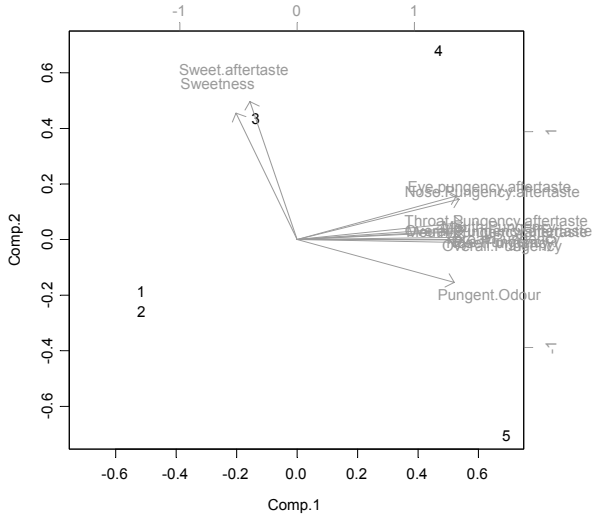


Figure 23 Principal component analysis of the thirteen sensory attributes (*Eye pungency*, *Eye pungency aftertaste*, *Mouth pungency*, *Mouth pungency aftertaste*, *Nose pungency*, *Nose pungency aftertaste*, *Overall pungency*, *Overall pungency aftertaste*, *Pungent odour*, *Sweet aftertaste*, *Sweetness*, *Throat pungency*, *Throat pungency aftertaste*).

Some of the pyruvate categories in this experiment possess different average levels of each sensory attribute. However it is difficult to visualise patterns of attributes that might be consistent within groups of pyruvate categories. The dimensionality of the data (5 pyruvate categories by 13 sensory attributes) makes the problem too complex.

PCA is a multivariate data analysis technique that reduces the dimensionality of data multivariate data set providing an interpretable overview of the relationship between individual attributes and pyruvate levels. Essentially, the 13 attributes or variables for each pyruvate level are mathematically reduced to a fewer number of orthogonal traits (usually two traits will summarise much of the variability in a multivariate data set). Each of the new traits is as highly correlated as possible with the original sensory attributes. Therefore, relationships between the pyruvate levels and each trait should reflect relationships between the pyruvate levels in terms of the original attributes. Each trait is orthogonal and is calculated in such a way that the first trait or principal component (PC) accounts for the largest or most obvious variability between the pyruvate levels. Similarly, the second PC accounts for the next most obvious level of variation in the data set not accounted for by the first PC and so on. Therefore, information contained in each successive PC is associated with increasing levels of subtlety. The reduction from 13 sensory attributes per pyruvate level to two traits per pyruvate level enables creation of the following scatter plot (Figure 24).

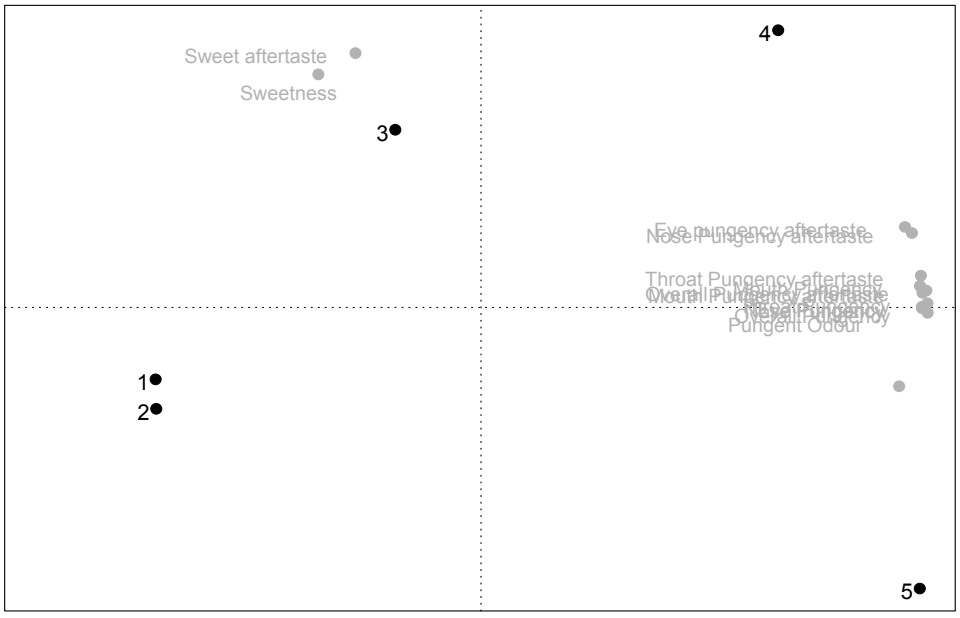


Figure 24 Clustering of the 13 trained panel sensory attributes in the principal component analysis and superimposed is the association of the five different pyruvate classes (1 = < 4 $\mu M.mL^{-1}$ pyruvate; 2 = 4 – 5 $\mu M.mL^{-1}$ pyruvate; 3 = 5 – 6 $\mu M.mL^{-1}$ pyruvate; 4 = 6 – 7 $\mu M.mL^{-1}$ pyruvate; 5 = > 7 $\mu M.mL^{-1}$ pyruvate)

This mapping of 13 original sensory attributes to two traits implies that pyruvate categories and sensory attributes that are spatially close in the plot will have a degree of association in terms of the original data. The main observations from Figure 24 include:

Horizontal axis (PC 1):
Most of the sensory attributes appear towards the right hand side of the plot while the *sweetness* and *sweet aftertaste* attributes appear towards the left. The horizontal axis discriminates between the pyruvate levels on this basis. Pyruvate categories appearing in the right hand quadrants of the plot are more likely to be high in everything apart from *sweetness* than pyruvate categories in the left hand quadrants.

Vertical axis (PC 2):
This axis separates pyruvate levels according to *sweetness* / *sweet aftertaste* against all the other *pungency* attributes. Onion pyruvate categories 3 and 4 (i.e. 5-6 and 6-7 $\mu M.mL^{-1}$ pyruvate) appear in the upper quadrants are more likely to have high concentrations of physiochemical components perceived as *sweetness* (thought to be SSC%); whilst pyruvate categories (1, 2, and 5) in the lower quadrants (< 4, 4-5 and > 7 $\mu M.mL^{-1}$ pyruvate) are more likely to have higher concentrations of physiochemical components contributing to attributes perceived as pungency.

Associations between different onion pyruvate categories:
The highest category of pyruvate (> 7 $\mu M.mL^{-1}$ pyruvate) is isolated from all other categories in the lower right quadrant. Therefore it rates highly on all the *pungency* scales and is particularly low in perceived *sweetness*.

Tight clusters of categories in Figure 24 indicate strong associations. Onions in pyruvate categories 1 and 2 (< 4 and 4-5 $\mu M.mL^{-1}$ pyruvate) would be expected on average, to be similarly perceived.
Eye and *nose pungency aftertaste*s are separated from the other *pungency* attributes. The other nine *pungency* attributes are closely clustered, implying that they are perceived in the same way by the trained panel.

5.2.6.5 Comparison with Literature Trained Panel Analysis (Crowther *et al*., 2005)

A recent study by Timothy Crowther *et al*. in 2005 assessed the flavour of onions by taste panels (shown below). This work was conducted in the United Kingdom over several years.

Journal of the Science of Food and Agriculture *J Sci Food Agric* **85**:112–120 (2005)
DOI: 10.1002/jsfa.1966

Assessment of the flavour of fresh uncooked onions by taste-panels and analysis of flavour precursors, pyruvate and sugars

Timothy Crowther,[1] Hamish A Collin,[2] Brian Smith,[1] A Brian Tomsett,[2]
David O'Connor[3] and Meriel G Jones[2]*

The method of Crowther *et al*. (2005): "For the test varieties, each panellist tasted a separate onion so that the variability between tasters would be confounded with the variability between onions but the ***average*** over all 28 panellists would give a better measure of the variety", was applied to our data in this project.

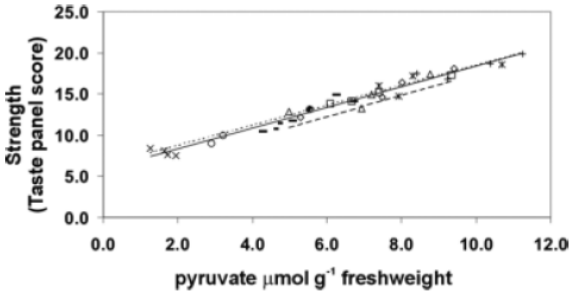


Figure 3. The relationship between onion flavour strength and pyruvate levels in all sessions of the first series of taste-panels. Solid line = regression line fitted by least squares method to data points from all nine tasting sessions. Dashed line = regression line fitted by least squares method to data points from first three tasting sessions only. Dotted line = regression line fitted by least squares method to data points from last six tasting sessions only.

Crowther *et al*. (2005)
J.Sci.Food Agric. **85**, 112-120

However, unlike the design of Crowther *et al*., onion varieties were not identified in our study. The pyruvate reading of each onion was used to assign it to a class (1 = < 4 $\mu M.mL^{-1}$ pyruvate; 2 = 4 – 5 $\mu M.mL^{-1}$ pyruvate; 3 = 5 – 6 $\mu M.mL^{-1}$ pyruvate; 4 = 6 – 7 $\mu M.mL^{-1}$ pyruvate; 5 = > 7 $\mu M.mL^{-1}$ pyruvate).

To replicate Crowther's method, mean pyruvate readings and *overall pungency* measurements across the nine panellists were calculated for the five classes in each of the six sessions.

Regression of *overall pungency* against pyruvate reading was performed. The accumulated analysis of variance is shown in Appendix 4.

As the effect of session was not significant, the simpler regression of *overall pungency* against pyruvate reading is described by this simple linear equation (below).

$$\textit{Overall pungency} = 30.67\ (\pm\ 3.80) + 4.93\ (\pm\ 0.66) * \text{pyruvate} \qquad (r^2 = 0.66)$$

where pyruvate is measured objectively by modified 'Schwimmer and Weston' method.

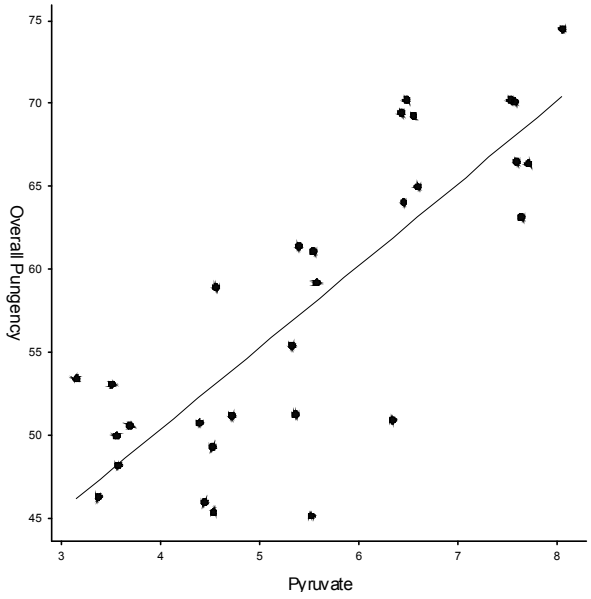


Figure 25 Relationship between perceived *overall pungency* and pyruvate levels ($\mu M.mL^{-1}$ pyruvate) following the method of Crowther *et al.*

This is a relatively good fit of the data ($r^2 = 0.66$) and illustrates the relationship between the integrated sensory attribute of *overall pungency* and pyruvate level.

5.3 Consumer assessments

5.3.1 Background

Consumers of onions were used to provide subjective-level measurements of onion *pungency*. This approach was used to generate measurements of *pungency* that accounted for both cognitive (perceptual) and affective factors pertinent to consumer preference, choice and opinion. In a similar manner to trained panel assessments, consumer measurements can be subject to psychologically biasing factors if confronted with the evaluation of vaguely understood attributes and/or attributes with possible negative connotation (e.g. *pungency*). Therefore, the consumer questionnaire was designed to instil a relevant context for the routine-like assessment of integrated attributes such as *flavour intensity* and *liking*.

The objective of using naïve consumer panel assessment was:

- Generate subjective-level information that could be combined with corresponding trained panel measurements to determine the relationship between perception (*pungency*) and the subjective conception of such and ensuing affective response.
- Combine subjective and affective information with corresponding physicochemical measurements to determine the relationships between a psychophysical stimulus (pyruvate / *pungency*) and the ensuing affective response (*like/dislike, mild/strong* etc.).

A hypothetical example of this relationship is depicted graphically in Figure 19 (previous).

5.3.2 Consumer Sample and Product Evaluation

Consumers (n = 106) were recruited from the Sydney metropolitan area. The consumer sample was required to conform to the following demographic and psychographic criteria:

- Approximate equal split 50% male: 50% female
- Approximate equal split between the age groups; 18-35, 36-50, 51-65 years
- Non-rejecters of onions:
    - Consume onions (alone or as a part of a meal) at least twice per week
    - Consume raw onions (alone or as a part of a meal e.g. salad) at *least* once per week

Onion samples were evaluated in individual booths under red lighting in the sensory laboratory at Food Science Australia's Centre in Sydney. Upon arrival at the sensory test facility, respondents were required to complete a disclaimer and a short demographic and usage questionnaire. Respondents were briefed on the test (including the use of palate cleansers) and the use of questionnaires prior to commencing the research.

Respondents had free access to tap water (room temperature), unsalted crackers, full cream milk or buttermilk, cranberry juice, cream cheese, banana, parsley and coconut to aid in palate cleansing during the 'wash-out' period between each sample. Furthermore, a six minute washout-out period was enforced between each sample to allow respondent palates' to return to a baseline level (desensitise) after onion sensitisation.

Flavour strength and *liking* were scored on unstructured 100 mm continuous line scales anchored at both ends (at 5% and 95%) with extremes of each term (Appendix 4.3). Respondents were also asked to classify the flavour of each sample as 'mild', 'medium' or 'strong'. The complete sensory vocabulary along with corresponding line scale anchors is outlined in Appendix 4.3. Data were recorded and stored using the Compusense five sensory data acquisition programme (Guelph, Ont., Canada). Strict controls were in place to ensure that all differences identified were the result of true product differences rather than any competing extraneous factors.

5.3.3 Experimental Design

A design of 20 5x5 Latin Square designs were used to balance the order of tasting, however the design became unbalanced due to consumer drop outs and was analysed accordingly.

In a similar manner to the trained panel assessments, a Latin-square design was used to accommodate a comparison of all pyruvate treatments by each consumer respondent during the evaluation session. Five samples, each of a designated pyruvate level and a specific instrument measurement of pyruvate, were assigned to each respondent according to the design. Samples were evaluated in a sequential monadic order, according to the balanced design to reduce the effect of positional bias (order).

5.3.4 Results

5.3.4.1 Consumer assessments of onion *flavour intensity* and *liking*

Pyruvate level was treated as a factor (1-5) and consumer respondents' measurement was fitted as a random effect in a mixed model analysis of *flavour intensity* using ASReml program. *Flavour intensity* was defined as the intensity of the onion flavour from 'low' to 'high' as measured on a continuous scale from 0 to 100. There was a significant effect of pyruvate category on *flavour intensity* responses for consumers. Consumers could not detect any significant differences in *flavour intensity* between the three lowest pyruvate categories (Table 20). The *flavour intensity* of onions in pyruvate category 4 was significantly higher than the first three categories. Pyruvate category 5 had the highest *flavour intensity*.

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	Predicted Value
1	(< 4 μM)	40.5 a
2	(4 – 5 μM)	42.9 a
3	(5 – 6 μM)	44.1 a
4	(6 – 7 μM)	58.6 b
5	(> 7 μM)	65.4 c

Table 20 *Flavour intensity* of onions as assessed by consumer panel (n = 100). *Flavour intensity* was defined as the intensity of the onion flavour from 'low' to 'high' as measured on a continuous scale from 0 to 100. (*Least Significant Difference* = 5.6)

Liking was defined as the intensity of the *liking* from *Dislike extremely* to *Like extremely*. It was also a continuous measure on a scale of 0-100. Analysis of *liking* was conducted in a similar manner to the *flavour intensity* analysis (outlined above).
Consumers rated pyruvate categories 1, 2 and 3, the highest for *liking* and these were not different from each other (Table 21). However, onions from pyruvate categories 4 and 5 were rated lower for *liking* responses (i.e. *liked the least*) and were not different from each other.

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	Predicted Value
1	(< 4 μM)	61.4 a
2	(4 – 5 μM)	59.9 a
3	(5 – 6 μM)	59.5 a
4	(6 – 7 μM)	54.0 b
5	(> 7 μM)	51.6 b

Table 21 '*Liking*' of onions as assessed by consumer panel (n = 100). '*Liking*' was defined as the intensity of the *liking* from *Dislike extremely* to *like extremely*. It was assessed on a continuous measure on a scale of 0-100. (*Least Significant Difference* = 5.1)

5.3.4.2 Consumer Onion Flavour Classification and Pyruvate Category

After assessing the onion for *onion flavour*, consumers (n = 100) were asked a purpose-of-use for each assessed onion. For each onion the consumer was asked the following question:

> In a commercial situation (e.g. grocery, supermarket ect.), do you think this onion should be labelled as having: *(please tick one answer only)*
>
> ❍ A. Mild flavour
>
> ❍ B. Medium flavour
>
> ❍ C. Strong flavour

Results

The number of consumer responses in each onion flavour category is presented in Table 22.

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	Onion Flavour		
		1 Mild	2 Medium	3 Strong
1	(< 4 μM)	63	25	12
2	(4 – 5 μM)	49	39	12
3	(5 – 6 μM)	40	42	17
4	(6 – 7 μM)	17	41	42
5	(> 7 μM)	10	37	53

Table 22 Classification of onions as assessed by the consumer panel (n = 100). Panellists were asked to classify each onion into a flavour category.

Statistical analysis using a chi-square test was used to investigate any association between flavour classification and pyruvate level and showed there was a significant association ($p < 0.001$) between the two factors, i.e. low onion flavour classifications are associated with low pyruvate levels and *vice versa*.

However it is worth noting that of the 100 consumers presented with an onion with less than < 4 $\mu M.mL^{-1}$ pyruvate, 12 consumers believed this onion had a strong flavour. This means that 12% of the consumers in this study believed that the onions with the lowest pyruvate level still tasted strong. Conversely, 10% of the consumer panel believed that the onions with the highest pyruvate concentration (> 7 $\mu M.mL^{-1}$ pyruvate) were mild in taste. Notwithstanding the possibility that this task may have been cogitatively challenging for respondents, it reflects the sort of human variability that exists amongst consumers, and must be acknowledged when discussing acceptability and *liking*. The consumer panel were not trained or guided in their interpretations of onion flavour. Therefore, it can be difficult to interpret the consumer responses, as each consumer may have a different interpretation of mild, medium, and strong.

5.3.4.3 Consumer Perception of *Mildness* and Pyruvate

- **Instrument pyruvate reading**

To relate the consumer classification of *mildness* to the instrument or machine pyruvate reading, the flavour classification was then re-assigned into two classes: either mild or strong. The *mild* and *medium* flavour responses (Table 22) were combined to make a single *mild* classification.
The following model is proposed:

Log(p / (1-p)) = a + b.pyruvate reading + *error*

Where p = probability of an onion being classified as *mild*

A generalised linear model (GLM) with binomial error distribution and logit link function was used to relate the probability of *mildness* to machine pyruvate reading (Appendix 4.3).
The predicted pyruvate value, and a 95% confidence interval, corresponding to a particular probability of an onion being *mild* (= *mild* plus *medium* flavour responses) can be calculated by inverting the regression equation and is summarised in Table 23. For example an onion of pyruvate 6.06 μM.mL^{-1} pyruvate (95% confidence interval= 5.65, 6.46 μM.mL^{-1} pyruvate) will be classed by consumers as mild with probability 0.7.

Probability	Predicted pyruvate	Lower 95% confidence limit	Upper 95% confidence limit
0.3	9.21	8.27	10.15
0.4	8.39	7.64	9.13
0.5	7.63	7.04	8.22
0.6	6.88	6.42	7.34
0.7	6.06	5.65	6.46
0.8	5.05	4.57	5.53
0.9	3.55	2.77	4.32

Table 23 Predicted pyruvate level for given probabilities of an onion being perceived as *mild* (*mild* + *medium* flavour responses).

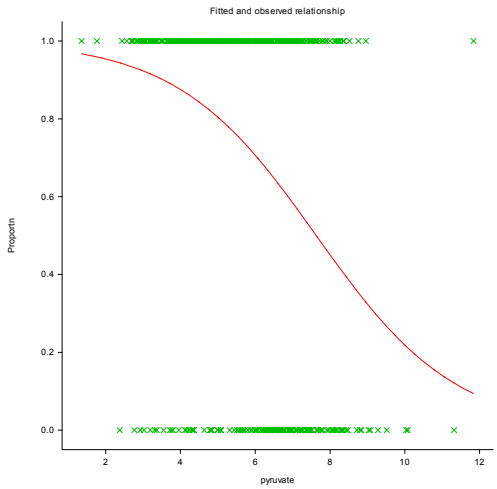


Figure 26 Graphic representation of the probability of an onion being perceived as *mild* (= *mild* + *medium*) with the level of pyruvate (μM.mL^{-1} pyruvate)

- **Pyruvate Category**

Determination of the proportion of onions classified as *mild* in each pyruvate category

The relation between the proportion of onions classified as *mild* and pyruvate category was examined using a generalised linear model in a similar manner to the previous analysis. However this time pyruvate category was used instead of pyruvate reading. The proportions of the onions considered *mild* from this analysis are summarised in Table 24.

In the lowest three pyruvate categories (< 6 $\mu M.mL^{-1}$ pyruvate) the proportion of onions being classified by consumers as *mild* was statistically similar. Whilst in the fourth and fifth pyruvate category (> 6 $\mu M.mL^{-1}$ pyruvate) the proportions of onions perceived as *mild* were lower than the other pyruvate categories but not significantly different from each other.

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	Proportion considered mild	SE
1	(< 4 μM)	0.88 a	0.032
2	(4 – 5 μM)	0.88 a	0.032
3	(5 – 6 μM)	0.82 a	0.038
4	(6 – 7 μM)	0.58 b	0.049
5	(> 7 μM)	0.47 b	0.050

Table 24 Predicted proportion of onions in each pyruvate category meeting consumer expectations of being *mild* (where *mild* is *mild* plus *medium* flavour responses). SE is the standard error of the predicted proportion

An alternative analysis which used the single *mild* response alone as a distinct flavour (rather than *mild* plus *medium*) was done to determine the probability of *mildness* related to machine pyruvate reading and is described in Appendix 4.3.

5.3.4.4 Appropriate Use of Onions from each Pyruvate Category

After assessing each onion for onion flavour, each consumer was asked what the appropriate use of the assessed onion would be used for, i.e. for cooking or eaten raw. The relationship between appropriate use and pyruvate level is summarised in Table 25.

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	Cooking	Consumed Raw
1	(< 4 μM)	28	72
2	(4 – 5 μM)	41	59
3	(5 – 6 μM)	41	58
4	(6 – 7 μM)	59	41
5	(> 7 μM)	66	34

Table 25 Appropriate use of onions in each pyruvate category.

A chi-square test showed there was a significant ($p < 0.001$) association between the consumer's opinion of appropriate use and onion pyruvate level. Onions in the lower pyruvate classes are thought more likely by consumers to be suitable for raw consumption. Conversely, onions in the higher pyruvate classes were thought by consumers to be more suitable for cooking.

The link between the probability of an onion being classified as suitable for raw consumption and pyruvate level was investigated using a generalised linear modelling approach. GenStat was used to fit a generalised linear model with binomial errors and logit link function to the proportions of onions classified as suitable for raw consumption. The results in Table 26 shows that a significantly higher proportions of onions in pyruvate categories 1, 2 and 3 (<4, 4-5 and 5-6 $\mu M.mL^{-1}$ pyruvate) were considered as suitable for raw consumption compared to onions in levels 4 and 5 (6-7 and > 7 $\mu M.mL^{-1}$ pyruvate).

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	Proportion suitable for raw consumption	SE
1	(< 4 μM)	0.72 a	0.051
2	(4 – 5 μM)	0.59 a	0.056
3	(5 – 6 μM)	0.59 a	0.057
4	(6 – 7 μM)	0.41 b	0.057
5	(> 7 μM)	0.34 b	0.054

Table 26 Proportion of onions in each pyruvate category considered to be suitable for raw consumption.
SE is the standard error of the predicted population.

5.3.4.5 Ancillary Consumer Results - Relationship Between Perceived *Flavour Intensity* or *Liking* and Consumer Background Information

To investigate the possibility of inherent relationships between consumer background and measures of *flavour intensity* and *liking*, consumers were asked five preliminary background questions before assessing the onion samples:

Consumer Background Information

1. Your age group? (18-34 years; 35-49 years; 50-65 years)

2. Your gender? (male; female)

3. Are you the main grocery buyer at your current residence?
   (yes; no; I buy some but not all of the groceries)

4. How often do you eat onions (cooked) as part of a meal?
   (most days during the week; occasionally (2-3 times) during the week; once per week)

5. How often do you eat raw onions (alone or as part of a meal)?
   (most days during the week; occassionally (2-3 times) during the week; once per week)

GenStat was used to fit a REML linear mixed model to the data. The significance of fixed terms was assessed with Wald statistics. Of the five background factors (above), only gender had a statistically significant effect.

Results

- **Gender and *Flavour Intensity***

Males rated the *flavour intensity* lower than females (Table 27), i.e. females generally rate *flavour intensity* higher than males. There was no interaction between the effect of gender and pyruvate levels (Table 28). This indicated that the relationship between gender and *flavour intensity* was present across all pyruvate levels (Table 29).

	Gender	
	Male	Female
Flavour Intensity	47.59	52.99

Table 27 Effect of gender on consumer *flavour intensity* (Standard error of differences: 2.49)

Pyruvate Level	1	2	3	4	5
	<4 μM.mL^{-1} pyruvate	4-5 μM.mL^{-1} pyruvate	5-6 μM.mL^{-1} pyruvate	6-7μM.mL^{-1} pyruvate	<7μM.mL^{-1} pyruvate
Flavour Intensity	40.35	43.15	43.86	58.64	65.43

Table 28 Pyruvate level on consumer *flavour intensity*. *Flavour intensity* was defined as the intensity of the onion flavour from 'low' to 'high' as measured on a continuous scale from 0 to 100.

Pyruvate Level		1	2	3	4	5
		<4 μM.mL^{-1} pyruvate	4-5 μM.mL^{-1} pyruvate	5-6 μM.mL^{-1} pyruvate	6-7μM.mL^{-1} pyruvate	<7μM.mL^{-1} pyruvate
Gender	Male	40.73	41.88	41.52	53.21	60.60
	Female	39.96	44.42	64.08	64.08	70.27

Table 29 Gender, pyruvate level and *flavour intensity*.
Flavour intensity was defined as the intensity of the onion flavour from 'low' to 'high' as measured on a continuous scale from 0 to 100.
The interaction was not significant.

- **Gender and *Liking***

Although the overall effect of gender was not significant (Table 30), there was a significant interaction between gender and pyruvate level. Males tended to give onions in the highest pyruvate levels higher *liking* scores compared to the scores given by women. At the lower pyruvate levels there was no difference between the two genders.
Onions in the 3rd pyruvate class (i.e. 5-6 $\mu M.mL^{-1}$ pyruvate) were given a significantly higher *liking* score (63.9) by women compared to the scores given by males (55.1) (Table 31).

	Gender	
	Male	Female
Liking	58.38	56.20

Table 30 Effect of gender on consumer *liking*
No significant effect

Pyruvate Level		1	2	3	4	5
		<4 $\mu M.mL^{-1}$ pyruvate	4-5 $\mu M.mL^{-1}$ pyruvate	5-6 $\mu M.mL^{-1}$ pyruvate	6-7$\mu M.mL^{-1}$ pyruvate	<7$\mu M.mL^{-1}$ pyruvate
Gender	Male	61.79	61.33	55.12	57.35	56.31
	Female	61.05	58.41	63.89	50.70	46.93

Table 31 Interaction of gender and pyruvate level on consumer *liking*.
Liking was defined as the intensity of the liking from *dislike extremely* to *like extremely*. It was assessed on a continuous measure on a scale of 0-100. (*Least Significant Difference* = 8.09)

- **Age**

Ratings of onion *flavour intensity* and *liking* were not related to age (Tables 32 and 33). This suggests that all three age groups responded in a similar manner to different pyruvate levels.

	Age		
	18-34	35-49	50-56
Flavour intensity	49.80	51.38	49.70

Table 32 Effect of age on consumer *flavour intensity*
No significant effect

	Age		
	18-34	35-49	50-56
Liking	55.68	58.82	57.50

Table 33 Effect of age on consumer *liking*
No significant effect

- **Main Grocery Buyer**

There was no relationship between ratings of onion *flavour intensity* or *liking* and the main household grocery buyer status of the respondents (Tables 34 and 35). Similarly, there was no relationship between ratings of onion *flavour intensity* or *liking* and the eating habits of consumers in terms of the consumption of cooked or raw onions most days of the week, 2 -3 times per week, or only once per week (Tables 36, 37, 38 and 39).

	Main grocery buyer		
	Yes	No	Sometimes
Flavour intensity	50.23	49.23	50.77

Table 34 Effect of main grocery buyer on consumer *flavour intensity*
No significant effect

	Main grocery buyer		
	Yes	No	Sometimes
Liking	57.68	61.26	55.19

Table 35 Effect of main grocery buyer on consumer *liking*
No significant effect

- **Cooked Onion Consumption**

	Frequency of eating cooked onions (per week)		
	most days	2-3	once
Flavour intensity	52.48	48.34	51.87

Table 36 Effect of frequency of eating cooked onions as part of a meal on consumer *flavour intensity*
No significant effect

	Frequency of eating cooked onions (per week)		
	most days	2-3	once
Liking	56.92	58.34	51.74

Table 37 Effect of frequency of eating cooked onions as part of a meal on consumer *liking*
No significant effect

- **Raw Onion Consumption**

	Frequency of eating raw onions (per week)		
	most days	2-3	once
Flavour intensity	46.67	50.68	50.65

Table 38 Effect of frequency of eating raw onions as part of a meal on consumer *flavour intensity*
No significant effect

	Frequency of eating raw onions (per week)		
	most days	2-3	once
Liking	58.66	57.38	56.82

Table 39 Effect of frequency of eating raw onions as part of a meal on consumer *liking*
No significant effect

5.3.4.6 Combined Trained Panel and Consumer Results

Investigating the relationship between the trained panel object-level of sensory assessment and the consumer panel subjective-level of sensory assessment is an important means to validate the relationship between perceived onion *pungency* and consumers' perception of onion flavour. To investigate this relationship means for each level of pyruvate were calculated for the trained panel measure attribute *throat pungency* and consumers' response of *flavour intensity*. This "paired" aggregate data is shown in Figure 27 and attempts to link the two levels of sensory measurement. The relationship is strong (r^2= 0.95), however it is an 'ecological' correlation, because the unit of analysis is not an individual onion but a group of onions (Freedman, 1999). It is possible that this relation may be spurious due to some unknown 'lurking variable' and must be acknowledged. Not withstanding this, it is pertinent that consumer perception of onion flavour can to a large extent, be accounted for by a perceived sensory attribute (i.e. *throat pungency*), thereby establishing a link between a physicochemical measurement and consumer response.

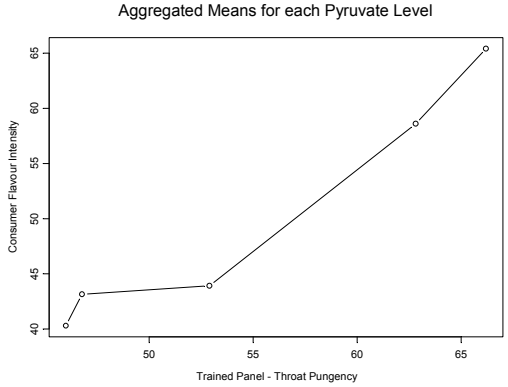


Figure 27 Relationship between the consumer panel (*flavour intensity*) and trained panel (*throat pungency*) and using aggregate means for each pyruvate level. (r^2= 0.95).

Similarly, the relationship between consumers' affective response to onions and a perceived sensory attribute was measured by investigating the relationship between *throat pungency* and onion *liking*. The relationship between *throat pungency* and consumers' response of *liking* for each level of pyruvate is shown in Figure 28. This relation is also strong (r^2= 0.95) indicating that the affective response (in the context of a controlled environment) is to a large extent related to the perceived intensity of an attribute describing onion *pungency*.

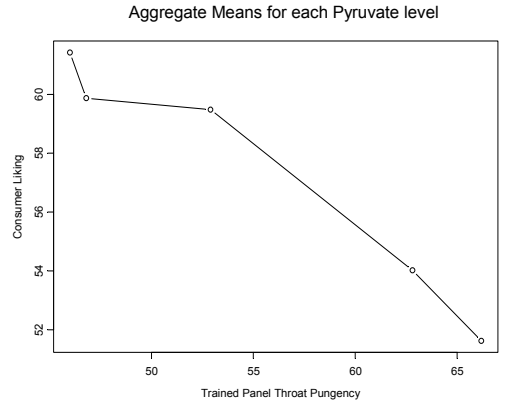


Figure 28 Relationship between the consumer panel (*liking*) and the trained panel (*throat pungency*) using aggregate means for each pyruvate level. (r^2= 0.95).

5.3.4.7 Modified Food Choice Questionnaire (FCQ)

Once the organoleptic consumer questionnaire was complete, consumer respondents were required to complete a modified food choice questionnaire (FCQ). This questionnaire was designed to gather insight about consumer attitudes and beliefs towards onions. Specifically, the questionnaire investigated the importance of each of the following onion-related choice or quality criteria: sensory quality, use / convenience, price, product knowledge and loyalty (Table 40). While the information generated by a modified FCQ is not definitive and somewhat open to broad and subjective interpretation, it is a useful means of gathering quantitative data pertinent to matters of consumer opinion and attitude. Notwithstanding this, it is prudent to combine this level of investigation with other quantitative (e.g. conjoint analysis) and qualitative (e.g. moderated focus groups) research techniques to further deduce areas of importance / interest identified by the modified FCQ. The criteria and statements pertaining to each onion-related criterion were prepared by Food Science Australia.

A projective questioning technique was used to measure each criterion. In this situation, consumers were asked to rate their agreement or disagreement with statements pertaining to each criterion. All responses were measured using a 9-point agreement scale. The full questionnaire is presented as Section 3 of the consumer questionnaire in Appendix 4. Statements were presented in randomised order.

Results

The onion choice criteria (A. Sensory quality, B. Use / convenience, C. Price, D. Knowledge and E. Loyalty) and associated statements, as well as a representation of the average consumer's level of agreement or disagreement with each criterion (i.e. responses averaged across statements) are outlined in Table 40. Not all consumers successfully completed this additional questionnaire, in total 94 consumers completed all components of the FCQ. The mean values are true means, each with a possible range of 1 to 9, corresponding to the 9-point modified FCQ measurement scale (Appendix 4.3).

A. Sensory Quality

On average, consumers showed a high level of agreement with sensory quality statements 3 and 4. This would indicate that consumers are aware of differences in flavour between onions and would welcome more information pertaining to the strength of onion flavour when making purchase decisions. Consumers showed some level of agreement with sensory quality statement 1. This might reflect a number of trends; for example, consumers may not be intimately annoyed by inconsistencies in the flavour of onions but taken together with sensory quality statements 3 and 4 they would welcome more assurances in this regard. Finally, there was a high level of disagreement with sensory quality statement 1 confirming that consumers are aware of onion flavour quality and are likely to be motivated by matters pertaining to onion flavour (sensory quality).

Consumers were relatively consistent in their reply to the sensory quality statements and no interactions for age, gender, main grocery buyer (MGB) status or frequency of raw or cooked onion consumption were identified. In other words, consistency in the level of agreement / disagreement was observed within and between sample population criteria such as male and female, different age cohorts, MGB status and frequency of raw or cooked onion consumption.

Variable	Statement	Label	Mean*(SD)*
Sensory Quality	(1) 'The flavour of onions is inconsistent, sometimes they are intensely flavoured (strong), other times the flavour is poor (weak)'	Sensory Quality 1	6.33 *(1.67)*
	(2) 'Onions are onions…. I never consider differences in flavour'	Sensory Quality 2	2.73 *(1.73)*
	(3) 'Sometimes onions can be '*sweeter*' in flavour while others are more '*pungent*' and intensely flavoured'	Sensory Quality 3	8.01 *(1.08)*
	(4) 'Information about the strength of onion flavour (e.g. mild, medium and strong) would help me choose the right type of onion of the meal(s) I plan to prepare (e.g. salad V cooking)'	Sensory Quality 4	7.72 *(1.53)*
Use / convenience	(1) 'I consider onions to be an important ingredient of most cooked meals'	Use 1	7.74 *(1.48)*
	(2) 'I usually choose my onions based on the type of meal I am preparing, e.g. brown onions for frying, red onions for salads etc'	Use 2	7.78 *(2.02)*
Price	(1) 'Onions are good value for money'	Price 1	7.28 *(1.64)*
	(2) 'I would be willing to pay a little extra if the strength of the onion flavour (e.g. strong V weak) was assured before purchase'	Price 2	6.00 *(2.04)*
Knowledge	(1) 'I am familiar with different varieties of onions, e.g. Wallon Brown V Golden Brown'	Knowledge1	7.72 *(1.42)*
	(2) 'There are differences between the flavour of brown, red and white onions'	Knowledge2	3.89 *(2.10)*
Loyalty	(1) 'When I buy onions, I always try to choose Australian onions over imported onions regardless of price'	Loyalty 1	5.34 *(2.61)*

Table 40 Onion choice criteria and associated statements.
Consumers used a 9 point discrete scale; where 1 = completely disagree with the statement, 5 = neither agree nor disagree, 9 = agree completely with statement.
The means and standard errors are shown in *italicised brackets*

B. Use / Convenience

On average, consumers showed a high level of agreement with use / convenience statements 1 and 2. Therefore, onions are considered to be an important meal ingredient and to a large extent, consumers attempt to match the type of onion with the type of meal they plan to prepare (although there was some noticeable variation in consumer response to this statement).

In terms of trends in the sample population, those consumers who use onions for cooking most days during the week agreed with use / convenience statement 1 more than those consumers who use onions less frequently during the week (most days = 8.39 vs. occasionally = 7.41 and once per week = 6.57). Similarly, the female sample agreed with statement 1 more than the male sample (female = 8.06 vs. male = 7.41).

C. Price

Overall, consumers consider onions to be good value for money (price statement 1). In terms of paying a 'little' extra if the flavour of the onions was assured prior to purchase, there was more of a mixed response (as reflected by the size of the standard deviation) among consumers. It is likely that consumers differed with regard to the value of the proposition presented in price statement 2. Notwithstanding this, providing a valid answer to this question is difficult as consumers currently do not have a suitable frame of reference to evaluate such a proposition with regard to onions.

Consumers were relatively consistent in their reply to price statement 1 and no interactions for age, gender and MGB status were identified. However, those consumers who use onions for cooking once per week did not agree with price statement 1 as much as those consumers who use onions more frequently during the week (once a week = 5.57 vs. occasionally = 7.37 and most days = 7.47). Similarly, the male sample agreed with price statement 2 more than the female sample (male = 6.48 vs. female = 5.54). This would indicate that males are prepared to spend a little extra for assurances and variety in terms of onion flavour. Taken together with the results for use / convenience (outlined above) it is prudent to not that while the male population would consider paying a little extra for more benefits in terms of flavour, they did not rate the importance of onions as a meal ingredient as high as females.

D. Knowledge

On average, consumers showed a high level of agreement with knowledge statement 1. In other words, consumers appear to be familiar with different varieties of unions. Conversely, there was a high level of disagreement with knowledge statement 2, but it is noteworthy that responses to this statement also had a high standard deviation. Knowledge statement 2 was included to provide an indication of consumer association of variety with flavour. However, it is likely that some confusion may have existed with the word '*flavour*' as some consumers may have used *flavour intensity* (i.e. *pungency*) as a frame of reference, whereas others may have used actual differences in flavour quality (i.e. generic onion flavour) when providing their response. Therefore, it would be prudent to investigate this knowledge criterion further before any conclusions are made.

Consistency in the level of agreement / disagreement was observed within and between sample population criteria. In other words no trends between male and female, different age cohorts, MGB status and frequency of raw or cooked onion consumption were observed for knowledge statements 1 and 2.

E. Loyalty

In terms of loyalty, there was an indifferent response (neither agree nor disagree) among consumers with regard to the purchase of 'Australian' onions. Notwithstanding this, there was also a high level of variation between consumers in response to the loyalty statement (as reflected by the size of the standard deviation).

In terms of trends in the sample population, the older age cohort (i.e. 50 – 65 years) agreed with the loyalty statement more than the younger age cohorts (50 – 65 years = 6.53, vs. 35 - 49 years = 5.17 and, 18 – 34 years = 4.44).

Considering the observed variation in opinion among consumers, PCA was used to provide an interpretable overview of FCQ data to study the inherent variation at an individual consumer level in more detail. Furthermore, PCA mapping of individual FCQ information was used to the relative relationships between onion quality criteria in the form of a sample population opinion data map (Figure 29).

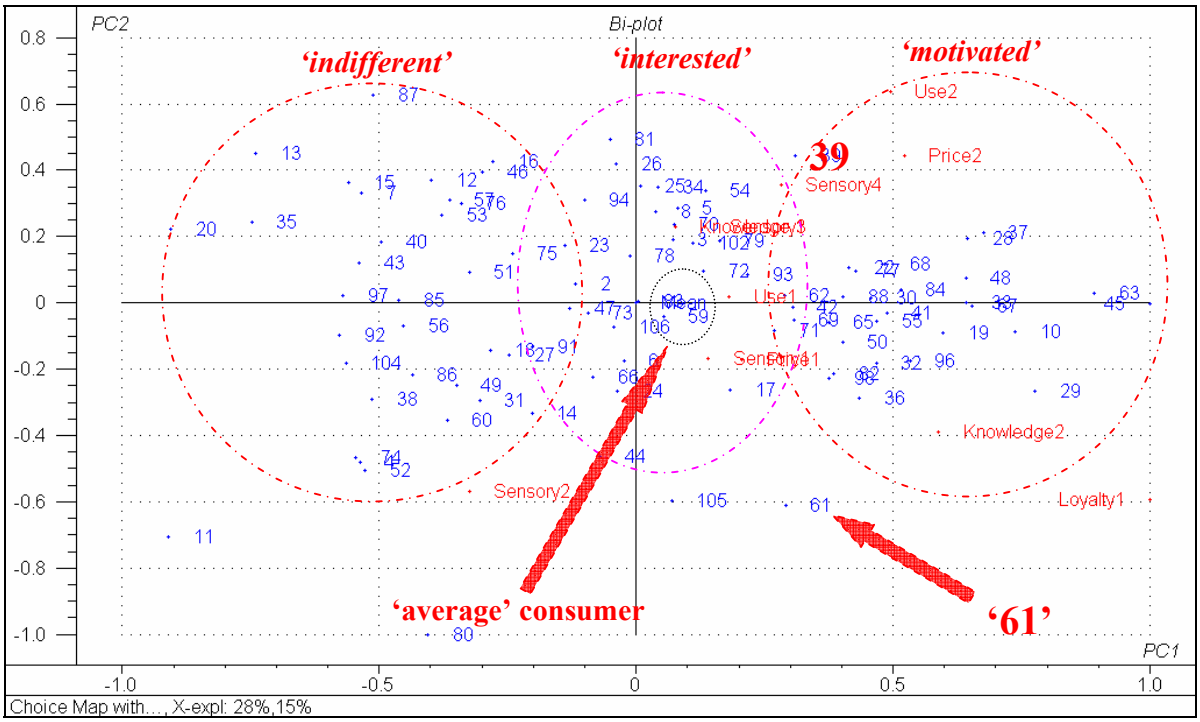


Figure 29 Modified FCQ sample population opinion map depicting the direction and strength of individual consumer's (n = 94) overall agreement or disagreement with onion quality-related statements (Table 40).

The overall opinion of each individual consumer is represented by a discrete point (respondent number) on the map. For example, as outlined in the map consumer 61 is positioned in lower right quadrant of the map. The direction and distance of a consumer from the mid-point of the plot illustrates the intensity and clarity of that consumer's opinion towards the 11 FCQ statements (highlighted in red). For example, consumer 39 (upper right quadrant) agreed strongly with sensory quality statements 3 and 4, use / convenience statement 2 and price statements 2 (Figure 29). Conversely, this consumer disagreed strongly with sensory statement 1 and interpretation of their overall position on the map would indicate that this particular consumer is quite passionate about onions. The overall position of the mean or 'average' consumer (i.e. data averaged across all consumers) is also identified on the map. It is interesting to note how individual consumers varied around this mean point and how the mean value does not necessarily represent the opinion of all consumers.

PC1 (read horizontally) accounted for 28% of the variation in consumer opinion, while PC2 (read vertically) accounted for a further 15% of the variation in consumer opinion, not accounted for by PC1.

From the sample population 'opinion' map it is apparent that there was some variation among consumers with regard to their agreement or disagreement towards the onion quality statements. As outlined by the elliptical illustrations on the map, consumers could be broadly divided into three 'clusters', which we have anecdotally described as those consumers who appear to be either 'indifferent', 'interested' or 'motivated' by onion-related quality matters. It is important to bear in mind that such a classification is subjective and based purely on the feedback received by means of the modified FCQ. Confirmation of such conclusions involves further market analysis. The challenge of the Australian onion industry is to successfully engage these consumers, to increase consumer satisfaction and repeat mild onion sales.

6. Discussion

To ensure the Australian Onion Industry could confidently support the further development of the mild onion industry, it was necessary to ensure there was a reliable and cost-effective basis to measure onion pungency. In addition it was necessary to determine whether the Australian palate could distinguish between different levels of onion pungency and finally determine at what pyruvate concentration is satisfactory for Australian consumers to enjoy raw mild onions. This project had three clear objectives:

1. Construct an onion juice press, establishing a recognised testing facility that will enable rapid and cost effective sampling of onion pungency,
2. Develop a reliable and reproducible pungency test utilising the modified 'Schwimmer and Weston' method, and
3. Calibrate the 'Schwimmer and Weston' method against the Australian palate utilising extensive taste panel comparisons.

Onion Press

An onion press was constructed from plans adapted from the University of Georgia (Bill Randle). This was done at NSW DPI, Wagga Wagga Agricultural Institute. The press was locally constructed and commissioned and is supervised and currently run by Richard Meyer. The onion press pneumatically crushes the onion (under constant pressure) to extract the juice which is used for pungency testing. The pneumatic pressing of the onion immediately releases the juice from the flesh under normal room temperatures. The juice can then be immediately used for the determination of pyruvate (pungency).

Pyruvate Testing using the Modified 'Schwimmer and Weston' Method

The current testing procedure for the measurement of pyruvate (pungency) was initially developed by Schwimmer and Weston (1961). This was adapted (Randle and Bussard, 1993) and is currently used by National Onion Labs Inc. to certify sweet onions. The State of Georgia have legislated this method as the only method for determining pyruvate levels for certifying Vidalia onions in the USA. Following a visit to Georgia, this method was adapted and is currently used in the NSW DPI Diagnostic and Analytical Services (DAS) at the Wagga Wagga Agricultural Institute. A significant improvement to the University of Georgia pyruvate method was the development of Flow Injection Analyser (FIA) technology into the method. This technology removes the human element in adding, incubating and measuring pyruvate. The FIA instrument at Wagga Wagga can automatically add the juice, substrates, stop the chemical reactions and measure the pyruvate level. In addition this laboratory is accredited with the National Associations of Testing Authorities which means all systems and results are quality assured from the national laboratory testing authority ensuring the reproducibility, quality and rigour of the results. This assurance and external auditing is essential for any commercial analytical testing service. The NSW DPI Diagnostic and Analytical Service is committed to providing reliable, accurate and cost-effective analytical services to the Australian onion industry.

When preparing onion samples for sensory analysis, a preliminary survey of onion pyruvate levels showed significant differences between sample batches of the same onion variety. Differences in pyruvate concentrations of up to two times were measured on different batches of the same variety. This is a salient lesson for both researchers and industry in not assuming variety will give similar levels of pungency between batches and reinforces a dominant role that climatic and geographic factors play in determining onion pyruvate content.

Sensory Calibration of Onion Pyruvate Levels to the Australian Palate

The sensory calibration work was completed in two parts; a trained panel and a consumer (untrained) panel. The trained sensory panel was used to provide objective measurements of onion *pungency*. The trained panel of ten specialist tasters were previously screened for sensory acuity and were trained to profile the sensory characteristics of food products. This approach was used to ensure the generation of purely perceptual measurements of *pungency*, without the influence of biasing psychological factors commonly associated with consumers such as prior 'unpleasant' onion experiences etc. The trained panel assessed 13 different sensory attributes thought to be important in onion flavour. An experimental design was used to accommodate a comparison of all pyruvate treatments by each panellist during each evaluation session. This balanced design was used to reduce the effect of positional bias (order of presentation). The results showed that the trained panel could distinguish between onions of different pyruvate levels in all *pungency* sensory attributes (*overall pungency*, *eye pungency*, *eye pungency aftertaste*, *mouth pungency*, *mouth pungency aftertaste*, *nose pungency*, *nose pungency aftertaste*, *overall pungency aftertaste*, *pungent odour*, *throat pungency*, *throat pungency aftertaste*). *Overall pungency* is an integrated sensory attribute that relates a range of different *pungency* attributes and the results show that the trained panel could not distinguish between onions in the two lowest categories of pyruvate (less than 4 $\mu M.mL^{-1}$ pyruvate and 4-5 $\mu M.mL^{-1}$ pyruvate). However the trained panel could reliably detect those onions in pyruvate category 3 (5-6 $\mu M.mL^{-1}$ pyruvate) and these were different to those onions greater than 6 $\mu M.mL^{-1}$ pyruvate. The trained panellists could not distinguish between onions that were between 6 – 7 $\mu M.mL^{-1}$ pyruvate and those onions greater than 7 $\mu M.mL^{-1}$ pyruvate. The panellists thought these onions were most pungent. The results of the other *pungency* attributes produced similar results. This shows that the palate could detect differences in mid-range of pyruvate concentrations. However the taste panel could not detect differences in *sweetness* (or *sweetness aftertaste*) between onions that had been classified according to their pyruvate concentration. This was not unexpected, and demonstrates that onions with low levels of pyruvate are not necessarily also perceived as *sweet*.

Having demonstrated that the trained panel could reliably differentiate onions based on their pyruvate concentration, the next step was to calibrate this to the untrained regular consumer. Consumers of onions were used to provide subjective-level measurements of onion pungency such as *flavour intensity* and *liking*. This approach was used to generate measurements of *pungency* that accounted for both cognitive (perceptual) and affective factors pertinent to consumer preference, choice and opinion.

One hundred consumers were recruited from the Sydney metropolitan area. The consumer sample was required have 50% male: 50% female and an approximate equal split between the age groups; 18-35, 36-50, 51-65 years. The consumers must have also been non-rejecters of onions, i.e. consume onions (alone or as a part of a meal) at least twice per week and eat raw onions (alone or as a part of a meal e.g. salad) at least once per week. Similar to the trained panel assessments, a Latin Square design was used to balance the order of tasting, such that each consumer sampled the five different onion pyruvate categories in a balanced design to reduce the effect of positional bias (order).

There was a significant effect of pyruvate category on *flavour intensity* responses for consumers. Consumers could not detect any significant differences in *flavour intensity* between the three lowest pyruvate categories (i.e. less than 6 $\mu M.mL^{-1}$ pyruvate). The *flavour intensity* of onions with pyruvate levels between 6 – 7 $\mu M.mL^{-1}$ pyruvate were significantly higher than the first three categories, whilst those onions with greater than 7 $\mu M.mL^{-1}$ pyruvate had the highest *flavour intensity*.

The results of consumer *liking* of the onions of different pyruvate classes were similar to those responses of *flavour intensity*, in that consumers assigned the highest onion *liking* responses to those onions possessing less than 6 $\mu M.mL^{-1}$ pyruvate. Onions with levels of pyruvate greater than 6 $\mu M.mL^{-1}$ pyruvate were liked the least. After assessing each onion, the consumers were asked a purpose-of-use question, i.e. 'In a commercial situation (e.g. grocery, supermarket etc.), Do you think this onion should be labelled as having mild, medium or strong flavour?' The results showed that the consumers could accurately classify onion flavour with the different pyruvate concentrations. This is an important result for industry as it clearly demonstrates that consumers can distinguish onions of different pyruvate concentrations and can reliably classify those onions. This was used to conduct further analysis to determine the probability of consumer perception of mildness to pyruvate concentration. This analysis revealed it was possible to determine the probability (and their confidence intervals) that an onion of known pyruvate concentration would be considered by consumers as 'mild'.

A further survey to link consumer *liking* and *flavour intensity* to consumer age, gender, main grocery buyer and frequency of onion eating was also conducted and showed there is a difference in gender of perception of *flavour intensity liking*, but other factors such as age, main grocery buyer and frequency of onion eating did not affect consumer perceptions of *flavour intensity* and *liking*.

An additional consumer questionnaire at the end of the assessments was designed to gather insight about consumer attitudes and beliefs towards onions. Specifically, the questionnaire investigated the importance of each of the following onion-related choice or quality criteria: sensory quality, use / convenience, price, product knowledge and loyalty. While the information generated by these sort of questionnaires are not definitive and somewhat open to broad and subjective interpretation, it is a useful means of gathering quantitative data pertinent to matters of consumer opinion and attitude. The main results of this questionnaire suggest that consumers consider onions an important meal ingredient and are aware of differences in flavour between onions. However consumers would welcome more information and assurances of the strength of onion flavour when making purchase decisions. However when asked if they would pay a 'little' extra if the flavour of the onions was assured prior to purchase, there was more of a mixed response. However providing a valid answer to this question is difficult as consumers currently do not have a suitable frame of reference to evaluate such a proposition with regard to onions.

Further statistical analysis of the consumer questionnaire showed that consumers could be broadly divided into three 'clusters', which we have anecdotally described as those consumers who appear to be either 'indifferent', 'interested' or 'motivated' by onion-related quality matters. It is important to bear in mind that such a classification is subjective and based purely on the feedback received by means of this consumer questionnaire but this maybe used by industry to begin further market analysis and promotion for the mild onion industry.

Conclusions

The outcomes and results of this project have been:

1. Construction of an onion juice press, establishing a recognised testing facility that has enabled the rapid and cost effective sampling of onion pungency,
2. Development of a reliable and reproducible pungency test utilising the modified 'Schwimmer and Weston' method, and
3. Calibration of the 'Schwimmer and Weston' method against the Australian palate utilising extensive taste panel comparisons.

These results will provide the basis of the development of a consumer-driven mild onion industry.

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Appendix 1

Original Horticulture Australia Expression of Interested for the 'Onion Pungency Testing and Consumer Calibration' Project December 2004

ONION PUNGENCY TESTING AND CONSUMER CALIBRATION

PROPOSAL BRIEF (Project Number: VN04002)

BACKGROUND

Horticulture Australia Limited (HAL) on behalf of the Australian Onion Industry Association Inc. is calling for expressions of interest to develop an onion pungency testing and calibration capability in Australia.

Onion bulbs vary in their pungency according to their genetic makeup and the environment under which they have been grown and stored. The compounds that create this pungency, which are well documented, make up the complex of flavour, odour and the ability to create tears.

Not all consumers like a pungent onion. In particular, less pungent onions may be preferred when consumed raw in salads and other dishes. Such a type of onion has been promoted and is well established in markets such as the USA and the United Kingdom. In these markets they are referred to as "Sweet" or "Sweet Mild".

In the US and United Kingdom markets the modified "Schwimmer and Weston" method for testing onion pungency has been calibrated to consumers tastes and is now widely accepted as an accurate measure of an onions pungency characteristics, traditional, sweet or sweet mild.

There is no such established "Sweet" or "Sweet Mild" market in Australia. With no recognized pungency test and testing facility that has been calibrated to the Australian palate, the definition of mild remains both subjective and inconsistent. Consumers are therefore unable to make an informed buying decision and marketers are unable to demonstrate unique product qualities and characteristics.

TERMS OF REFERENCE

Project activities are to include

- Review of literature/knowledge and technology being utilized in applying the modified "Schwimmer and Weston" method in the USA and United Kingdom
- The successful applicant will liaise with Horticulture Australia Ltd and the Onion Industry Advisory Committee (IAC) responsible for the administration of the national onion research and development levy.

Project Outcomes

- Develop a reliable and reproducible pungency test utilizing the modified "Schwimmer & Weston" method
- Calibrate the "Schwimmer & Weston" method against the Australian palate utilizing extensive taste panel comparisons
- Import or construct an onion juice press, establishing a recognized testing facility that will enable rapid and cost effective sampling of onion pungency

HAL understand that a single service provider may not be in a position to provide all of the required project outcomes in house. If necessary the project may be carried out by multiple service providers however HAL will only contract a single lead service provider.

PROJECT OUTPUTS AND TECHNOLOGY TRANSFER

Throughout the project there is a need to:

1. Produce full written and electronic reports including:
   - The assumptions (and other relevant considerations) made in conducting the project
   - The approach taken in conducting the project
   - Any difficulties encountered and how they were resolved
   - A list of all sources of information and personal communication with other horticultural industries
   - Recommendations (if appropriate)
   - Any other documentation that has formed a requirement for the brief

2. Hold meetings with the Industry Advisor Committee to discuss progress of the project
3. Provide regular project updates in the industry newsletters and magazine
4. Conduct annual formal industry presentations

Development of Test Method - Timetable

What	Input sought from	Summary/points	Finalised by
1. Select researcher	IAC	• Researcher selected on basis of selection criteria following advertising of Brief	End February 2005
2. Evaluation of method	IAC Overseas contacts	Generate standard curve using sodium pyruvate and spectrophotometer. Import or construct juice press. Test bulbs from a range of Australian sources	End April 2005
3. Brief progress report	IAC	Reporting of project progress to IAC at R&D committee meeting. Researcher will have collaborated with/conducted taste panel comparisons	June 2005
4. Method ready for commercial adoption	Researcher	• Presentation of results from research at annual levy payers meeting • Decision made on test method	November 2005

Calibration of Method against the Australian Palate - Timetable

What	Input sought from	Summary/points	Finalised by
1. Select researcher	IAC	• Researcher selected on basis of selection criteria following advertising of Brief	End February 2005
2. Initial project briefing	IAC	Provide researcher with an initial briefing and answer questions regarding industry requirements for a reliable method for determination of mild flavour in onions	End April 2005
3. Brief progress report	IAC	Reporting of project progress to IAC at R&D committee meeting. Taste panel will have rated pungency against new test method	June 2005
4. Method ready for commercial adoption	Researcher	• Presentation of results from research at annual levy payers meeting • Decision made on test method	November 2005

PROJECT MANAGEMENT RESPONSIBILITIES

The researchers will report to the Onion Industry Advisory Committee and the Horticulture Australia Program Manager, Mr Simon Drum.

The final report will be provided to HAL, with 2 hard copies (1 bound, 1 unbound) and an electronic copy.

RESOURCE ALLOCATION TO THE PROJECT

Researchers will provide their own administrative support, including word processing and printing requirements. Researchers will be responsible for the collation of data and the analysis of the results.

The Horticulture Australia contact will provide assistance in accessing relevant Horticulture Australia documents and appropriate Horticulture Australia and industry representatives as may be agreed to.

Research personnel allocated to the project cannot be changed throughout the project without the concurrence of Horticulture Australia.

GENERAL CONDITIONS OF THE CONTRACT

Horticulture Australia expects that:

- Confidentiality will be maintained at all times.
- All intellectual property (including but not limited to the copyright in all reports) developed, as the result of a project, will be negotiated between Onions Australia, HAL and the project researchers.
- The project is undertaken in an impartial, objective and professional manner.
- EEO principles will be applied in both the selection of personnel for the project and in the conduct of the project.
- The consultant has insurance cover for property damage and public risk, public liability and accident or injuries to employees of their company.
- Any areas of potential conflict of interest be identified at the time of the researcher's response to the brief and updated during the course of the project should potential conflicts arise.
- The researcher's contract may be terminated or the work content reduced, with a fair and reasonable monetary adjustment determined by Horticulture Australia, subject to the consultant being given notice in writing.
- Any material provided by Horticulture Australia for this project will be used only for this project and remains the property of Horticulture Australia.
- A formal Research Agreement will be entered into at the commencement of the project. The general conditions as stated in the brief and the specific conditions as stated in the Research Agreement will apply.
- The decision as to which, if any, proposal will be pursued further will be made by Horticulture Australia at its absolute discretion. No legal relations with regards to any proposal will arise unless a legal agreement with Horticulture Australia has been executed.

RESEARCHER'S PROPOSAL

The researcher's response to the brief must address:

1. Methodology:
   a) Demonstration of a detailed understanding of the project requirements
   b) A detailed description of the proposed methodology to address the specific project outcomes and associated timeframes.
2. Costing and payment:
   a) A total job cost with breakdown of anticipated costs for each major phase or milestone of the project, including allocation of the researcher's time, material and other costs
   b) A detailed outline of when project payments are due.
3. Qualifications and expertise of researchers:
   a) A statement of the names, role, qualifications and experience of personnel allocated to the project must be provided.
   b) Current references, which would demonstrate the experience of both the organisation and personnel nominated for this project, must also be provided.
   c) Contact details for all personnel nominated for involvement in the project.
   d) Clearly identify the project leader, the main contact for correspondence.

CRITERIA FOR SELECTION

The various criteria for selection will include:

- Competence of the researcher/s to undertake the work
- Availability of the researcher/s to undertake the work
- Past history in the field of research
- Feed back from referees
- Other criteria considered applicable by HAL and Onions Australia

OTHER REFERENCES

Researcher to provide any other relevant reports or documentation that is available

LODGEMENT OF RESPONSE

To respond to this brief please submit a proposal including acknowledgment that all terms and conditions stated in this brief are accepted. Three paper copies and one electronic version of the proposal must be lodged in the tender box during normal business hours by 5.00 pm Friday December 24th 2004.

Late proposals or faxed and e-mailed proposals will NOT be considered.

Please address all responses marked "Confidential" as follows:

Proposal for: Development of a reliable test method for the assessment of mild flavour in onions and/or Development of a taste panel to calibrate a reliable test method for the assessment of mild flavour in onions.

Three copies of the proposal to:

Mr Simon Drum
HAL Level 1
50 Carrington Street
Sydney NSW 2000

Ph: 02 8295 2300

Appendix 2

Travel Report to the University of Georgia (USA) for discussions on Mild Onion Pungency Testing

Travel Report to the University of Georgia (USA) for discussions on Mild Onion Pungency Testing

30 April – 8 May 2005

Visit to the University of Georgia (USA) to meet with Professor Bill Randle and colleagues to discuss mild onion pungency testing in the USA, and the scope and direction of the Horticulture Australia funded project; 'Onion Pungency Testing and Consumer Calibration' (VN 04016)

Dr. John Golding
Research Horticulturalist (Postharvest)
Gosford Horticultural Institute
NSW Department of Primary Industries
Locked Bag 26 Gosford NSW 2250
e-mail john.golding@agric.nsw.gov.au
Telephone 02 4348 1926 Facsimile 02 4348 1910

NSW DEPARTMENT OF PRIMARY INDUSTRIES

Executive Summary

Pungency is responsible for the hot flavour when eating raw onions. Mild onions have low levels of pungency and are eaten raw in salads etc. The onion industry believes there is a considerable market for mild onions in Australia, but the lack of a reliable, rapid and cost effective test for pungency is limiting the development of this industry. Onions Australia and Horticulture Australia Ltd. funded a project (April 2005) for the development of a rapid and cost effective method for the assessment of onion pungency (pyruvate), and to calibrate this test to the Australian palate. It was recommended by the onion research and development committee that John Golding (Project leader) and Trevor Twigden (Chair Onion R&D Committee) meet with Professor Bill Randle at the University of Georgia (USA) for detailed discussions about the plans and scope of the project.

These discussions and visits were invaluable for the directions and scope of the project and the development of the Australian mild onion industry. Apart from learning the exact laboratory details of measuring pyruvate and the procedures for extracting juice, this visit also revealed important additional factors that should be taken into account for the research project and to the Australian onion industry.

I believe the most important factor for pyruvate (pungency) testing is sampling. There is huge field variation in pungency which determines the level of sampling required to ensure the onion population can be classified as 'mild'. Research over many years at the University of Georgia has concluded that two (10 onion bulb) samples per acre are required to assess the pungency of the crop. A commercial onion testing laboratory in south Georgia ('National Onion Labs Inc.') run a service for growers in precision agriculture, using GPS to relate soil nutrition to pungency. This technique is very powerful and has been successful for the growers in the scheme not only with reducing pungency and field variation, increasing size and yields but with premiums being paid by US supermarkets.

Other important outcomes of the visit that directly impact on the current project include the importance of the lachrymatory factor (LF) in onion pungency. LF is a chemical compound that is responsible for the mouth burn and tears production when eating some onions. The validity and ability to routinely quantify LF is not obvious in the current scientific literature and was not considered in this initial HA proposal (Feb 2005). However recent developments at the University of Georgia and the National Onion Labs Inc., have increased the importance of LF in onion pungency testing. We now plan to measure LF, as well as pyruvate and sugar levels in this project.

Another important outcome of this visit was the potential to use the same onion for both the pungency testing and the sensory analysis. We were planning to sample different onions from the same onion population for the chemical and sensory analysis. This was because the literature stated you could not use the same onion due to chemical changes in the cut onion. But the potential to same onion for both tests significantly increases robustness and reliability of the data.

This visit to the University of Georgia was invaluable to this HA project and the Australian industry. The outcomes of the visit will fast-track the development and reliability of the pungency test and assist the development of a mild onion industry in Australia.

Introduction

The Australian onion industry believes there is a significant potential for a mild onion industry in Australia. The mild onion (or Vidalia onion) industry in the state of Georgia (USA) generates over A$120 million each year. Mild onions are not pungent (hot) and are generally eaten raw in salads, sandwiches etc. However to guarantee that mild onions are not pungent, the development of a rapid and cost effective method for the assessment of onion pungency is critical for the Australian Onion Industry.

Onions Australia and Horticulture Australia Ltd. funded a research and development project (April 2005) for the development of a rapid and cost effective method for the assessment of onion pungency using the modified "Schwimmer & Weston" method. The project will also calibrate this pungency assessment method to the Australian palate utilizing comprehensive taste panel comparisons.

Project Title
'Onion Pungency Testing and Consumer Calibration' (VN 04016)

Project Objectives

- Develop a reliable and reproducible pungency test utilizing the modified "Schwimmer & Weston" method
- Calibrate the "Schwimmer & Weston" method against the Australian palate utilizing extensive taste panel comparisons
- Construct an onion juice press, establishing a recognized testing facility that will enable rapid and cost effective sampling of onion pungency

This is a collaborative project with NSW Department of Primary Industries at Gosford Horticultural Institute with the pungency testing being conducted through NSW DPI Diagnostic and Analytical Services (DAS) at the Wagga Wagga Agricultural Institute. Richard Meyer is the chief chemist at DAS Wagga running the pungency testing. This project will also utilize the extensive practical expertise of Food Science Australia (Dr. Patrick O'Riordan) at North Ryde to calibrate the pungency assessment to the Australian palate utilizing comprehensive taste panel comparisons. Food Science Australia is the Australian leader in sensory and consumer research and application, and can successfully conduct well planned taste panel comparisons.

It was recommended by the onion research and development committee that John Golding (Project leader) and Trevor Twigden (Chair Onion R&D Committee) meet with Professor Bill Randle at the University of Georgia (USA) for detailed discussions about the plans and scope of the project.

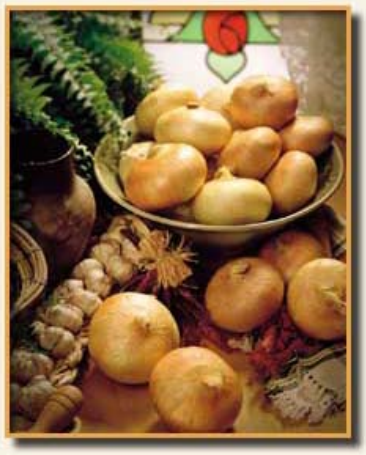
We spent five days with Dr. Randle's laboratory and associated visits in May 2005 (30 April – 8 May). These discussions and visits with industry were crucial in the development, planning of the project and future directions for the Australian onion industry.

Vidalia Onions in Georgia (USA)

Background The Vidalia onion industry in Georgia generates $US95 million ($A120 million) each year (2000). Vidalia onion industry is highly regulated where 'Vidalia Onions' can only be grown in 20 counties in South Georgia. In 1986 Georgia's state legislature defined the 20-county production area as follows: All of these counties can grow and sell Vidalia Onions - Emanuel, Candler, Treutlen, Bulloch, Wheeler, Montgomery, Evans, Tattnall, Toombs, Telfair, Jeff Davis, Appling, and Bacon. (Portions of the following counties can also grow and sell Vidalia Onions - Jenkins, Screven, Laurens, Dodge, Pierce, Wayne, and Long).
In 1989, Vidalia onion growers united to form Federal Marketing Order No. 955. This USDA program established the Vidalia Onion Committee, extended the definition of a 'Vidalia' onion to the federal level and provided a way for growers to jointly fund research and promotion programs.

The Vidalia Sweet Onion Industry is based around the town of Vidalia in South Georgia (USA)

Production There are about 15 seed varieties of onions approved for planting as Vidalias by the Georgia Agricultural Commission. The yellow Granex seed varieties selected have survived two consecutive years experimental testing, subjected to chemical analysis and taste test by a panel of trained experts.
The planting process starts by producing seedlings that are allowed to geminate in a controlled environment. The seedlings are then planted into the low sulphur soil by hand. Georgia's 130 registered growers plant over 6,000 ha of Vidalia Onions. 70,000 plants are produced on each acre and are typically planted in 25cm rows between 11 – 15cm apart. Irrigation is used when necessary. During the growing season temperatures average around 12C in the winter and 24C in the spring and rainfall averages



90mm. This combination produces a sweet, mild Vidalia onion with a somewhat flat top and bottom.

Harvesting Harvesting of Vidalia onions typically occurs from late April through mid-June. Standard practices in onion harvesting include undercutting the onions, allowing them to cure (air dry) for two to three days, clipping the tops and roots, bagging in burlap sacks, transporting to a warehouse, drying, grading, bagging or boxing, and shipping. The delicate nature of the onions requires that they be harvested by hand. To ensure continued quality Georgia's Department of Agriculture Commissioner created and implemented the Vidalia Onion Quality Control Inspection Service.

Under-cut Vidalia onions near Lyons, GA (May 2005)
Onions are allowed to air 'cure' before hand harvesting.

Storage Vidalia Onions can be stored for several months to prolong the marketing season well into November and December by using Controlled Atmosphere (CA) storage. Vidalia Onions can be stored for several months in an atmosphere of 5% CO_2 and 3% O_2 with the air temperature maintained at approximately 0.5C with 70% humidity. However CA storage is being less utilised due to counter-seasonal imports of fresh mild onions from South America (eg Peru).

Vidalia onion facts at a glance

The Vidalia onion is a Georgia-grown, yellow Granex hybrid known for its sweet, mild flavour. Vidalia onions have given themselves the reputation as the "world's sweetest onion." Their mild flavour is due to the combination of soils and climate in the 20-county production area.

About 140 growers cultivate Vidalia onions on 5,820 ha with about 104 handlers grade, pack and distribute Vidalia onions.

An average of 300, 50-pound (23kg) bags are produced per acre. 3.6 million onions were sold at the end of the 2001 growing season. About 70 percent of the Vidalia crop is distributed

through grocery stores as a specialty item. The remaining 30 percent is distributed through roadside stands and mail order businesses and as an added-value product. Generally recognized Vidalia onion sizes are small (2.5 to 5.7cm; 1 to $2^{1/4}$ inches), medium (5 to 9.5cm; 2 to $3^{3/4}$ inches) and large or jumbo (7.5cm; 3 inches or larger). Vidalia onions are harvested from late April through mid-June. Retailers usually have fresh Vidalia onions available through mid-July. Controlled atmosphere storage research makes Vidalia onions available through December. Farmers plant Vidalia onions from September through February. About 70,000 plants are produced per acre (173,000 plants per ha).
The delicate nature of the Vidalia onion requires that they be harvested by hand, thoroughly dried and treated gently during grading and packaging. Migrant labour is not a problem in Georgia, where large labour gangs harvest the under-cut crop.

AMERICA'S FAVORITE SWEET ONION

Vidalia Onion Committee
100 Vidalia Sweet Onion Drive
P.O. Box 1609
Vidalia, Georgia 30474 USA http://www.vidaliaonioncommittee.com

Trevor Twigden (Onions Australia), Dr Davey Kopsell (National Onion Labs Inc) and Dr Bill Randle in a Vidalia onion field in South Georgia (May 2005)

Pungency Testing Procedure

Pungency Background

The characteristic flavour of onions develops when the tissue is cut or damaged. The enzyme alliinase which is localised in the vacuole, is released to hydrolyse the flavour precursors, collectively known as the *S*-alk(en)yl cysteine sulfoxides (ACSOs), which are localised in the cytoplasm This gives rise pyruvate, ammonia and the many volatile sulfur compounds associated with flavour and odour (Figure 1). The reaction of the enzyme and substrate to produce sulphur volatiles is the central point of onion flavour biochemistry.

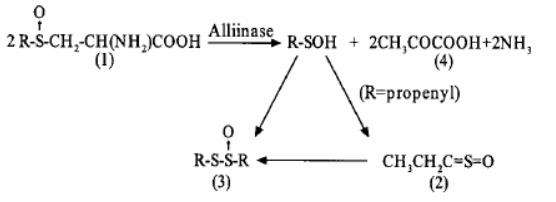


Figure 1. Flavour reaction in onion

(1) alkenyl cysteine sulfoxides:
R= CH_3 (methyl); **R=** CH_3-CH_2-CH_2 (propyl); **R=** CH_3 CH_3-CH=CH (propenyl) ;
(2) thiopropanal *S*-oxide ;(3) thio- CH_3-CH=CH sulfinates ; and (4) pyruvic acid
(from Lancaster *et al*., 1998)

In onions there are three ACSOs ((*1) above*), (+)-*S*-methyl-L-cystine sulfoxide (MCSO), (+)-*S*-propyl-L-cystine sulfoxide (PCSO) and *trans* (+)-*S*-(1-propenyl)-L-cystine sulfoxide (1-PRENCSO), however PRENCSO generally predominates (Randle *et al*., 1995). The unstable sulfenic acids re-arrange over time to produce disulfides and other sulfur compounds.

(*Z, E*) Propanethial *S*-oxide or the lachrymatory factor (LF) arises from the hydrolysis of 1-propyl cysteine sulfoxide (1-PRENCSO) and is responsible for the tear producing, mouth burn and heat associated with eating onions. Sensory attributes from the LF can be overwhelming and can dominate the experience of eating onions with high levels of 1-PRENCSO.

The current testing of pungency in Georgia is based on measuring pyruvate (a breakdown product of the ACSOs) by spectrophotometry (see page 9). This method is relatively rapid, although sample preparation can restrict sample through put.

If Vidalia onions are measured to have a pungency less than 3 $\mu M.mL^{-1}$ pyruvate, then they can marketed as Certified Extra Sweet™. If the pungency is between 3 and 5 $\mu M.mL^{-1}$ pyruvate, then the onions can be marketed as Certified Sweet™. If the onions are above 5 $\mu M.mL^{-1}$ pyruvate, then the onions can not be marketed as Vidalia onions, as they are considered too pungent to classify as sweet Vidalia onions. See sweet onion certification scheme news article (page 16) and National Onion Labs Inc (page 11).

Laboratory

Pungency Chemistry

The testing procedures and regulations adopted by the State of Georgia were developed by Dr. Bill Randle using the modified "Schwimmer & Weston" method. We visited Dr. Randle for discussions of the pyruvate test and its application to the Australian onion industry. Dr. Randle's technical officer (Jim Gegogeine) and graduate students (Tim Coolong and Pai-Tsang Chang) were conducting pungency testing on Vidalia onions when we were visiting, so was an excellent opportunity to observe and note how the pungency test was done.

The standard pungency analysis testing procedure (as prescribed the Georgia State Agriculture Department) is described in Appendix 2 and is summarised as:

- A core sample or wedge is cut from the onion (top)
- The sample is squeezed in the Randle press (middle)
- 0.5 ml of the juice is put into a 40 ml test tube
- The slurry is allowed to sit for 10 minutes
- 1.5 ml of 5% trichloroacetic acid is added to each test tube and vortexed
- 18 ml of deionized water is added to each test tube, which is vortexed and capped. 7. 1 ml of the solution is added to a 20 ml test tube
- 1 ml of 2,4-Dinitrophenylhydrazine and 1 ml of deionized water is added to each test tube and vortexed
- The test tubes are placed in a water bath at 37ºC and allowed to incubate for 10 minutes
- 5 ml of 0.6 N sodium hydroxide is added to each test tube and vortexed (bottom)
- The samples are run on a spectrophotometer set at 420 nm. Standards are made and run at the same conditions to create a standard curve

Initial literature suggested that background pungency was an issue in the determination of onion pungency; however this was not considered a significant contributor to *overall* pungency (Yoo and Pike, 2001) and is not measured. The work of Yoo and Pike (2001) has been recognised and is now accepted in routine pungency (pyruvate) testing in Georgia.

Pungency Sampling

Sampling will be a significant issue for the development of the Australian mild onion industry.

It was initially 'planned' (hoped) that a single 10 bulb sample from a grower's lot would be sufficient to certify the whole lot as 'mild'. However the results and experience from Georgia do not support this (Appendix 3). The Georgia Vidalia industry sample two (10 bulb) samples per acre to predict with 95% confidence an onion field's true mean and variance. This is high level of sampling is to counter the very high levels of field variability. The field variability comes from a variety of local and environmental sources, however Dr. Randle and the National Onion Labs Inc., believe that nutrition can account for most field variability in their conditions.

We were encouraged to conduct similar field variability and sampling studies in Australia as our soils and environment are different to south Georgia. However it was clear that a single 10 bulb sample from a composite lot from a grower will not be satisfactory.

National Onion Labs Inc

National Onion Labs, Inc.
Certifying the World's Best Onions!

National Onion Labs Inc. was formed in 1998 to ensure Vidalia sweet onion flavour for consumer satisfaction. This private company now provides a wide range of customised services to sweet onion producers in many North (eg. Washington State, Texas etc), Central and South (eg. Peru etc) American locations. Since 1998 the company has used GPS field sampling and laboratory based testing of onion flavour. The same methodology was recently mandated by the Georgia Department of Agriculture if pungency levels are used in the promotion and marketing of Vidalia onions. In order to resolve any confusion between testing facilities, the Georgia Department of Agriculture published actual sampling and testing procedure (See Appendix 2).

Using precision farming techniques (essentially GPS), the National Onion Labs Inc. take two 10 bulb sample per acre and a single soil sample per acre for complete soil and nutrient analysis. The matching the onion yield, size and pungency data of each sample to the field soil nutrient analysis in each paddock is a very powerful agronomic technique to improve not only yield and onion quality, but more importantly profitability. The agronomic database collected over the years by National Onion Labs Inc. is a valuable asset and will continue to assist the company manage onion yield and quality for its clients.
Onions which pass the National Onion Lab Inc test are marketed as Certified Sweet™ or Certified Extra Sweet™ and are commercially available.
We visited the National Onion Labs, Inc in Collins GA with Dr Randle on 3 May 2005. We met David Burrell, President, and Dr. Davey Kopsell, Horticultural Research Specialist to discuss the work of their company, the Georgia Vidalia industry, the Australian onion industry and the scope and direction of the current Horticulture Australia project (VN 04016). These discussions were very stimulating and opportunities for similar research and application in Australia should be developed, not only for the onion industry, but for other Australian horticultural industries such as potatoes.

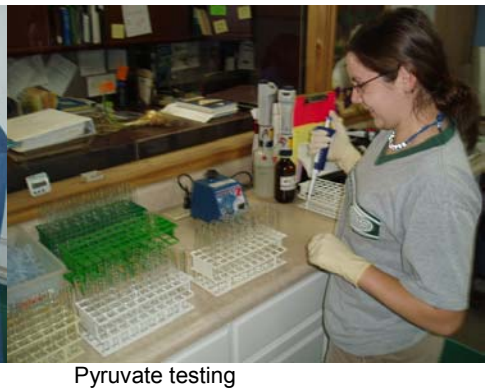
(L-R) Dr. Randle (UGA), Trevor Twigden (Onions Australia) David Burrell (President) and Dr. Davey Kopsell (Research) at National Onion Labs Inc, Collins Georgia

National Onions Labs Inc Collins, Georgia USA

Vidalia onion pungency testing preparation National Onions Labs, Inc. Collins GA

Sample preparation

'Randle' onion press



Pyruvate testing

Field bulb and soil sampling using GPS

Certified 'Certified Extra Sweet™' Vidalia onions

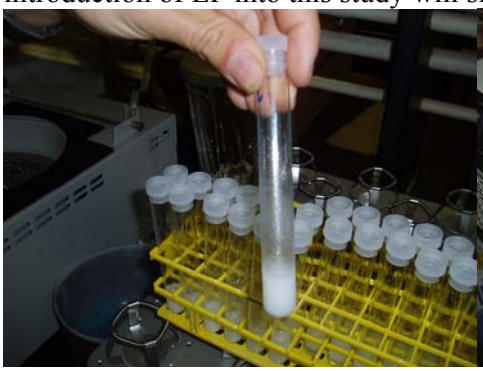
Lachrymatory Factor (LF)

During extensive discussions with both Dr. Randle and the National Onion Labs Inc. it became clear that the lachrymatory factor (LF) is an important aspect of onion pungency. LF ((*Z, E*) propanethial *S*-oxide) arises from the hydrolysis of 1-propyl cysteine sulfoxide (1-PRENCSO) and is responsible for the mouth burn and heat associated with eating onions. Sensory attributes from the LF can be overwhelming and can dominate the experience of eating onions with high levels of 1-PRENCSO. This is in addition to the pyruvate levels.

The validity and ability to routinely quantify LF is not obvious in the current scientific literature and was not considered in the initial HA pungency proposal (Feb 2005). However recent developments at the University of Georgia and the National Onion Labs Inc., have increased the importance of LF in onion pungency testing.

Traditionally LF has not been quantified in any quality or sensory assessment, but it is now strongly believed that the LF is a significant contributor to pungency, especially in 'borderline' pungent onions (around 4-5 $\mu M.mL^{-1}$ pyruvate), and in onions that are not from the Granex type. This would be the case in the majority of mild onions grown in Australia where it is believed that these onions would have high concentrations of 1-PRENCSO (LF precursor). It was thought that both the pungency (pyruvate) and LF of onions grown in the southern areas of Australia would be a significant factor in consumer acceptability.

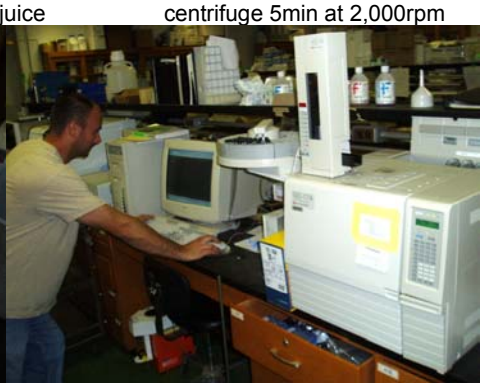
It was noted in numerous discussions that two onions both with similar pyruvate concentrations (eg. 4 $\mu M.mL^{-1}$ pyruvate) can have significantly different perceived pungencies, due to differences in LF. One onion which may have low LF will taste mild and sweet, whilst the high LF onion (although having similar pyruvate concentrations) can taste extremely pungent. High LF will cause extreme mouth burn and tear production. It is now thought that the sometimes poor correlations in the scientific literature between perceived pungency and pyruvate concentration will be significantly improved with the inclusion of the LF into the equation. The relationship between pyruvate, LF and TSS and sensory analysis has not been explored. Although previous studies have made good correlations between pyruvate and sensory perceived pungency (eg Wall and Corgan, 1992), it is expected that the introduction of LF into this study will significantly improve on this relationship.



2mL methylene chloride + 2mL onion juice

centrifuge 5min at 2,000rpm

collect LF in methylene chloride fraction



analyse on GC/FID

Taste testing

Discussions with Prof. Rob Shewfelt in the Department of Food Science and Dr. Randle have refined the technique for sensory analysis for the project. Dr. Shewfelt is a leading sensory scientist and is about to commence consumer taste test panels on sweet onions from Dr. Randle's breeding program. Dr. Shewfelt was highly informative on some insights of conducting taste tests with onions. Some suggestions included:

- Testing should be limited to three samples at a time to reduce palate / sensory overload
- Assess onion sweetness first by chewing and keeping the mouth closed, before opening the mouth and assessing pungency.
- Dr. Shewfelt preferred 5 (or even 3) -point scales for scales rather than continuous scales (eg 1. tastes good, 2. acceptable, or 3. unacceptable).

The most significant development from discussions with Dr. Randle was the potential to store and transport cut onions for sensory or biochemical analysis. Apparently it is possible to longitudinally cut the onion in half, seal the cut surface with paraffin wax and keep the onion half in storage (0C) / transport for later analysis. Dr. Randle believed that onions which had been correctly handled and stored could be kept for several months. This is a significant development for this project as the current literature suggests this may have caused problems with pungency degradation. This problem was further compounded as the chemical analysis was to be conducted at NSW DPI Wagga Wagga, and the sensory analysis was to be conducted at Food Science Australia North Ryde (Sydney). To counter this problem pungency testing and sensory analysis were to be done on different onions from the same population (same field, and place in field). This methodology had obvious limitations, particularly having recently learnt of the *huge* natural pungency variation within a same population (up to 5 fold differences in pungency from the same paddock). An appropriate experimental design was developed with the NSW DPI biometricians (Feb 2005) to take this sample and variation into consideration. However this may now not be necessary with the new onion sampling procedure. The improved sampling procedure whereby chemical pungency assessment (pyruvate and LF) can be conducted on the exact same onions presented for sensory analysis, will add significantly robustness and confidence to the project outcomes.

These suggestions will be discussed and implemented into the sensory analysis at Food Science Australia.

Vidalia Sweet Onion Certification Scheme

Whilst visiting Georgia in May 2005, a long running legal battle was continuing between Vidalia sweet onion growers. The background of the legal action is outlined in the following Associated Press article by Russ Bynum (14 April 2005).

'Sweet onions, bitter feud: Vidalia growers in court over sweetness labels'
By RUSS BYNUM The Associated Press Published: Apr 14, 2005

CLAXTON, Ga. (AP) - A bitter feud among growers of sweet Vidalia onions has resurfaced over whether farmers can legally market their crops with a sweetness guarantee offered by a private company - for a hefty fee. For the past seven years, Georgia-based National Onion Labs has been prohibited by a court order from contracting with growers to market Vidalia onions as certifiably sweet. But a judge ruled Wednesday that the order has expired. The ruling came just before Vidalia onions - reputed to be mild enough to be eaten like apples - go to market April 28. But some farmers oppose paying an outside company for scientific testing to label their onions as sweet or extra sweet.

Opposing growers, who say the program costs about $100 an acre, call it a gimmick to milk profits from the $75 million crop. They fear those who don't pay for the voluntary labels won't be able to compete. "National Onion Labs is telling produce buyers that if you don't certify these onions, you can't be sure they're really good Vidalias," said Delbert Bland, who owns one of Georgia's largest Vidalia onion farms. "They're trying to say a certified Vidalia is better than a regular Vidalia - and they're really one-in-the-same," said Bland, who grows onions on 1,800 acres in Reidsville.

The company debuted its guarantee labels in 1998, using tests developed by the University of Georgia that measure pyruvic acid in onions. The lower the acid content, the sweeter the onion.

Several growers sued. They said the for-profit combo of science and marketing misleads buyers and makes unlabeled onions harder to sell. A judge ordered a stop to the labeling while the case was pending. The lawsuit was never resolved, and Superior Court Judge Robert Russell ruled Wednesday that the order lapsed after five years. Growers have filed a new suit, hoping to halt the labels again, but Russell declined to impose a new restraining order.

David Burrell, president of National Onion Labs, said the testing and labels help farmers compete by giving produce buyers and consumers more confidence in their crops. The company guarantees sweet onions in six other states and in Central and South America. "Flavor certification has become a best management practice for leading growers and marketers and is desired by many grocers nationwide," Burrell said. "It's simply a practice that Vidalia growers have not been able to use."

Georgia doesn't require flavor testing for Vidalia onions, a trademark of the state Department of Agriculture. Instead, the crop is limited to 13 southeastern Georgia counties located around Vidalia. Burrell said his company has contracted with five Vidalia onion growers for testing and labeling this year.

R.T. Stanley, who grows onions on 1,000 acres in Vidalia, hired the company two years ago to test his onion fields after some customers complained his crop wasn't as sweet as it used to be.

While he couldn't use the results to market his crop, Stanley said the company's help improved the taste of his onions. "I need to do something to assure the customer I'm giving them the very best quality and taste," Stanley said. "Last year, I didn't get any complaints. I did get letters from these little old ladies about how sweet they were and, whatever I was doing, to keep doing it."

Vidalia Sweet Onion Certification Scheme

The certification of Australian mild onions will be an significant issue for the Australian onion industry. Several major issues will include:

- consumer acceptance and market share
- consistency and acceptance of certification
- price premium
- reliable cost effective testing scheme
- market acceptance
- industry acceptance
- others

This is a foundation to a mild onion industry in Australia and will be have to be thoroughly considered and actively managed by the Australian onion industry.

Pyruvate Biosensor

Several days before our visit to the University of Georgia, Prof. Randle had several visitors from the United Kingdom. One of these visitors was Dr. Leon Terry from Cranfield University at Silsoe (UK) where he gave an interesting session on biosensors. Dr. Terry is involved in a project to develop a biosensor for the measurement of pyruvate in onions.

This project is a collaborative project managed by 'The Allium and Brassica Centre' through the UK government (Defra, UK Department for Environment, Food and Rural Affairs) with 11 private companies and Cranfield University. The ultimate aim of this project is to develop a hand-held prototype 'grower friendly' biosensor using existing knowledge of chemical markers for pungency (pyruvate) and new markers for sweetness to be identified in the project. The project commenced in 2003 with a total cost of £725,000 ($A1.76 million), with 50% of the investment from industry. A sub-program of the larger project, 'Defining quality assurance for sweet onions with rapid Biosensor analysis - HL0164LFV' identified that the current spectrophotometric pyruvate test was slow and costly. The current pyruvate test takes a week and costs £20-30 per analysis. Researchers from the medical sector who work with biosensors at Cranfield University claim biosensors would revolutionise pyruvate analysis costing much less than a laboratory analysis. They predict a 15 fold reduction in cost per assay [£20-30 ($A50-75) to £2 ($A5) per sample] and 12-fold reduction in assay time (from 60 minutes to 5 minutes). They claim a good correlation between biosensor response and pyruvate concentration in the onion, but this has yet to be commercially demonstrated and adopted. Dr Terry says the sensor is going into commercial trial this harvest (May 2005).

This project, HortLink 186 "Fundamentals for Mild Onion production" has also resulted in the formation of a Sweet Onion Development Group and launch through J.S. and Safeway of the "Supasweet" (Trademark) onion. This group is moving forward from pyruvate analysis to examine sweetness. They believe there is a strong market for mild and sweet onions rather than mild onions only. The project demonstrated that mildness is a prerequisite for sweetness but the biochemical basis of sweetness is not understood. The project is now focussing on sweetness of mild onions. Dr Terry is looking at using LC-MS and LC profiling of onions in order to separate the issue of sweetness and mildness (i.e. low pungency).

Appendix 4 briefly details the UK Department for Environment, Food and Rural Affairs project; HortLink 186 "Fundamentals for Mild Onion production"

Implications for the Australian Onion Industry

With the final pungency and sensory analysis of this Horticulture Australia project complete, the Australian onion industry will have several crucial decisions consider, manage and implement. I believe the issue of field sampling for pungency will be critical in confidently determining the 'true pungency' (within 95% confidence). It appears a single postharvest sample of 10 bulbs will not be satisfactory and a sampling procedure for the Australian industry will be required.

Another major issue for the onion industry will be mild onion certification. Salient and potentially costly lessons can be learnt from the Georgia Vidalia industry. Although the two onion industries have completely different regulatory and commercial drivers, I believe the Australian industry could learn from the experience of the Vidalia onion industry. The role of the onion industry, its representatives, HA and the supermarkets will be crucial in determining the fate of the mild onion industry in Australia.

Recommendations

Project recommendations

From discussions with Professor Bill Randle and others, some fundamental changes to the current project are required to improve the confidence and reliability of the results. Some of these main issues include:

- Removing the necessity to measure background pyruvate (Yoo and Pike, 2001)
- In addition to pyruvate and SSC(%), also measure the lachrymatory factor (LF) using GC/FID
- The ability to use the same onion for both the chemical and sensory analysis (with precautions)

Other minor changes and developments from discussions and demonstrations at the University of Georgia of the current pyruvate method will be implemented in the project.

Industry recommendations

The Australian onion industry must carefully consider the management of the crucial issues of field sampling and mild onion certification.

Future work

Some future research and development work that is essential to start the process of establishing an Australian mild onion industry should include:

- Quantifying and understanding Australian field and genetic variability for pungency (pyruvate and LF). The sampling recommendations of the Vidalia onion industry are specific to the unique soils and environment to south Georgia. Pungency uniformity / variability trails need to be conducted in different regions of Australia with typical soils and conditions, eg sand. This will allow the Australian industry to develop its own sampling protocol.
- Further integrating and understanding the interaction of pyruvate, LF and sweetness into a workable and reliable certification scheme
- Inviting Prof. Randle's graduate student (Tim Coolong) to Australia to complete some research work with NSW DPI that would benefit his PhD program and the Australian industry. His current research is on the role of calcium in Vidalia onion quality. Tim is very knowledgeable and his experience would be an invaluable long term asset to the Australian onion industry.
- Assess the potential for the pyruvate biosensor in Australia. This would require extensive calibration to the current spectrophotometric method

Acknowledgments

I wish to thank the financial contribution and support of the following organisations and individuals:

- NSW Department of Primary Industries
- Horticulture Australia Limited.

This project has been funded by onion levies, facilitated by HAL in partnership with Onions Australia. The Australian Government provides matched funding for all HAL's research and development activities.
['Onion Pungency Testing and Consumer Calibration' (VN 04016)]

I would also like to sincerely thank Professor Bill Randle from the Department of Horticulture at the University of Georgia for his time, invaluable expertise, discussions and organisation. I would also like to thank the following at the University of Georgia for their helpful discussions, demonstrations and debate:

Prof. Stan Kays
Prof. Rob Shewfelt
Jim Gegogeine
Tim Coolong
Pai-Tsang Chang

I also appreciate the time and discussions with National Onion Labs Inc. who took time out of their very busy schedule during harvest to show us around and talk with us about their program and the Georgia Vidalia onion industry.
I would also like to thank Trevor Twigden for sharing his vast knowledge and experience.

Appendix 2.1

Itinerary for visit to University of Georgia (Athens)

Saturday 30 April	Depart Gosford / Sydney / Los Angeles
Sunday 1 May	Arrive Atlanta, Georgia USA – travel Athens (University of Georgia main campus)
Monday 2 May	Meet and discuss mild onion research and development with Dr. Bill Randle *et al.* at the University of Georgia
Tuesday 3 May	Travel to Vidalia onion growing region (South Georgia) to visit and talk with 'National Onion Labs Inc' and local onion growers / packing shed
Wednesday 4 May	Meet and discuss taste and sensory analysis of mild onions with Prof. Robert Shewfelt, Department of Food Science
Thursday 5 May	Discussions with Bill Randle and graduate students in onion laboratory
Friday 6 May	Discussions with Bill Randle (morning). Travel Atlanta / Los Angeles
Saturday 7 May	Travel Sydney
Sunday 8 May	Arrive Sydney / Gosford

Appendix 2.2

Georgia Department of Agriculture (USA) Onion pungency testing procedures
(as of May 2005)

GEORGIA DEPARTMENT OF AGRICULTURE: ONION PUNGENCY TEST PROCEDURES

The following standard procedures will be required to analyse and evaluate pungency levels in Vidalia Onions. These procedures must be used when there is the desire to publish such findings and utilise the same in promoting and / or marketing Vidalia® onions based on their pungency analysis results. This regulation is in two sections:

- Section 1 establishes the test methodology required for the determination of pungency levels of Vidalia onions.
- Section 2 establishes the sample collection method that must be utilised if pungency values are to be used in the promotion and/or marketing of Vidalia onions.

Section 1.0: Standard Method for Onion Pungency Analysis

The following standard analysis method will be required when conducting pungency analyses on Vidalia Onions.

Section 1.1: Preparation of the Required Chemical Reagents:

Reagent 1: Trichloroacetic acid (TCA)

Trichloroacetic acid (TCA)
Source: Fisher Scientific (A322-500)

5% Solution
Dissolve 50 grams of TCA in 1000 mL of distilled water using a volumetric flask.
Using a powder funnel, add 50 grams of TCA to volumetric flask add ~ 200 mL of water and stir until dissolved. Bring to volume (1000 mL) with distilled water. The solution can be stored in a closed amber bottle at room temperature for no more than six months.

Comment: TCA is used to stop the enzymatic activity of alliinase by precipitating and deactivating the enzyme.

Reagent 2: 2,4 dinitrophenylhydrazine (2,4 DNPH)

2,4 dinitrophenylhydrazine (2,4 DNPH)
Source: Sigma Chemical Company (D 2630)
Hydrochloric acid (HC1) 36-38%
Source: J T Baker (9535-33)

First:
2N HCl
Dilute 166 mL of ~ 38% HCl in 1000 mL of distilled water using a volumetric flask.
Using a standard funnel, gradually add HCl to ~ 500 mL of water and stir until dissolved. The solution will heat slightly as HCl is added, which can change volume. Bring volume (1000 mL) with remaining distilled water, making sure the funnel is washed of any remaining HC1.

Second:
Prepare 0.0125% 2,4 DNPH
Transfer ~ 500 mL of the 2N HCl to a clean beaker. Weigh out exactly 0.125 grams of 2,4 DNPH on a scale that reads to four decimal places (e.g. 0.0001 grams). Use a Fisher brand 1 ⅝ inch weigh boat. On a hot plate/stir plate combination, add 0.125 grams of 2,4 DNPH to the HCL remaining in

the volumetric flask. Use 2N HCl to wash any 2,4 DNPH sticking to the weigh boat into the flask. Set the temperature on the hot plate to a low setting and place a magnetic stir bar in the bottom of the flask to help dissolve the 2,4 DNPH. When the 2,4 DNPH is fully dissolved, add the remaining 500 mL of 2N HCl to make 1000 mL. Let the solution cool to room temperature before using.

Precautions:

- **2,4 DNPH is very toxic, and should be handled with extreme care**
- **The 2,4 DNPH solution must be used out of and stored in an amber bottle**
- **The solution if stored in the refrigerator, is good for six months**
- **If the solution is refrigerated, it must be brought to room temperature before being used in the pyruvic acid method. A cold solution could affect the reaction in the water bath, because the reaction is temperature and time sensitive**
- **If a precipitant is observed in the solution, it has gone bad, and should be disposed of properly. Check for a precipitant every time the 2,4 DNPH solution is used by holding the bottle up to a light source.**

Reagent 3: Sodium Hydroxide (NaOH)

Sodium Hydroxide (NaOH)
Source: J T Baker (3722-05)
0.6 N NaOH
Dissolve 24 grams of NaOH in 1000 mL of distilled water.
Using a powder funnel, add the NaOH pellets to a 1000 mL volumetric flask. Add approximately 500 mL of distilled water and dissolve the pellets. Then add the remaining water to make 1000 mL. Immediately put the solution in an amber bottle before dispensing.

Precautions:

- **NaOH solutions degrade in a very short period of time, and must be made daily or only on the days that this procedure is performed**
- **NaOH that has gone bad will cause the solution from the final reaction to appear dark yellow, when it should be a rust colour**
- **NaOH pellets will absorb water readily from air, and will change weight quickly. When weighing out the NaOH, make sure it is done as quickly and accurately as possible so the pellets do not absorb water. Immediately close the NaOH container once the pellets have been removed for the same reason**
- **Only make up enough NaOH to be used for the current days analyses. Estimate the volume of 0.6 N NaOH to be used, including that for the standard curve, and adjust the NaOH pellet weight and distilled water to accommodate.**

Reagent 4: Sodium Pyruvate (Used in making the standard series)

Sodium Pyruvate (Used in making the standard series)
Source: Sigma Chemical Company (P 2256)

Section 1.2: Preparation of a Standard Series for Pyruvic Acid measurement

Prepare 0.1 M sodium pyruvate stock solution

Dissolve 1.1 grams of sodium pyruvate in 100 mL of distilled water.
Add to a 100 mL volumetric flask 1.1 grams of sodium pyruvate. Wash the weight boat containing the sodium pyruvate with distilled water and pour into the flask. Then bring the flask to volume (100 mL) with distilled water.

Pyruvate Standard Series: Seven Concentrations suitable for Vidalia Onions

Concentration 1: 0.25 µ moles pyruvate/mL
2.5 mL of 0.1 sodium pyruvate stock solution brought to 1000 mL with distilled water in a volumetric flask

Concentration 2: 0.2 µ moles pyruvate/mL
2 mL of 0.1 sodium pyruvate stock solution brought to 1000 mL with distilled water in a volumetric flask

Concentration 3: 0.15 µ moles pyruvate/mL
1.5mL of 0.1 sodium pyruvate stock brought to 1000 mL distilled water in a volumetric flask

Concentration 4: 0.1 µ moles pyruvate/mL

50 mL of 0.2 µ moles pyruvate stock solution brought to 100 mL distilled water in a volumetric flask

Concentration 5: 0.05 µ moles pyruvate/mL

25 mL of 0.2 µ moles pyruvate stock solution brought to 100 mL distilled water in a volumetric flask

Concentration 6: 0.025 µ moles pyruvate/mL

12.5 mL of 0.2 µ moles pyruvate stock solution brought to 100 mL distilled water in a volumetric flask

Concentration 7: 0.010 µ moles pyruvate/mL

5 mL of 0.2 µ moles pyruvate stock solution brought to 100 mL distilled water in a volumetric flask.

Precautions and Comments:

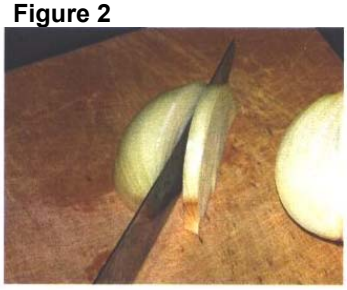
- **Extreme precision should be exercised when measuring the sodium pyruvate salt, and dispensing volumes when constructing the standard series. The prediction of unknown pyruvate concentrations from onion juice is only as accurate as the standard series established.**
- **The sodium pyruvate series will degrade over time, and significant loss can occur in a 24 hour period. While the sodium pyruvate stock does not degrade as quickly, it should be made fresh each time a new series is established.**
- **Once made, each of the standards can be dispensed into 1.5mL plastic vials and frozen (-20 to -80^{o}C) until needed. *This is the preferred method.* The above dilutions are sufficient for making 60 units of the standard series if each standard is dispensed in 1.5mL aliquots. Once frozen, the standards are good for up to a year if they are not thawed. This approach adds consistency to the pungency evaluation by establishing a uniform standard series across evaluation dates. Prior to use, the standards need to be brought to room temperature.**
- **A new standard series should be used for pyruvate quantification each time a new reagent stock solution is made and used during pungency analysis.**
- **When constructed as prescribed above, the standard series results in a straight line (a linear relationship) when the results are graphed. However, on some spectrophotometers with lower powered light sources, the higher standard concentrations may begin to fall below the predicted line. If this occurs, the series will overestimate low pungency unknowns and underestimate higher pungency unknowns. Therefore, the power of the spectrophotometer should be considered when establishing the high standard in the series, making sure the line predicted is linear.**
- **The absorbance from the highest standard in the series should always exceed the absorbance (Spectrophotometric measurement) of the highest unknown (onion sample). Otherwise those points beyond the highest standard are being extrapolated, and are unreliable.**
- **If the unknown samples are consistently reading above the highest standard, the onion juice containing the unknown pyruvate content should be further diluted to bring their concentration within the linear range of the standard series. Further dilution should occur at the water addition step in the preparation of the juice. Subsequently, the multiplication factor needs to be adjusted accordingly.**

Section 1.3: Obtaining Onion Tissue Samples:

Each "sample" must consist of the tissues obtained from 10 individual bulbs. This is required in order to account for bulb-to-bulb flavour variability.
Tissue samples from each bulb must be obtained in one of two established ways.
Method A: Obtain a wedge from each bulb. First, cut the bulb in half, top to bottom (Figure 1). Second, cut a wedge from one of the halves which represents the entire bulb (Figure 2).

Figure 1

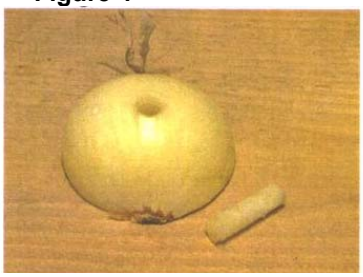
Figure 2



Method B: Obtain tissue cores from each bulb. Tissue cores must be taken just below the equator of the bulb. Whole bulbs or bulbs that have been halved can be used. A cork bore is positioned just below the equator of the bulb, and is inserted through the tissue (Figure 3). The tissue core is then pushed out of the bore and collected for analysis (Figure 4).

Figure 3

Figure 4



Regardless of the method used above, the combined tissues of ten bulbs are collected in a disposable weigh dish for juicing. Adjust the size of the wedge or the diameter of the coring device so that the combined volume of the 10 tissue samples results in a complete maceration of the tissue sample during the pressing process. The tissue samples must be pressed within 15 minutes after collection.

Section 1.4: Obtaining Onion Juice from the Tissue Samples:

Onion juice is to be extracted from the combined tissues of 10 bulbs through the use of a pneumatic press with a press plate and barrel specially designed for onion tissue (Figure 5).

Mechanical drawings which detail the press components and exact dimensions of the plate and barrel are available from the University of Georgia, Horticulture Department. The pneumatic press must be operated at air pressure of 90 pounds per square inch. Two screens lie on top of the press plate. Screen 1, which is made from disposable window screening, lies on top of Screen 2, which is made from stainless steel wire stock. The dimensions of both screens are outlined in the mechanical drawings. Following maceration, Screen 1 should be discarded, whereas Screen 2 can be reused after first being rinsed with fresh water, and completely dried. After maceration, the plunger must be wiped clean with a dry cloth or towel. Also after maceration, the plate and barrel assembly must be rinsed in fresh water and dried prior to reusing. All components are to be at room temperature and free of onion debris and moisture.

Figure 5

Step 1. Macerate the onion tissue through operation of the press. The juice extracted is to be collected in a weigh dish.
Step 2. Within 5 minutes of juicing, 0.5 mL of juice is to be pipetted into a 25mm diameter by 150mm test tube (40 mL). The 0.5 mL of juice is allowed to incubate at room temperature for not less than eight minutes and not more than ten minutes after pressing.
Step 3. Following incubation, for the specified length of time, 1.5 mL of 5% TCA is dispensed into the juice and the solution is immediately mixed thoroughly on a vortex apparatus.
Step 4. Eighteen mL of distilled water is then added to the test tube, and that solution is immediately thoroughly mixed on a vortex apparatus.
Step 5. The test tube is then capped with a #4 rubber stopper, and can sit at room temperature for up to eight hours before continuing with the pyruvate analysis.

Precautions:

- **It is necessary to pipette the 0.5 mL of juice within five minutes of juicing, as occasionally the onion juice will congeal to a gelatin like consistency. If congealing occurs after the juice is pipetted into the test tube, the results are not compromised.**
- **All pipettors used in the analysis should be calibrated daily. This is done by pipetting distilled water into a weigh boat that has been tared, or zeroed, on a balance. One mL of distilled water is equal to one gram. The pipette calibration should be repeated until the mL dispensed is equal to the weight equivalent (e.g. 1 mL = 1 gram). Anytime a pipette is accidentally dropped, its calibration needs to be checked immediately for accuracy by using the above method.**
- **When repeating dispensers are used for dispensing the stock solutions, these dispensers should be calibrated weekly. The same method of water to weight calibration is used for dispensers.**
- **The diluted juice with TCA should not be held overnight for analysis.**

Section 1.5: Pyruvic Acid Development and Qualification

The use of spectrophotometer set at 420mm is required. The spectrophotometer must be turned on and allowed to warm for a minimum of 10 minutes. This time required for "warm-up" may vary depending on the specific machine and manufacturer used.
The use of a water bath which is able to maintain a temperature of 37^{o}C is required. The water bath must be turned on and allowed to warm for a sufficient time for the water to reach 37^{0}C (+/- 0.5^{o}C). Water depth must be maintained at a level sufficient to submerge the solution volumes when the test tubes are placed in the water bath. A test tube rack is to be used to hold the test tubes upright.
Step 1: Pipette one mL of the diluted onion solution (with the TCA – from the 40 mL test tube) into a 16mm diameter by 125mm test tube (Fisherbrand 14-962-10G).
Step 2: Add one mL of 0.0125% 2,4 DNPH and then add 1 mL of distilled water. After adding the distilled water, vortex the mixed solution.
Step 3: Place the test tubes containing the mixed solution in a test tube rack. Place the test tube rack into a re-circulating water bath set at 37^{o}C (+/- 0.5 ^{o}C) for exactly 10 minutes. This period must be timed with a countdown clock. After 10 minutes, remove the rack from the water bath.
Step 4: Within one minute, dispense five mL of 0.6 N NaOH into each test tube. Thoroughly mix these with a vortex device.

Step 5: From each tube, pour a sample into a disposable cuvette that fit the spectrophotometer used, and the absorbance is read and recorded within 15 minutes of adding the NaOH. The solutions are then disposed of properly.

Precautions and Comments:

- **Repeating dispensers are used for the 2,4 DNPH, distilled water, and 0.6 N NaOH.**
- **If the solutions are not thoroughly mixed, inconsistent results can be obtained.**
- **Time in the water bath and its temperature are extremely important. The reaction of the 2,4 DNPH and pyruvic acid is temperature and time dependent. For consistent results, these should be closely monitored.**
- **Once the NaOH is added, time is critical. Do not exceed 15 minutes before the absorbance is determined, or the values will begin to decrease.**
- **Batches of 15 to 20 samples can be done efficiently and accurately. If the number of samples exceeds 20 in a batch, the absorbance may begin to decrease, as the reactants begin to precipitate out of solution.**

Section 1.6: Zeroing the Spectrophotometer and Establishing a Standard Series

Step 1: If the standards have been frozen, remove from the freezer and thaw to room temperature.
Step 2: One mL of each of the standard series stocks is added to a 16mm by 125mm test tube. One mL of distilled water is also added to a test tube which will be used to zero the spectrophotometer.
Step 3: To each of the standards and the water zero, one mL of 2,4 DNPH and one mL of distilled water are added and the solutions are mixed.
Step 4: The solutions are to be placed in the 37^{o}C water bath for exactly 10 minutes and then removed.
Step 5: Five mL of NaOH is added to each standard and water zero, and mixed.
Step 6: First, the water zero is pored into a disposable cuvette, placed in the spectrophotometer, and the absorbance is adjusted to zero. The standard series is then dispensed into cuvettes and their absorbance is determined and recorded.
Step 7: Plot these values using a simple linear regression equation. These results will be used to determine the pyruvic acid content in the onion juice.

Precautions and Comments:

- **The standards should be analysed before the unknowns in the onion juice.**
- **Absorbance values should be close to the µ moles pyruvate values in each of the standards if the water/2,4 DNPH solution is used to zero the spectrophotometer (e.g. the 0.10 µ moles pyruvate should have an absorbance close to 0.10).**
- **Each time a new reagent stock solution is used, a new standard series should be established and used to predict the unknown pyruvate samples.**
- **Because the NaOH is made daily, a new standard series needs to be established daily.**
- **The colour of the final solution, after the NaOH is added, should be rust coloured. The intensity of the colour will depend on the amount of pyruvate in the solution. More pyruvate will cause a darker colour to develop. If the solutions are bright yellow, one of the stock solutions is bad. Most often, NaOH made up incorrectly or a solution that has gone bad will cause a bright yellow colour to develop. On occasion, bad 2,4 DNPH will cause a bright yellow colour to develop.**
- **The pH of the final solution should be close to 12 for the proper rust colour to develop.**

Section 1.7: Calculating the Pyruvic Acid Content in the Onion Juice

µ Moles pyruvic acid of the onion juice is determined by multiplying the predicted value from the regression equation by 40. The dilution factor of the raw onion juice as written is 40x. A spreadsheet, such as EXCEL, can be used for these calculations. The values determined through the simple linear regression should be reduced by 0.4 µ moles in order to allow for "normal" background pyruvate. Values are reported as µ moles pyruvic acid per mL of onion juice.

Disclaimer:

The following disclaimer must be printed on all pungency analysis reports when the samples **ARE NOT** collected in accordance with Section 2 of these procedures:

> **"The pungency results reported were obtained using the pungency analysis method specified by the Georgia Department of Agriculture. The samples tested are not indicative of the flavour characteristics of any onions not tested, and have no value in predicting the flavour characteristics of the field or shipment from which they were collected".**

Section 2.0: Sample Collection Procedures

To better inform consumers of the flavour intensity they might be purchasing, field sampling and pungency testing must be used. This section of the regulation establishes the sample collection method that must be utilised if pungency values are to be used in the promotion and/or marketing of Vidalia onions.

Section 2.1: Onion Samples must be Collected From the Field Prior to or During Harvest

Onion samples must be collected no earlier than 7 days prior to harvest, and up to the time that the onions are removed from the field. Harvest is defined as undercutting of the onion roots. Removal from the field is defined as the onions being loaded onto or into a truck or a bulk transport vehicle. Onions can not be sampled after the onions have been removed from the field.

Onions must be tested for pungency within 5 days of the sample date. If onions are held during the 7 days allowed prior to pungency testing, they should be held at room or refrigerated temperatures. At no time should the sample onions be frozen or exposed to temperatures above 120 degrees Fahrenheit.

Section 2.2: Onion Sample Lots Must be Identified and Not Co-mingled with Onions Samples of Another Lot

Individual lots must be identified and tested separately. A lot is defined as a single variety harvested within a single field within a 7 day period. A change in lot is required when there is a change in variety and / or a change in harvest dates of more than 7 days and / or a change of fields. Example: One variety planted in one field harvested within a 7 day period would be considered one test lot. Two varieties planted within one field, even if they are harvested within the same 7 day period, would be considered two lots.

Section 2.3: Onion Sample Lots Must be Tracked and Segregated

The grower/packer must maintain lot integrity throughout all handling and packing processes to ensure that "tested" lots are not co-mingled with untested lots. Records of the movement of tested lots from the field through the packing, and storage and re-packing process must be maintained through all product handling steps so that "tested" lots are not co-mingled with untested lots.

Section 2.4: Onion Samples Must be Collected Using a Statistically Valid Sampling Density

In the Vidalia onion production region, it has been determined that two 10-bulb samples must be collected from each acre of any commercial lot. Sampling density in a given field lot was established for Vidalia onions through a statistical sampling study conducted by the University of Georgia and published in HortTechnology (1998, Volume 8, pages 329-332).

Samples must be collected on a stratified grid basis which equally represents the characteristics of the field lot. Samples can not be taken from a single geographical location within a lot. If a lot size is less than 3 acres, six 10-bulb samples must be collected on a stratified grid basis which equally represents the spatial characteristics of the lot.

Section 2.5: Each Onion Sample Must Consist of 10 Bulbs which are Size Representative of the Marketable Onions in the Field

A single sample is defined as a 10-bulb composite selected from adjacent plants in a single location within the field lot. The 10-bulb sample should be size representative of other plants within reasonable proximity. Only disease-free and marketable bulbs should be collected.

Section 2.6: Onion Samples must be Tested in Accordance with Section 1 of this Regulation

Section 2.7: Pungency Testing Results Have a Limited Length of Validity

As bulb pungency changes during long-term storage, the test values are considered valid for 50 days. If any lot remains in storage for a period longer than 50 days after harvest, the onions will need to be retested. Onion lots will need to be re-sampled on a lot basis. Two 10- bulb samples will be needed per acre equivalent from stored onions lots. For example, if onion yield from a lot was 500 50 pound units, then two 10-bulb samples would need to be retested per 500 50 pound units coming out of any lot in storage longer than 50 days.

Section 2.8: Disclaimer

"The pungency results reported were obtained using the sample collection and pungency analysis method specified by the Georgia Department of Agriculture. This is the method that must be utilised if pungency values are utilised in the promotion and/or marketing of Vidalia onions".

Appendix 2.3

Abstract

Field Sampling Short-day Onions for Bulb Pungency

W.M. Randle, D.A. Kopsell, D.E. Kopsell, R.L. Snyder, and R. Torrance

HortTechnology (1998) *8*, 329-332

The marketing of onions (*Allium cepa* L.) based on bulb pungency as a measure of overall flavor intensity is being considered by the onion industry. Pungency is highly variable within and among fields due to genetic and environmental factors. Therefore, a study was undertaken to develop a sampling procedure to estimate onion pungency means and variances from field-grown onions with predetermined degrees of accuracy and confidence. Two short-day onion cultivars, commonly grown in the Vidalia, Ga., area, were each randomly sampled from four different fields. The sampled bulbs were analyzed for enzymatically formed pyruvic acid (EPY) and soluble solids content (SSC) to assess pungency and sugars, respectively. EPY concentration and SSC varied between the two cultivars, among the four fields within cultivars, and among the fifty samples within each field. In a combined analysis of all eight fields, at least 1.3 ten-bulb samples would be needed per acre to come within ±0.5 μmol EPY of a field's true EPY mean with 95% confidence. If the accuracy of the estimation was lowered to ±1.0 μmol EPY of a field's true mean, then at least 0.4 ten-bulb samples would be needed per acre. Because SSC was less variable than EPY, the number of ten-bulb samples needed per acre to estimate a field's true mean was lower than the number required to estimate EPY. Establishing a sampling method to estimate an onion field's EPY and SSC will provide the mechanism to standardize onion flavor in the market place and instill greater consumer confidence in purchasing onions.

UK Department for Environment, Food and Rural Affairs project
HortLink 186 "Fundamentals for Mild Onion production"

Improving the market for sweet onions through tests for sweetness and mildness

A simple biosensor would allow standardisation of both strength and sweetness in onions enabling UK growers to exploit to the full the consumer interest in sweet onions

Benefits of research

As a result of a previous HortLINK project (186: UK fundamentals for mild onion production) a Sweet Onion Development Group has been formed and the trade marked 'Supasweet' onion introduced into supermarkets.

From correlations with taste panels, a pyruvate test was found to be a reliable indicator of mildness, but there is no standard for sweetness. Standardisation of both strength and sweetness is needed to avoid 'Sweet' onions being sourced inside and outside the development group that do not meet the flavour requirements of the 'Supasweet' onion.

The development of low-cost tests using biosensor technology will advance the mild onion market in the UK and help the substitution of imports by home production.

Aims of the research

- Research and develop a prototype biosensor measuring device for on-farm testing of the mildness of onions based on the pyruvate test developed in HortLINK project 186
- Identify the differences in flavour chemistry between mild onions that taste sweet and mild onions that do not taste sweet as found in HortLINK project 186
- Develop a second biosensor capable of giving a rapid, cheap, on-farm quality control test of the sweetness of onions
- Investigate environmental factors that may influence the sweetness of onions and their shelf-life

Field trials are being used to investigate the environmental factors involved in onion sweetness

"There is a developing market for sweet onions, which UK growers will be better able to exploit."

David O'Connor
Allium and Brassica Centre Ltd

HortLink

Research methods

Grower partners are growing onions under commercial and trial conditions to produce bulbs of varying strengths and levels of sweetness. Biochemical profiling of selected onions is then proceeding alongside taste panel analysis. The aim is to identify biochemical differences that correlate with differences in sweetness reported by taste panels.

The trials are also generating data relating to agronomic factors and storage which may cause variations in sweetness.

Cranfield University with instrumentation partners has now produced a prototype biosensor which measures pyruvate in onion juice in 100 secs, well ahead in the project timetable.

As information becomes available on the biochemical basis of sweetness, further progress will be made on the development of a laboratory prototype of a sweetness biosensor.

A biosensor is being developed as a technique for measuring sweetness in onions

Benefits for industry

Sweet onions command a higher price than UK brown onions; any market growth and import substitution will bring a substantial financial benefit to the UK onion industry.

No major capital outlay will be required by growers to take advantage of the biosensor technology which will also be an invaluable tool for onion breeders. There is also an international market for the technology.

Consumers will then benefit from a more reliable supply of sweet onions which, because they can be eaten raw, will provide the full range of health benefits.

"We are confident biosensing equipment for use by growers can be developed"

Dr Lewis Terry
Cranfield University

LINK is a UK Government mechanism for supporting collaborative research partnership between UK industry and the research base.

The HortLink programme is currently open to bids to 2007.

The aims of the programme are:

- To improve the sustainability of the horticultural industry
- To improve knowledge and understanding of processes and factors which determine the performance of the horticultural industry
- To enable access by the horticultural industry to innovative ideas and technology by involving a wide range of research institutes and university departments
- To promote wider industry awareness of the benefits of advanced horticultural techniques/methods.

Further information from the programme co-ordinator.
E-mail:
david.coles@defra.gsi.gov.uk

LINK collaborative research

Project details	Project Liaison Officer	Participants	Government sponsor
Defining quality assurance for sweet onions with rapid biosensor analysis	David O'Connor Allium and Brassica Centre Ltd Wash Road Kirton Boston Lincolnshire PE20 1QD Tel: 01205 723414 Email: abcentre@aol.com	Allium & Brassica Centre Ltd Applied Enzyme Technology Ltd Bedfordshire Growers Ltd Cranfield University, Silsoe ID's Marketing Ltd Gwent Electronic Materials Ltd Moulton Bulb Co Ltd FB Parrish & Son Rustler Produce Ltd Sainsbury's Supermarket Ltd Tesco Stores Ltd	Defra
Reference number HL0164			

Appendix 2.5 Contact list The University of Georgia

Professor William M. Randle
Department of Horticulture 1111 Plant Sciences Building
University of Georgia Athens, GA 30602-7273
Phone: 706-542-2471, Fax: 706-542-0624
Email: wrandle@uga.edu
Research Interests: Breeding of vegetable crops with emphasis on the vegetable Alliums and Brassicaceae; understanding the flavour chemistry of the Alliums and Brassicaceae and the factors which influence flavour intensity; and the investigation of selenium substitution in plant sulphur metabolic pathways which affect mammalian health.

Technical Officer
Jim Gegogeine

Graduate Students
Tim Coolong
Ph.D. Candidate Email: tcoolong@uga.edu

Pai-Tsang Chang
M.S. Candidate Email: mize@uga.edu
Thesis Title "Changes of flavour components of onion in a saline environment"

Other Technical Specialists
Professor Stan Kays
Telephone number: 706-542-2471
Email: kaysstan@arches.uga.edu
Research Interests: Flavour and insect resistance chemistry, developmental physiology of vegetable crops, postharvest physiology of horticultural products.

Professor Robert Shewfelt
Telephone number: 706-542-5136
118F Food Science
Department of Food Science and Technology
Email: shewfelt@arches.uga.edu
Research interests: Flavour and colour quality of foods as evaluated by instrumental techniques, sensory analysis and consumer testing; postharvest physiology of fresh fruits and vegetables.

National Onion Labs Inc.

270 NW Main Street
Collins Georgia 30421 USA

David Burrell
President

Dr. Davey Kopsell
Horticultural Research Specialist

National Onion Labs, Inc. is an independent agricultural testing laboratory and is not affiliated with any government agencies.

Appendix 3

Pungency Testing

3.1 Considerations in Selecting the Flow Injection Analysis Technology

3.2 Determination of Pyruvate Concentration in Onion Juice

3.3 Determination of Lachrymatory Factor Concentration in Onion Juice

3.4 Determination of Soluble Solids Content (SSC%) in Onion Juice by Refractometer

3.5 Determination of Pyruvate Recovery in FIA Method

3.6 Verification for Pyruvate Method

3.7 Measurement Uncertainty for Pyruvate Method

Appendix 3.1 Considerations in Selecting the Flow Injection Analysis Technology

The following considerations were made in development of the manual spectrophotometric method into an automated FIA technique:

1. Thermal stability of pyruvate species. Early indications suggested that pyruvate should be stable up to 80°C. This is a fundamental consideration for the success of an FIA method, as the manual method required an incubation time of 10 minutes at 40°C. As a rough guide, reaction rates normally double for additional 10°C of temperature. Therefore the requirement for an approximate equivalent reaction completion would require a temperature of 60°C for approximately 2.5 minutes (a realistic residence time within the FIA). The stability of pyruvate species is evident in the fact that co-workers have used microwave heating techniques (80°C +) in order to destroy the alliinase enzyme to determine background levels of pyruvate. These levels are similar to those reported using trichloroacetic acid to destroy alliinase enzymes.

2. Organoleptic panels typically can perceive a difference of between 0.5 - 1.0 $\mu M.mL^{-1}$ pyruvate. This effectively 'sets' the framework for the required limits of detection for the FIA method.

3. The FIA typically requires sample volumes within the order of 5 - 10mL.

4. The FIA has both 520nm and 420nm filters available. Anthon and Barrett (2003) reported that a greater response and better signal to noise ratio (there was less overlap with other absorbing species) was achieved by using a wavelength of 520nm rather than the 420nm originally proposed by Schwimmer and Weston (1961).

5. Samples following initial preparation must be analysed within an 8 hour period.

6. Following work by Yoo and Pike (2001), background pyruvate was found to be at a level that was relatively constant across species and cultivars. Therefore it has been suggested that background determinations can be eliminated by allowing for 0.4 $\mu M.mL^{-1}$ pyruvate subtraction in the calculation.

Appendix 3.2 Determination of Pyruvate Concentration in Onion Juice

Document No: LMOP 2.1120 Version No: 2-14/03/06

Pyruvate Concentration in Onions

1.0 Introduction

This method is used to determine the level of naturally occurring pyruvate in onions. Pyruvate concentrations are frequently used to estimate the pungency of onions. Pyruvic acid is a by-product of the enzymatic reaction of S-alk(en)yl cysteine sulfoxides (ACSOs) with alliinase, to produce chemical species which contribute to the pungency of onions. The reaction proceeds only when the onion cells are disrupted, and the enzyme is free to come in contact with the ACSOs.

2.0 Principle of Procedure

In this method, based on a modified method by Randle and Bussard (1993), onion juice is firstly extracted from the onion using a pneumatic press. The onion juice is then left at ambient temperature on the bench, so the reaction can proceed to completion (between 8-10min). Trichloroacetic acid solution (5%) is added to an aliquot of the juice to halt any further enzymatic reactions, and some water is added for dilution.

The solutions are then analysed by Flow Injection Analysis (FIA) using a HCl and 2,4-Dinitrophenyl hydrazine solution at 60°C followed by alkalinisation using NaOH at 60°C . The method is specific for reaction of the carbonyl group in pyruvate . The colour intensity is measured at 520 nm.

3.0 Scope and Application

The range and use of this method extends to all onion species. It is critical that a suitable sampling methodology is used when measuring pyruvate and pungency in onions. Typically a sub-sample of 10 onions per measurement, at 2 measurements per acre should be employed to build a picture of pungency trends across a cultivar.

All chemicals used in this method are (AR) grade quality unless specified otherwise. All chemicals used in this method will be referred to as their common name and chemical formula, water used is type II or better where appropriate.

4.0 Safety Procedures

Refer to the Material Safety Data Sheets (MSDS) for each chemical.

Refer to the Risk Assessment for each chemical, each work method and each compartment, Refer to the Compulsory use of Protective Clothing in the Laboratory workplace 3-1

Trichloroacetic acid is corrosive. Inhalation and/or skin contact may produce health damage. This chemical is a suspected carcinogen. Use only in well ventilated areas. Dispose of this chemical in chlorinated waste container.
2,4-dinitrophenylhydrazine is toxic by inhalation, in contact with skin and if swallowed. There is danger of cumulative effects. This product is flammable.
32% Hydrochloric Acid is corrosive. Always ensure that the correct PPE including gloves and eyewear is used when handling this chemical.
Sodium Lauryl Sulfate (SLS) is irritating to the skin, eyes and respiratory system. Always wear gloves, glasses and avoid breathing fine particles of this substance.

5.0 Apparatus and Reagents

5.1 Apparatus

- **ANALYTICAL BALANCE (0.1 MG, CAPACITY ≥ 100 G)**
- Top-pan Balance (0.1 g, capacity ≥ 2 kg).
- Magnetic Stirrer, variable speed.
- Flow Injection Analyser (FIA) Lachat or equivalent
- Stirring magnet.
- Volumetric Flasks (5 L, 1 L, 200 mL, 100 mL).
- Glassware:
  - Beakers (100 mL, 5 L).
  - Measuring cylinder (100 mL).
  - Storage Bottle Schott (1 L).
  - Storage Bottle amber glass (1 L).
- Racks to hold plastic tubes
- Dispenser (20 mL)
- Plastic Tubes with Lids (30 mL)
- Variable Pipette 100-1000µL

5.2.0 Reagents

5.2.1 Sodium Lauryl Sulphate (SLS) Carrier solution 0.1%:
Add 2.0 ± 0.05g of sodium lauryl sulphate needles to 2.0 ± 0.05L of water, and dissolved by stirring on a heater stirrer over medium - strong heat.

5.2.2 5% Trichloroacetic acid (TCA) Solution.
Dissolve 50 ±0.1 g of TCA in approximately 200 mL of water and stir until dissolved. TCA is solid at room temperature. Melt the TCA by ensuring the lid is loosened slightly to prevent vapour build up, and immersing the storage vessel in water between 60- 80°C. This should be carried out in a fume- cupboard, as TCA vapours are toxic. Use a respirator to when weighing to prevent respiratory exposure. Bring to volume (1000 mL) with distilled water in a volumetric flask. The solution can be stored in a closed amber bottle at room temperature for no more than six months.

5.2.3 2N HCl Dilute 200 ± 0.5 mL of ~ 32% HCl in approximately 500 mL of water and stir until dissolved. The solution will heat slightly as HCl is added, which can change volume. Bring volume (1000 mL) with remaining distilled water in a volumetric flask.

5.2.4 2,4 dinitrophenylhydrazine (2,4 DNPH) in 2N HCl

Weigh out exactly 0.250 ± 0.002 g of 2,4 DNPH on an analytical balance. On a hot plate/stir plate combination, add 2,4 DNPH to approx. 600mL of 2N HCl and dissolve using low heat. When the 2,4 DNPH is fully dissolved, make up the solution to 1 L in a volumetric flask with 2N HCl.

Precautions: • 2,4 DNPH is very toxic, and should be handled with extreme care • The 2,4 DNPH solution must be used out of and stored in an amber bottle • The solution if stored in the refrigerator, is good for six months • If the solution is refrigerated, it must be brought to room temperature before being used in the pyruvic acid method. A cold solution could affect the reaction in the water bath, because the reaction is temperature and time sensitive • If a precipitant is observed in the solution, it has gone bad, and should be disposed of properly. Check for a precipitant every time the 2,4 DNPH solution is used by holding the bottle up to a light source.

5.2.5 3.0N Sodium Hydroxide (NaOH)

Dissolve 120 ± 0.1 g of NaOH in approx. 800 mL of distilled water. Make up to volume with remaining water to make 1000 mL.

Precautions: • NaOH solutions degrade in a very short period of time, and must be made daily or only on the days that this procedure is performed • NaOH that has gone bad will cause the solution from the final reaction to appear dark yellow, when it should be a rust colour • NaOH pellets will absorb water readily from air, and will change weight quickly. When weighing out the NaOH, make sure it is done as quickly and accurately as possible so the pellets do not absorb water. Immediately close the NaOH container once the pellets have been removed for the same reason. Only make up enough NaOH to be used for the current days analyses. Estimate the volume of 0.6 N NaOH to be used, including that for the standard curve, and adjust the NaOH pellet weight and distilled water to accommodate.

5.2.6 0.100 M Sodium Pyruvate (Stock) :

Dissolve 1.100 ±0.005 g of sodium pyruvate in 80 mL of distilled water. Make to volume in a 100 mL volumetric flask.

5.2.7 2.5 mM Sodium Pyruvate (Stock) :

Add 2.50 ± 0.03mL of 0.100N stock solution to a 100mL volumetric flask and make up to volume.

5.2.8 Sodium Pyruvate Working Standards 0.05 – 0.25 mM:

Add the following volumes of 2.5 mM stock solution to make up working standards:

Pyruvate Concentration (µmoles/mL)	Volume of 2.5 mM added (mL)	Final volume (mL)
0.000	0	100
0.025	1	100
0.050	2	100
0.100	4	100
0.150	6	100
0.200	8	100
0.250	10	100

Precautions and Comments: • Extreme precision should be exercised when measuring the sodium pyruvate salt, and dispensing volumes when constructing the standard series. The prediction of unknown pyruvate concentrations from onion juice is only as accurate as the standard series established. • The sodium pyruvate series will degrade over time, and significant loss can occur in a 24 hour period. While the sodium pyruvate stock does not degrade as quickly, it should be made fresh each time a new series is established. • Once made, each of the standards can be dispensed into 8 mL plastic analysis tubes and frozen (-20 to -80^{o}C) until needed. Once frozen, the standards are good for up to a year if they are not thawed. This approach adds consistency to the pungency evaluation by establishing a uniform standard series across evaluation dates. Prior to use, the standards need to be brought to room temperature. •

6.0 Procedure

6.1.0 Preparation

Obtaining representative onion tissue samples

6.1.1 Obtaining Onion Tissue Samples: Each "sample" must consist of the tissues obtained from 10 individual bulbs. This is required in order to account for bulb-to-bulb flavour variability. Tissue samples from each bulb must be obtained in one of two established ways.

6.1.2 METHOD A : Obtain a wedge from each bulb. First, cut the bulb in half, top to bottom. Second, cut a wedge from one of the halves which represents the entire bulb.

OR

6.1.3 METHOD B: Obtain tissue cores from each bulb. Tissue cores must be taken just below the equator of the bulb. Whole bulbs or bulbs that have been halved can be used. A cork bore is positioned just below the equator of the bulb, and is inserted through the tissue. The tissue core is then pushed out of the bore and collected for analysis

6.1.4 Regardless of the method used above, the combined tissues of ten bulbs are collected in a disposable weigh dish for juicing. Adjust the size of the wedge or the diameter of the coring device so that the combined volume of the 10 tissue samples results in a complete maceration of the tissue sample during the pressing process. The tissue samples must be pressed within 15 minutes after collection.

Obtaining onion juice sample using the pneumatic onion press
(CAUTION: Operator must have specific equipment competency to operate onion press)

6.1.5 Onion juice is then extracted through the use of a pneumatic press with a press plate and barrel specially designed for onion tissue. Two screens lie on top of the press plate. Screen 1, which has round punched holes, lies with the pressed side down on top of Screen 2, which is made from stainless steel wire stock.

6.1.6 Insert the pressure plate over the locating lugs, and then fit the two screens, ensuring that the disposable finer screen is above the coarser permanent screen. Then fit the barrel assembly over the locating lugs and fix in position with the two locking levers.

6.1.7 Ensure that a small beaker is located below the collection point on the plate, and then add the 10 onion sub-samples to the barrel assembly.

6.1.8 Make a final check that the necessary parts are in position, and then press the juice from the onions by pulling downward on the safety valve lever with the left hand, and simultaneously pull the operating lever downward with the right hand. The plunger will now move downward at a controlled rate into the barrel.

6.1.9 Once the plunger has come to rest in the barrel, wait for a period of ten seconds and then raise the plunger by pulling downward on the safety valve lever with the left hand, and simultaneously pushing the operating lever upward with the right hand. Remove the juice for processing.

6.1.10 Following maceration, rinse screens with fresh water, and completely dry. After maceration, the plunger must be wiped clean with a dry cloth or towel. Also after maceration, the plate and barrel assembly must be rinsed in fresh water and dried prior to reusing. All components are to be at room temperature and free of onion debris and moisture.

Juice preparation following extraction

6.1.11 Within 5 minutes of juicing, 0.5 mL of juice is to be pipetted into a 25 mm diameter by 150 mm test tube (40 mL). The 0.5 mL of juice is allowed to incubate at room temperature for not less than eight minutes and not more than ten minutes after pressing.

6.1.12 Following incubation, for the specified length of time, 1.5 ± 0.05 mL of 5% TCA is dispensed into the juice and the solution is immediately mixed thoroughly on a vortex apparatus.

6.1.13 18.0 ± 0.05 mL of distilled water is then added to the test tube, and that solution is immediately thoroughly mixed on a vortex apparatus.

6.1.14 A reagent blank is prepared by substituting 0.5mL of water for juice in 6.1.11.

6.1.15 Pour approximately 5mL of each of the samples into a test tube, and store in FIA analysis rack.

6.1.16 The test tube is then capped, and can sit at room temperature for up to 8 hours before continuing with the pyruvate analysis.

Precautions: • It is necessary to pipette the 0.5 mL of juice within five minutes of juicing, as occasionally the onion juice will congeal to a gelatine like consistency. If congealing occurs after the juice is pipetted into the test tube, the results are not compromised

6.2.0 Analytical Finish – Flow Injection Analyser

6.2.1 Figure 1 shows the operation of the pyruvate manifold for use on the FIA. Refer to the specific operating guide of the instrument for routine use and troubleshooting where necessary.

6.2.2 Peristaltic pump tubing should be checked for excessive stretch or wear prior to each run. It is important that all type II water used in the method is degassed prior to use. If the standards have been frozen, remove from the freezer and thaw to room temperature.

6.2.3 Run the prepared extractions through the FIA. Set up a batch containing all the sample solutions prepared and calibrate using the prepared pyruvate calibration solutions.

6.2.4 Prime the auto-dilutor, and preview the baseline. Ensure that the heaters are set at the required temperature have stabilised at they're required temperature.

6.2.5 When the baseline has stabilised the batch can be started. Review the calibration curve once the standards have been ran, and confirm the correlation coefficient is an acceptable value (>0.99)

6.2.6 At the completion of the batch ensure that any necessary repeat samples are re-analysed, and the values of the QC standards are within their respective control limits.

6.2.7 Disposal – Test solutions should be stored in waste bottles labelled as Trichloroacetic acid waste. 0.5%.

7. Calculations and Reporting

Concentration of Pyruvate (μmoles/mL) = [(Raw FIA Value – blank)x 40] – 0.4

Report this value to 2 significant figures

8. Quality Control

All samples should be analysed in duplicate (from a common preparation). At least one spike recovery per batch should be processed.

- Duplicate samples should be analysed at the rate of 1 in 15 samples;
- An in-house standard, as well as a chemical pyruvate standard should be used in every batch. These standards are able to be kept in the freezer at -20°C for 12 months, and thawed just prior to use.
- At least one of each of the above standards should be used in each batch processed.
- Consult the chemist if a there is a problem with duplicates or standards meeting the quality criteria assigned to them.

9. Method Performance

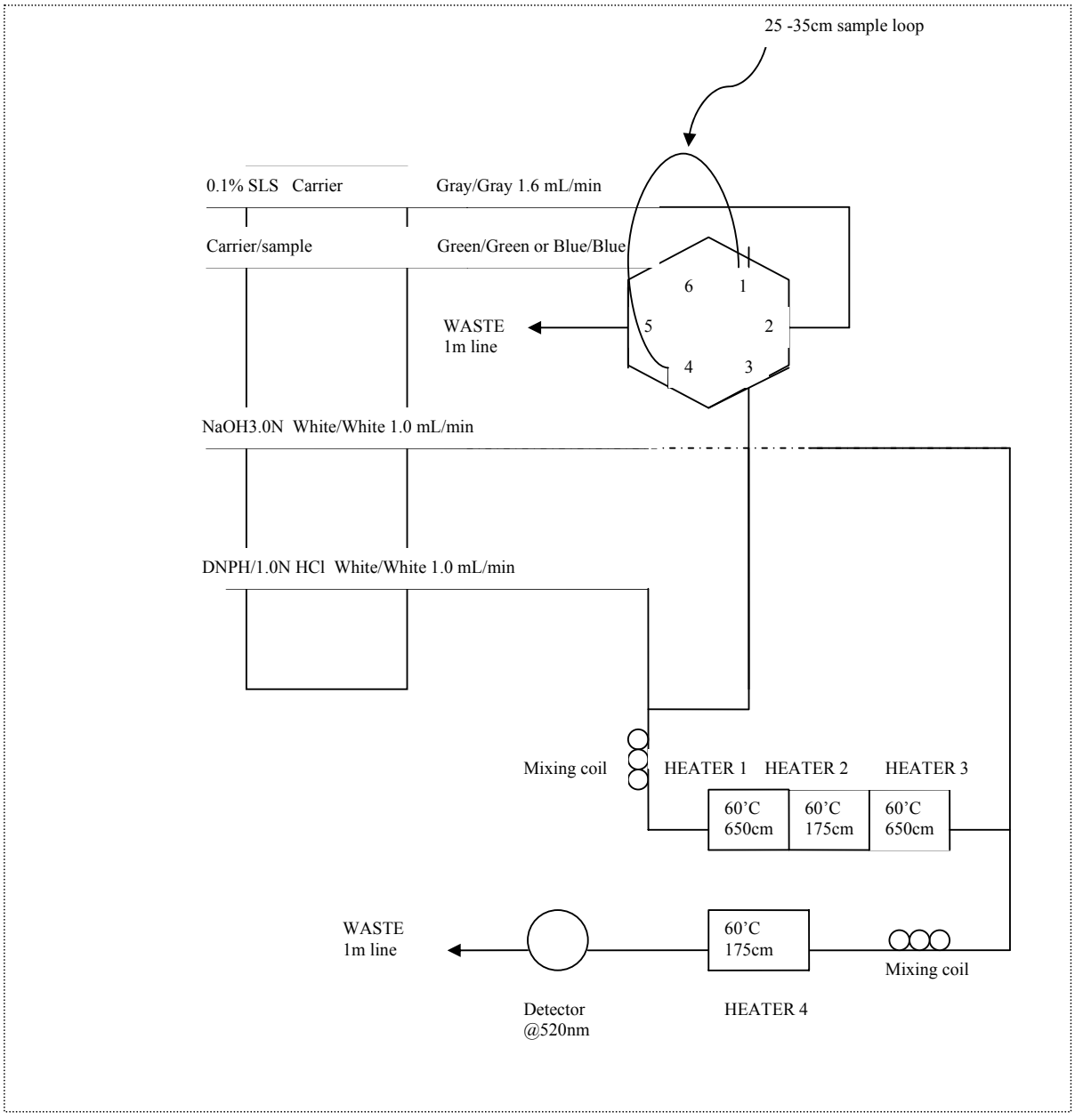
Document details regarding method sensitivity, precision, accuracy and uncertainty here. These details should include the:

- Limits of Reporting (LOR) – The LOR has not been investigated with this method.
- The accepted relative error between successive samples is 5% or 0.3 μmole/mL.
- Spike recovery control limits, and the Estimate of the Uncertainty of Measurement have not been investigated with this method at this time.

10 References

Georgia Department of Agriculture: Onion Pungency Test Procedures

Figure 1 Flow Injection Analyser Manifold



Appendix 3.3 Determination of Lachrymatory Factor Concentration in Onion Juice

Document Number: LMOP2.1121 Version No:1-26/10/05

Lachrymatory Factor in Onions

1. Introduction

This method is used to determine the level of naturally occurring lachrymatory factor (LF) in onions. (LF) is an important aspect of onion pungency. LF ((Z, E) propanethial S-oxide) arises from the hydrolysis of 1-propyl cysteine sulfoxide (1-PRENCSO) and is responsible for the mouth burn and heat associated with eating onions. Sensory attributes from the LF can be overwhelming and can dominate the experience of eating onions with high levels of 1-PRENCSO. This is in addition to the pyruvate levels.

2. Principle of Procedure

In this method, which is a direct adaptation of a method developed by the Georgia Department of Agriculture, onion juice is firstly extracted from the onion using a pneumatic press. The onion juice is then extracted using dichloromethane (DCM), which contains an internal standard, *m*-xylene, and the DCM is then further separated using a centrifuge. The chloroform layer is then collected and analysed by Gas Chromatography (GC).

3. Scope and Application

The range and use of this method extends to all onion species. It is critical that a suitable sampling methodology is used when measuring LF in onions. Typically a sub-sample of 10 onions per measurement, at 2 measurements per acre should be employed to build a picture of pungency trends across a cultivar.

All chemicals used in this method are (AR) grade quality unless specified otherwise. All chemicals used in this method will be referred to as their common name and chemical formula, water used is type II or better where appropriate.

4. Safety Procedures

Refer to the Material Safety Data Sheets (MSDS) for each chemical.

Refer to the Risk Assessment for each chemical, each work method and each compartment,
Refer to the Compulsory use of Protective Clothing in the Laboratory workplace 3-1
Dichloromethane is toxic by inhalation and skin absorption. Always use in a fume cupboard when not in a sealed vessel. Dichloromethane is a suspected carcinogen. Dispose of this chemical in a sealed bottle labelled chlorinated organic waste.
Xylene is flammable and a S6 poison. Xylene is harmful by inhalation and skin absorption. Xylene is also a suspected carcinogen, and may be harmful to foetus/embryo.

5. Apparatus and Reagents

5.1 Apparatus

- Top-pan Balance (0.1 g, capacity ≥ 2 kg).
- Gas Chromatograph with FID
- Pneumatic Onion Press.
- 2mL glass GC vials, with lids and inert PTFE septa.
- Vortex stirrer
- Fume cupboard
- Glassware:
  - Volumetric flasks (1000 mL).
  - Storage Bottle Schott (250 mL, 1 L) with foil to shield from light.
- Test tube racks
- Dispenser (2 mL)
- 14 mL standard test tubes
- 5 mL Variable pipetter

5.2.9 Reagents

5.2.10 Stock Internal Standard Solution 0.4% m-Xylene in dichloromethane

Weigh 0.40±0.01g of m-Xylene into a 100mL volumetric flask. Dilute to volume with dichloromethane. A stock solution of m-xylene in HPLC grade methylene chloride (0.4%) can be stored in the refrigerator for 1 month in the dark (aluminium foil). Dichloromethane is a very non polar compound and is difficult to pipette. Care must be taken when pipetting this solvent.

5.2.11 Extraction solvent containing m-Xylene internal standard.

Pipette 12.5 ± 0.05 mL of stock 0.4% m-Xylene solution into a 250mL volumetric flask and make to volume with dichloromethane. Store in the refrigerator prior to use. This solution should be used within a 2 day period. This volume is enough to accommodate 120 samples.

6.0 Procedure

6.1. Preparation

Obtaining representative onion tissue samples

6.1.1 Obtaining Onion Tissue Samples: Each "sample" must consist of the tissues obtained from 10 individual bulbs. This is required in order to account for bulb-to-bulb flavour variability. Tissue samples from each bulb must be obtained in one of two established ways.

6.1.2 METHOD A: Obtain a wedge from each bulb. First, cut the bulb in half, top to bottom. Second, cut a wedge from one of the halves which represents the entire bulb.

OR

6.1.3 METHOD B: Obtain tissue cores from each bulb. Tissue cores must be taken just below the equator of the bulb. Whole bulbs or bulbs that have been halved can be used. A cork bore is positioned just below the equator of the bulb, and is inserted through the tissue. The tissue core is then pushed out of the bore and collected for analysis

6.1.4 Regardless of the method used above, the combined tissues of ten bulbs are collected in a disposable weigh dish for juicing. Adjust the size of the wedge or the diameter of the coring device so that the combined volume of the 10 tissue samples results in a complete maceration of the tissue sample during the pressing process. The tissue samples must be pressed within 15 minutes after collection.

Obtaining onion juice sample using the pneumatic onion press (CAUTION: Operator must have specific equipment competency to operate onion press)

6.1.5 Onion juice is then extracted through the use of a pneumatic press with a press plate and barrel specially designed for onion tissue. Two screens lie on top of the press plate. Screen 1, which is made from disposable window screening, lies on top of Screen 2, which is made from stainless steel wire stock.

6.1.6 Insert the pressure plate over the locating lugs, and then fit the two screens, ensuring that the disposable finer screen is above the coarser permanent screen. Then fit the barrel assembly over the locating lugs and fix in position with the two locking levers.

6.1.7 Ensure that a small beaker is located below the collection point on the plate, and then add the 10 onion sub-samples to the barrel assembly.

6.1.8 Make a final check that the necessary parts are in position, and then press the juice from the onions by pulling downward on the safety valve lever with the left hand, and simultaneously pull the operating lever downward with the right hand. The plunger will now move downward at a controlled rate into the barrel.

6.1.9 Once the plunger has come to rest in the barrel, wait for a period of ten seconds and then raise the plunger by pulling downward on the safety valve lever with the left hand, and simultaneously pushing the operating lever upward with the right hand. Remove the juice for processing.

6.1.10 Following maceration, Screen 1 should be discarded, whereas Screen 2 can be reused after first being rinsed with fresh water, and completely dried. After maceration, the plunger must be wiped clean with a dry cloth or towel. Also after maceration, the plate and barrel assembly must be rinsed in fresh water and dried prior to reusing. All components are to be at room temperature and free of onion debris and moisture.

Juice preparation following extraction

6.1.11 Immediately following extraction with the pneumatic press, 2.0 ± 0.05 mL of juice is to be pipetted into a test tube and then 2.0 ± 0.05 mL of extracting solution containing the internal standard is then dispensed into the test tube.

6.1.12 The extract was vortexed for 5 seconds then immediately stored on ice in an insulated container. They can be stored at this point for up to an hour.

6.1.13 Centrifuged the samples at 3,000 rpm for about 5 minutes. You may need to weigh each centrifuge basket to achieve a balanced run.

6.1.14 Collect the lower organic phase carefully by inserting a Pasteur pipette and transferring the extraction to a glass GC vial then quickly cap with a PTFE septa and lid.

NOTE: Be careful not to collect any of the upper or intermediate phases. Care must be taken as DCM is very volatile and can eject from the end of the pipette. "Preloading" pipettes with some DCM a couple of times just prior to collection of the lower phase can help avoid this.

6.1.15 Samples should be kept in the deep freeze at -20± 5 °C until there are enough samples to run on the GC. In any case samples should be run on the same day to avoid any degradation of the LF prior to analysis.

GC Analysis

Separation is conducted using a DB-1 column (5 m x 0.53 mm, film thickness 2.65 mm, Aglient Technologies USA). A Shimadzu GC-17A GC was used for separation. The oven temperature is set at 60^{o}C for 0.3 min, then a gradient of 15^{o}C per min to 100^{o}C. The total run time of 3 min is used with an equilibrium time of 1 min between samples. The injector was set at 210^{o}C and FID detector was set at 250C. A split ratio of 10:1 was used with a total flow of 90 mL/min. The column flow was 8.2 mL/min with a velocity of 64 cm/sec. The set pressure is set at 100 kPa and measured flow of 3 mL/min.
The LF response was integrated and peak assignment was carried out by comparing an authentic LF standard. LF concentration was determined by comparing GC peak areas of the compound and m-xylene internal standard for the same sample. The response factor is 8.6.
At the completion of the batch ensure that any necessary repeat samples are reanalysed, and the values of the QC standards are within their respective control limits.

8.0 Calculations and Reporting

Concentration of LF = (LF Peak Area)/(*m*-xylene Peak Area) x 8.6
NOTE: The response factor from *m*-xylene to LF is 8.6.

Report this value to 2 significant figures

9. Quality Control

- Duplicate samples should be analysed at the rate of 1 in 15 samples;
- There are currently no LF standards that are available to check this method as they degrade quickly.
- Consult the chemist if a there is a problem with duplicates meeting the quality criteria assigned to them.

10. Method Performance

Document details regarding method sensitivity, precision, accuracy and uncertainty here.
These details should include the:

- Limits of Reporting (LOR) – The LOR has not been investigated with this method.
- The accepted relative error between successive samples is 5% or 0.3.
- Spike recovery control limits, and the Estimate of the Uncertainty of Measurement have not been investigated with this method at this time.

11. References

Georgia Department of Agriculture: Onion Pungency Test Procedures

Appendix 3.4 Determination of Soluble Solids Content (SSC%) in Onion Juice by Refractometer

1. Introduction

This method is used to determine the level of soluble solids content (SSC) within onion juice. % SSC is related to the perceived sweet flavour of an onion cultivar.

2. Principle of Procedure

In this method, the Brix(%) is read directly off the digital refractometer to give a measure of the %SSC associated with the extracted juice from the onion. The juice must be prepared according to the extraction techniques obtained in the pyruvate method. The juice should be measured within 5 minutes to obtain an accurate reading.

3. Scope and Application

The range and use of this method extends to all onion species. Typically a sub-sample of 10 onions per measurement, at 2 measurements per acre should be employed to build a picture of pungency trends across a cultivar.
All chemicals used in this method are (AR) grade quality unless specified otherwise. All chemicals used in this method will be referred to as their common name and chemical formula, water used is type II or better where appropriate.

4. Safety Procedures

Refer to the Material Safety Data Sheets (MSDS) for each chemical.
Refer to the Risk Assessment for each chemical, each work method and each compartment,
Refer to the Compulsory use of Protective Clothing in the Laboratory workplace 3-1

5. Apparatus and Reagents

5.1 Apparatus

- Pneumatic Onion Press.
- Lint free cleaning tissue
- Pastuer pipettes
- Deionised rinsing water bottle

- **% Soluble Solids Content (SSC) in Onions by Refractometer**

6.0 Procedure

6.1. Preparation

1. Turn the digital refractometer unit on, and ensure the window is clean.
2. Apply some deionised water to the sample window, and then press zero. “0.0” should now be shown on the display.
3. Refer to Pyruvate Method for procedure on juice extraction.
4. Ensure the juice is well mixed by stirring the sample gently.
5. Carefully draw up 1-2mLs of sample in a Pasteur pipette, and apply to the window of the hand-held refractometer. Press the READ button and wait a few seconds for the unit to give a result.
6. Rinse the window of the unit, and carefully wipe off with a lint free tissue.
7. Turn the units power off when finished.

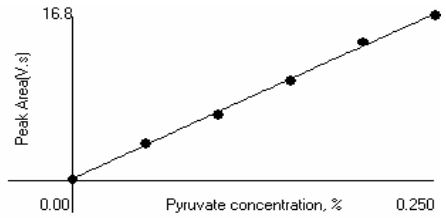
Appendix 3.5 Determination of Pyruvate Recovery in FIA Method

SAMPLE	Tube No.	*Raw pyruvate from FIA*	*Calculated Pyruvate (□moles/mL)*	Volume of 100 mM pyruvate spiked (mL)	recovery (%)		
Blank	tube 1	0.00399	-0.40	0	-		
Spike sample blank	tube 2	0.0604	1.86	0	-		
Spike sample blank	tube 3	0.0596	1.82	0	-		
Spike sample blank	tube 4	0.0598	1.83	0	-		
Spike sample blank	tube 5	0.059	1.80	0	-		
Spike sample blank	tube 6	0.059	1.80	0	-		
Spike sample blank	tube 7	0.06	1.84	0	-	Mean concentration pyruvate unspiked =	1.82
Spike sample blank	tube 8	0.0585	1.78	0	-	Std dev =	0.027
Spike 1	tube 9	0.0995	3.42	0.077	103		
Spike 1	tube 10	0.0989	3.40	0.077	103		
Spike 1	tube 11	0.0976	3.34	0.077	101		
Spike 1	tube 12	0.0992	3.41	0.077	103		
Spike 1	tube 13	0.0983	3.37	0.077	102	Spike level 1	
Spike 1	tube 14	0.0973	3.33	0.077	101	Mean concentration pyruvate unspiked =	3.37
Spike 1	tube 15	0.0968	3.31	0.077	100	Std dev =	0.041
Spike 2	tube 16	0.197	7.32	0.293	101	theoretical =	3.31
Spike 2	tube 17	0.197	7.32	0.293	101	Mean % recovery =	101.9
Spike 2	tube 18	0.198	7.36	0.293	101		
Spike 2	tube 19	0.197	7.32	0.293	101		
Spike 2	tube 20	0.198	7.36	0.293	101	Spike level 2	
Spike 2	tube 21	0.196	7.28	0.293	100	Mean concentration pyruvate unspiked =	7.34
Spike 2	tube 22	0.2	7.44	0.293	103	Std dev =	0.051
Spike 3	tube 23	0.278	10.56	0.467	103	theoretical =	7.25
Spike 3	tube 24	0.276	10.48	0.467	103	Mean % recovery =	101.2
Spike 3	tube 25	0.276	10.48	0.467	103		
Spike 3	tube 26	0.279	10.60	0.467	104		
Spike 3	tube 27	0.278	10.56	0.467	103	Spike level 3	
Spike 3	tube 28	0.276	10.48	0.467	103	Mean concentration pyruvate unspiked =	10.54
Spike 3	tube 29	0.279	10.60	0.467	104	Std dev =	0.056
						theoretical =	10.21
						Mean % recovery =	103.2

Table 1: Pyruvate

	Conc. (%)	Rep	Peak Area (Volt-s)	Peak Height (Volts)	% Residual	Detection Date	Detection Time
1	0.25	1	16.8	0.524	0.9	6/06/2006	11:03:26 PM
2	0.2	1	14	0.438	-3.1	6/06/2006	11:03:26 PM
3	0.15	1	10.1	0.322	1.8	6/06/2006	11:03:26 PM
4	0.1	1	6.68	0.208	3.5	6/06/2006	11:03:26 PM
5	0.05	1	3.71	0.113	-4.9	6/06/2006	11:03:26 PM
6	0.025	1	0.499	0.0161		6/06/2006	11:03:26 PM
7	0	1	0.0563	0.00183		6/06/2006	11:03:26 PM

Figure 1: Pyruvate



Appendix 3.6 Verification for Pyruvate Method

NSW DEPARTMENT OF PRIMARY INDUSTRIES

Diagnostic Analytical Services
Wagga Wagga Agricultural Institute

TEST VERIFICATION RECORD

Procedure	Repeatability	Accuracy	Recovery	IDL	MDL (PQL)	Linearity
Result 1	1.86	7.32	3.42	0.15	1.86	
Result 2	1.82	7.32	3.40	0.11	1.82	
Result 3	1.83	7.36	3.34	0.06	1.83	
Result 4	1.80	7.32	3.41	0.06	1.80	
Result 5	1.80	7.36	3.37	0.07	1.80	
Result 6	1.84	7.28	3.33	0.07	1.84	
Result 7	1.78	7.44	3.31	0.08	1.78	
Mean	1.82	7.34	3.37	0.09	1.82	
Standard Deviation	0.02	0.05	0.04	0.03	0.02	
COV (%)	0.01	0.01	0.01	0.35	0.01	
Expected Result		7.25	3.31			
Percent Recovery		101.23%	101.85%			0.0% residual
Confidence Interval	0.02	0.04	0.04	0.03	0.02	
Detection Level				0.10	0.08	
PQL					0.39	
Correlation Coefficient						0.99998

Reference:	pyruvate 100mMol/L Spikes	**Comments:**
Operator:	RM	
Date:	06.06.2006	

Appendix 3.7 Measurement Uncertainty for Pyruvate Method

Step 3. Combining Uncertainty for **Pyruvate**

Comined uncertainty at **6.922829225** **mmol/mL**

u_c = $y\sqrt{\left(\frac{u_{rm}}{rm}\right)^2+\left(\frac{u_{rs}}{rs}\right)^2+\left(\frac{u_{dup}}{dup}\right)^2+\left(\frac{u_{std}}{std}\right)^2}$

Type Units

= **0.27** mol/mL

Step 4. Expanded Uncertainty

U_C = $u_c \times k \left(\frac{\mu_{rs}}{rs}\right)$ = **0.54** **mmol/mL**

Expanded Uncertainty at Different Concentrations of **Pyruvate** **at a 95 % Confidence Level**

Concentration	*Expanded Uncertainty (U_c)*
0.1	**0.51**
0.7	**0.51**
1.4	**0.51**
6.9	**0.54**
13.8	**0.63**
27.7	**0.90**
69.2	**1.92**
173.1	**4.65**

Print this page.

Appendix 4 Sensory Methodolody and Planning

4.1 Sensory Sessions Plans

4.2 Trained Panel Sensory Assessment Sheets (FSA)

4.3 Consumer Panel Sensory Assessment Sheets and Modified Food Choice Questionnaire (FSA)

Appendix 4.1 Sensory Sessions Plans

ONION PUNGENCY (OCTOBER - NOVEMBER 2005)

Session Plan Onion Pungency–25/11/05 –01/12/05 – Getting full descriptions of Onion pungency, sweetness and LF factor

Food Science Australia Project Team:
Dr Patrick O'Riordan, Stephanie Kirchhoff, Ewa Orszulok

Planning overview of the training sessions

Date	Objectives / Main activities
25/11/05	- introduction to the project - description of *pungency* + *sweetness*
28/11/05	- review of *pungency* - review of palate cleansers - selection of attributes to describe *pungency* + definition + standard protocol for each attribute - validation of the method to assess the sample: time between samples (inter-stimulus rest period), palate cleanser order
29/11/05	- review of *pungency* - training on *pungency* with a focus on *Lachrymatory Factor (LF)*
30/11/05	- review of *pungency* - training on *pungency* with a focus on *sweetness*
01/12/05	- review of *pungency* and *sweetness* - pilot profile

SESSION ONE – Friday – 25/11/05: 13.00– 15.00
Introduction to the project
- The session begins with a formal introduction to the project.

Introduction to the main objectives
- Sweet & Acid tastes: the assessors are asked to begin the evaluation of the taste solutions: acid (pyruvic acid) and sweet (sucrose).
- *Pungency* and *sweetness*: It will be explained to the assessors that their objective is to develop a protocol to measure these 2 attributes. They will be asked to keep these aspects in mind and start to describe what they experience with the 1st sample tasted. They will describe their perception of *pungency* and *sweetness* in odour, then in taste (in-mouth) and finally in *aftertaste*.
- The first sample will be used to develop an appropriate method of sample evaluation. Order of modality evaluation will be odour / taste (sweetness) / flavour / *aftertaste*.
- Panellists will bite through the onion to get a representative taste of all the onion scales (layers).
- Different palate cleanser will be then presented to the assessors. They will be asked to judge the suitability each palate cleanser during the session.

Pungency: attributes generation
- Assessors will then be asked to proceed to the tasting booths for the first round of sample evaluation. Four samples will be evaluated in total. Assessors will receive one part of each sample in the same order.
- For each sample, they will be asked to write a description of *pungency* in their project note book.

- In the focus room, each assessor will read his/her full description of perceived *pungency*.
- Each sample will be described in the same manner and using round-table discussions.
- We will set up a vocabulary list of attributes related to *pungency* + appropriate definition

Samples
- 5 different samples of onion will be presented to the assessors.

	Onion (pyruvate level)	Code
Focus room	P 3	178
Booth	P 1	629
Booth	P 4	317
Booth	P 2	894
Booth	P 5	105

Palate cleansers
- We will propose to the assessors to try :
    - Tap water (room temperature) and unsalted crackers (as usual)
    - Sugar in water (7g/L)
    - Sparkling mineral water
    - Natural yoghurt
    - Cream cheese

- The assessors will decide which palate cleansers suit them and how they will use them. They will also be asked to propose other palate cleansers.

SESSION TWO - Monday –28/11/05: 10:00 – 12:00

Introduction to the session
- Particular focus on:
    - Review of training session 1 attributes and attitude towards palate cleansers
    - Onion *pungency* carry-over, the use of 'inter-stimulus interval' and different palate cleansers to minimise product carryover
    - Pungency attributes and order of attribute evaluation
    - Definition (by consensus of each attribute)
    - Profiling three onion samples (PY1, PY3, PY1) in booths

Inter-stimulus time and palate cleanser
Crowther *et al.* (2005) assessed the pungency of raw onion using 'trained panels'. They noticed that the sequence in which onions are tasted can influence results. In their observations a highly flavoured ('*pungent*') onion tasted before a milder one would make the second seem more *pungent* ('halo effect'). Consequently, in their study all the assessors received onions in the same order from the lowest level of pyruvate to the highest level to limit carry-over effects. From a sensory methodological perspective this approach was highly flawed as Crowther *et al.* could not account for psychologically biasing variables such as 'mere learning', where assessors develop a preconceived idea of attribute intensity based on order of product evaluation. Therefore, while carry-over may have been reduced from physiological perspective, their design would have ensured a strong psychological carry-over effect.

Dowell *et al.* (2005) pointed out that after spicy food an effect of sensitisation is followed by an effect of desensitisation before returning to the baseline. Therefore, we have to decide with the

Assessors the time needed between samples for their palate to go back to baseline before assessing the next sample. Furthermore, we have to determine how assessors will use the different palate cleansers to control the carryover effect.

Crowther, Collin, Smith, Tomsett, O'Connor and Jones (2005). Assessment of the flavour of fresh uncooked onions by taste panels and analysis of flavour precursors, pyruvate and sugar, *Journal of the Science of Food and Agriculture* 85:112-120.

Dowell, Chambers, Milliken and Chambers (2005). Predicting inter-stimulus intervals between samples for capsaicin-containing salsa with a range of heat levels, *Journal of Sensory Studies,* 20, 187-199.

Pungency: validation of protocol + definition of the inter-stimulus time and use of palate cleanser

- The assessors will be asked to begin with the evaluation of the taste solutions: sweet and acid.
- Between each taste solution, Assessors will receive water and crackers.
- Some time will then be spent reviewing the evaluation performed during session 1.
- Assessors will then be instructed on the need to reduce carry-over and we will explain the importance of reference standards and time between each sample.
- Assessors will then be asked to assess a 'strong' pungent onion (pyruvate level 4) blind in the focus group room. Assessors will use the provisional ballot sheet. No information re: level of pyruvate will be given to the assessors.
- Assessors will then be provided the opportunity to sample each palate cleanser (carbonated water, cranberry juice, normal water, parsley sticks, banana and crackers) with discussion.
- Once Assessors feel that their palates are suitably cleansed they will be then asked to assess a 'mild' pungent onion (pyruvate level 1) blind in the focus group room. No information re: level of pyruvate will be given to the assessors.
- We will then review the ranking of *pungency* (& *sweetness*) and discuss the suitability of palate cleansers and time between samples needed to thoroughly cleanse the palate.
- After cleansing their palates, assessors will then be asked to go to the booths to assess 3 onion samples **(PY 3, PY1 and PY1)**.
- They will be asked to focus on *pungency* and to use the attributes they have generated the day before. They will do their assessment on a paper ballot with 100mm line scales for all the attributes.
- They will evaluate the samples in the same way they have done in the 1st session. We will use a 20min break period between each sample. They will be asked to notice as well the palate cleansers they use, the amount and the order.
- In the focus room, each attribute to describe *pungency* will be reviewed including ranking of attributes for each sample. Attributes will be reviewed and a definition for each will be fixed.
- We will then narrow down the palate cleansers to use for the evaluation. With all this information, a method will be set up to clear the palate between samples.

Session preparation

Samples

- 5 samples of onion will be presented to the assessors **(PY1 + PY4 (FOCUS GROUP ROOM), PY3, PY1, PY2 (BOOTHS)**.
- The following table gives the details of each onion:

	Onion (pyruvate level)	Code
FG Room	P 4	896
FG Room	P 1	125
Booth	P 3	701
Booth	P 1	653
Booth	P 2	572

Palate cleansers

We will propose to the assessors to try:

- Tap water (room temperature) and unsalted crackers (as usually)
- Sugar in water (7g/L)
- Carbonated water
- Cream cheese
- Cranberry juice
- Banana
- Parsley sticks

SESSION THREE - Tuesday – 29/11/05 - 10:00 – 12:00

Introduction to the session

The session begins with the review of:

- Review of the previous session and the method to assess onion: time between samples, use of palate cleanser
- The attributes to assess *pungency*. The final vocabulary list will be presented.

Lachrymatory factor: validation of a protocol + pungency training

- In the booth, the assessors will be asked to assess the 3 onion samples for *pungency* and *sweetness*. They will be asked then to rank the 3 onions for all the attributes.
- In the focus room, each assessor will give his/ her ranking. As the pyruvic acid level should be equivalent in all the 3 samples and the LF different, we are expecting a consensual order for LF and various orders for the other attributes. It will help us to assign an appropriate definition for LC and finalise a standard procedure to assess LF.

Practice: pungency assessment

- Assessors will then go to the booth to assess 2 samples, practising the assessment methodology.
- In the focus room, we will check their agreement with the method.

Session preparation

Samples

- 5 different samples of onion will be presented to the assessors.

- The following table gives the details of each onion:

	Onion (pyruvate level)	Code
Booth – 1st assess	LF 1	896
Booth – 1st assess	LF 2	125
Booth – 1st assess	LF 3	701
Booth – 2nd assess	P 2	653
Booth – 2nd assess	P 3	572

Palate cleansers
We will propose to the assessors to try:

- Tap water (room temperature) and unsalted crackers (as usually)
- Sugar in water (7g/L)
- Sparkling mineral water
- Natural yoghurt
- Cream cheese

Data
For this training assessment, proposed parameters to check individual performance:
- Spearman coefficient for LF according to the LF level of the samples.

SESSION FOUR - Wednesday – 30/12/05: 10:00 – 12:00
Introduction to the session
The session begins with a review of:
- The method to assess onion: time between samples, use of palate cleanser
- The attributes to assess *pungency* and *sweetness*. The vocabulary list will be presented.

Training on pungency + special focus on sweetness
- The assessors are asked to begin with an evaluation of the taste solutions: sweet and acid (bitter if mentioned in the previous session).
- In the booths, the assessors will be asked to assess the 3 onion samples for *pungency* and *sweetness*. They will be asked to rank the 3 onions for all the attributes.
- In the focus room, each assessor will give his/ her ranking. As the pyruvic acid level should be equivalent in all the 3 samples and the level of sugar different, we are expecting a consensual order for *sweetness* and various orders for the other attributes. It will help us to check their ability to perceive and measure *sweetness*.

Practice of pungency assessment
- Assessors will then go to the booth to assess 2 samples, practising the assessment methodology.
- In the focus room, we will check their agreement with the method.

<u>Session preparation</u>
Samples
- 5 different samples of onion will be presented to the assessors.
- The following table gives the details of each onion:

	Onion (pyruvate level)	Code
Booth – 1st assess	SSC1	1
Booth – 1st assess	SSC2	2
Booth – 1st assess	SSC3	3
Booth – 2nd assess	P 4	4
Booth – 2nd assess	P 5	5

Order of presentation will be decide the previous session.

Product preparation and presentation

- Each assessor will be presented with one sample of onion in a leaded cup labelled with the corresponding code. 10 half onions will be prepared for the 10 assessors according to the procedure described in the 'Sensory research component of Onion Pungency test' doc.
- Samples will be prepared just before the assessment to prevent the lachrymatory factor.

Palate cleansers

We will propose to the assessors to use the palate cleanser they have selected in the previous session, as well as other palate cleansers they may have mentioned on an individual basis:

- Tap water (room temperature) and unsalted crackers (as usual)
- Full cream milk or buttermilk
- Cranberry juice
- Cream cheese
- Banana
- Parsley
- Coconut

Taste solutions

Sweet (sucrose), acid (pyruvate) solutions will be removed from the fridge at least one hour before evaluation.

Data

For this training assessment, proposed parameters to check individual performance:
- Spearman coefficient for *sweetness* according to the sugar level of the samples.

SESSION FIVE - Thursday – 01/12/05: 10:00 – 12:00

Introduction to the session

- The method to assess onion: time between samples, use of palate cleanser.
- The attributes to assess *pungency* and *sweetness*. The vocabulary list will be presented.

Onion assessment: training session

- In the focus room, all the definitions will be reviewed. The protocol to assess onion will also be reviewed.
- In the booths, assessors will assess the 5 levels of pyruvate using Compusense.
- After the assessment in the booth, all the recommendations will be given in the focus room to prepare for onion evaluation.

<u>Session preparation</u>

Samples

- 5 different samples of onion will be presented to the assessors.
- The following table gives the details of each onion:

	Onion (pyruvate level)	Code
Booth – 1st assess	P 5	875
Booth – 1st assess	P 3	681
Booth – 1st assess	P 2	197
Booth – 1st assess	P 1	344
Booth – 1st assess	P 4 or P 1	552

Product preparation and presentation

- Each assessor will be presented with one sample of onion in a plastic container labelled with the corresponding 3-digit code.

Palate cleansers

We will propose to the assessors the palate cleanser they have selected in the previous session:

- Tap water (room temperature) and unsalted crackers (as usually)
- Full cream milk or buttermilk
- Cranberry juice
- Cream cheese
- Banana
- Parsley
- Coconut

Taste solutions

Sweet (sucrose), acid (pyruvate) solutions will be removed from the fridge at least one hour before evaluation.

Data

For this training assessment, proposed parameters to check individual performance:

- Spearman coefficient for *pungency* according to the pyruvate level of the samples.
- Agreement between assessors (scaling and discrimination)

Appendix 4.1

TRAINED PANEL DESCRIPTIVE VOCABULARY: ONION PUNGENCY

ATTRIBUTE	DEFINITION	ANCHORS
Odour		
Pungent odour	Intensity of burning sensation in the *nose*, also affects the eyes (the sensation just before LF), start of salivation	A little to a lot
Flavour (taste & aroma)		
Sweetness	Sweet taste associated with the sucrose reference standard solution	A little to a lot
Mouth pungency	Intensity of burning sensation all over the mouth, especially on the tongue and on the roof of the mouth	A little to a lot
Throat pungency	Intensity of burning sensation on the soft palate and in the back of the *throat*	A little to a lot
Nose pungency	Sensation of irritation: prickling and tingling in the back of the *nose* from the inside to the outside as you exhale through your *nose*	A little to a lot
Eye pungency (LF-crying)	Sensation of watery eyes and irritation on the in the corner of the eye	A little to a lot
Overall pungency	Combined sensation of irritation	A little to a lot
Aftertaste		
Sweet	Intensity of the residual sweetness of the sample after swallowing	A little to a lot
Mouth pungency	Intensity of the lingering residual sensation: burning and tingling all over the mouth	A little to a lot
Throat pungency	Intensity of the residual burning sensation at the back of the *throat*	A little to a lot
Nose pungency	Intensity of the residual sensation remaining in the *nose* after swallowing	A little to a lot
Eye pungency (LF-crying)	Intensity of the residual sensation of watery eyes and residual irritation in the corner of the eyes	A little to a lot
Overall pungency	Intensity of the residual combined sensation of irritation	A little to a lot

Appendix 4.2 Trained Panel Sensory Assessment Sheets (FSA)

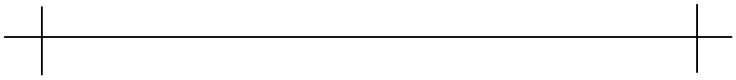
The characteristics of each onion will be evaluated as follows:

1. Odour
2. Taste/Aroma
3. *Aftertaste*

Please evaluate the union using the method of assessment discussed during training

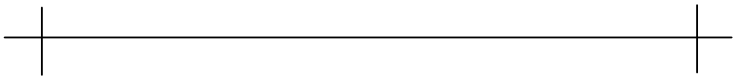
---

ODOUR
Pungent odour



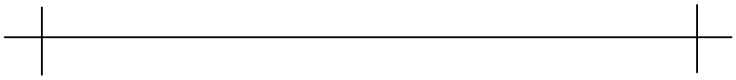
a little a lot

FLAVOUR
Sweetness



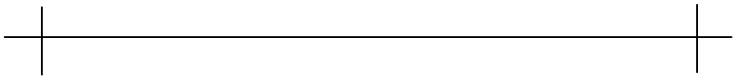
a little a lot

Mouth pungency



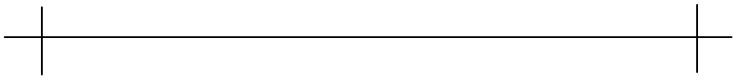
a little a lot

Throat pungency



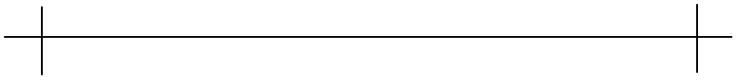
a little a lot

Nose pungency



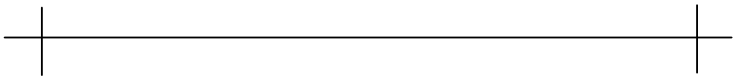
a little a lot

Eye pungency



a little a lot

Overall pungency

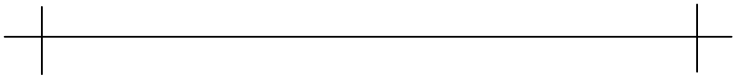


a little a lot

Please take two slow breaths as instructed

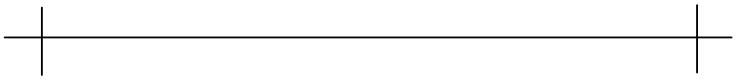
AFTERTASTE

Sweetness



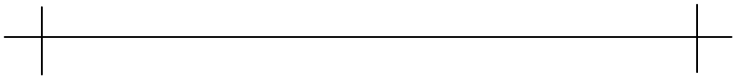
a little a lot

Mouth pungency



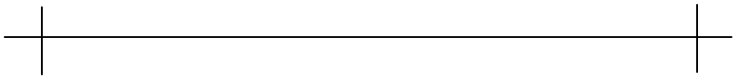
a little a lot

Throat pungency



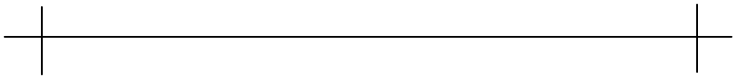
a little a lot

Nose pungency



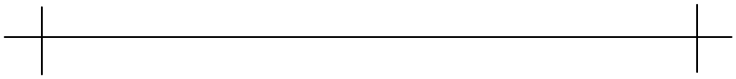
a little a lot

Eye pungency



a little a lot

Overall pungency



a little a lot

Please sample the relevant products to clean your palate

Appendix 4.3 Consumer Panel Sensory Assessment Sheets and Modified Food Choice Questionnaire (FSA)

WELCOME TO SENSORY

Today we are taste-testing raw onions

please follow all instructions
if you require assistance at any stage please ask one of the staff

THANK YOU!

---

the study is divided into 3 sections

in section 1 we ask some general questions about ***you***

section 2 involves ***tasting onion***...the fun part!

finally in section 3 we gather some information about ***your opinion*** towards onions

Section 1: Background Information

- Before the tasting please answer the following questions about your background.

Question # 1.

Please select your **age group?** *(please tick one answer only)*

- 18-34 years
- 35-49 years
- 50-65 years

Question # 2.

Please select your **gender?** *(please tick one answer only)*

- Male
- Female

Question # 3.

Are you the **main grocery buyer** at your current residence? *(please tick one answer only)*

- Yes
- No
- I buy some but not all of the groceries

Question # 4.

How often do you eat onions **(cooked)** as part of a meal? *(please tick one answer only)*

- Most days during the week
- Occasionally (2-3 times) during the week
- Once per week

Question # 5.

How often do you eat **raw onions** (alone or as part of a meal)? *(please tick one answer only)*

- Most days during the week
- Occassionally (2-3 times) during the week
- Once per week

thank you for completing section 1

Section 2: Product Evaluation

- You have been presented with a raw onion sample
- This is from a total 5 that you will taste today
- Please carefully follow the product evaluation instructions

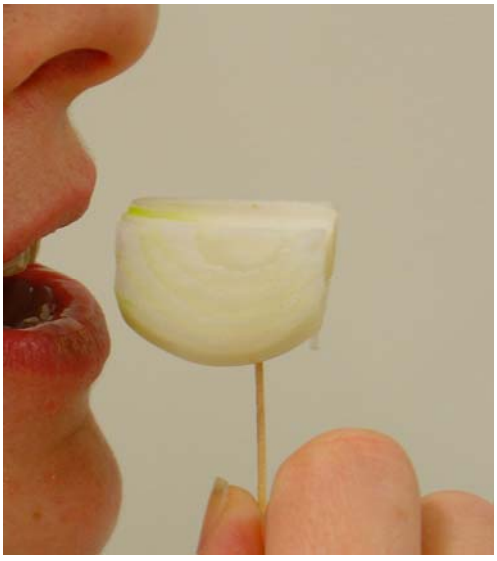
a brief questionnaire (section 3) will follow this section

Evaluation Instructions

Flavour Evaluation

1. Remove the lid from the container

2. Hold the piece of onion as shown:



3. Please taste the onion

Note: When tasting the onion please bite through the whole piece to ensure that all the onion scales are sampled

4. Please consume (and swallow) the part of the onion you have sampled

Question # 6

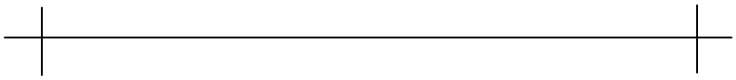
Considering the ***intensity (strength) of onion flavour***, would this onion be more suitable for *(please tick one answer only)*:

- A. Cooking (e.g. as part of a stir-fry, bbq, roast etc.)
- B. Raw consumption (e.g. as part of a salad, sandwich, etc.)

Question # 7

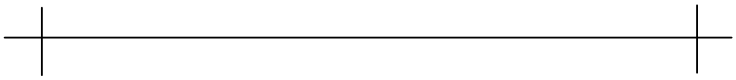
Onion flavour

Please rate the intensity (strength) of onion flavour?



Low High

Considering the flavour intensity, how much do you like or dislike this onion?



Dislike extremely **like** extremely

Question # 8

In a commercial situation (e.g. grocery, supermarket ect.), do you think this onion should be labelled as having: *(please tick one answer only)*

- A. Mild flavour
- B. Medium flavour
- C. Strong flavour

PALATE CLEANSE

Please cleanse your palate with some of the cream cheese, banana, cracker, milk and water provided...these products will help cleanse your palate before commencing the next sample

while waiting for the next sample to commence feel free to read the paper provided

thank you for completing section 2

Section 3: Your Opinion

- In this section we ask you questions about your opinion in relation to onions.

Instructions

- The following are a number of statements that **other people** have made about onions
- Please read each statement and using the scale provided please indicate how much do you **agree** or **disagree** with the following statements

Q1. "There are differences between the flavour of brown, red and white onions"(*please tick one box only*)

Disagree completely				Neither agree nor disagree				Agree completely
☐	☐	☐	☐	☐	☐	☐	☐	☐
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)

Q2. "I consider onions to be an important ingredient of most cooked meals" (*please tick one box only*)

Disagree completely				Neither agree nor disagree				Agree completely
☐	☐	☐	☐	☐	☐	☐	☐	☐
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)

Q3. "I usually choose my onions based on the type of meal I am preparing e.g. brown onions for frying, red onions for salad etc." (*please tick one box only*)

Disagree completely				Neither agree nor disagree				Agree completely
☐	☐	☐	☐	☐	☐	☐	☐	☐
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)

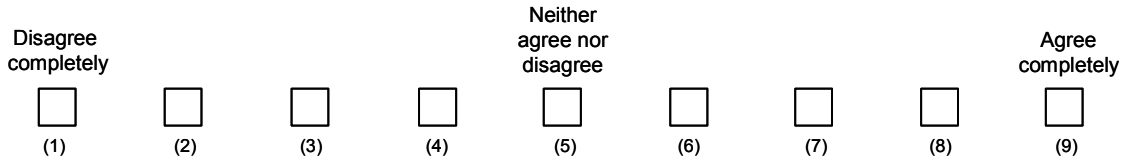
Q4. "The flavour of onions is inconsistent, sometimes they are intensely flavoured (strong) other times the flavour is very poor (weak)" (*please tick one box only*)

Disagree completely				Neither agree nor disagree				Agree completely
☐	☐	☐	☐	☐	☐	☐	☐	☐
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)

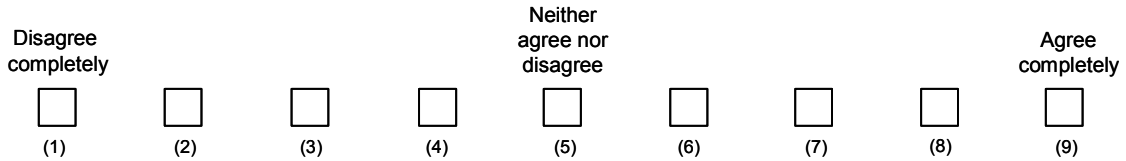
Q5. "I am familiar with different varieties of onion e.g. Wallon brown, Golden brown etc." (*please tick one box only*)

Disagree completely				Neither agree nor disagree				Agree completely
☐	☐	☐	☐	☐	☐	☐	☐	☐
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)

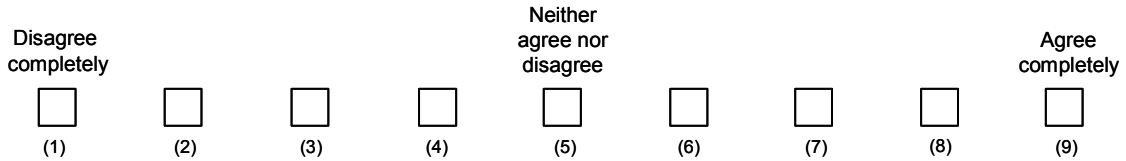
Q6. "Onions are good value for money" (*please tick one box only*)



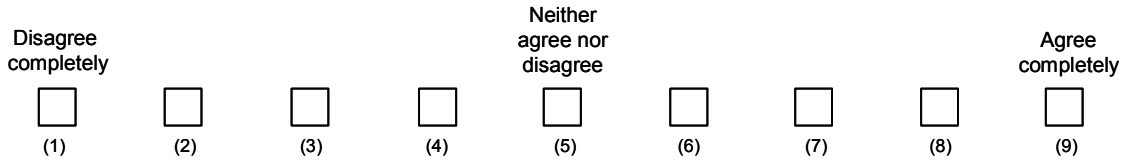
Q7. "Onions are onions…I never consider differences in flavour" (*please tick one box only*)



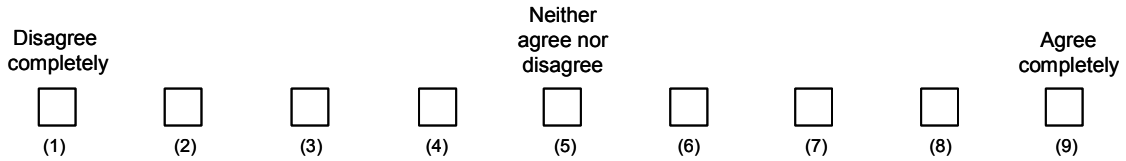
Q8. "Sometimes onions can be '*sweeter*' in flavour while others are more '*pungent*' and intensely flavoured" (*please tick one box only*)



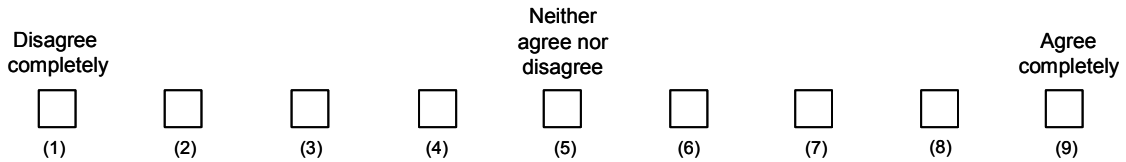
Q9. "I would be willing to pay a little extra if the strength of onion flavour (e.g. strong vs. weak) was assured before purchase" (*please tick one box only*)



Q10. "When I buy onions, I always try to choose Australian onions over imported onions regardless of the price" (*please tick one box only*)



Q11. "Information about the strength of onion flavour (e.g. mild, medium, strong) would help me choose the right type of onion for the meal(s) I plan to prepare (e.g. salad vs. cooking)" (*please tick one box only*)



Appendix 5 Supplimentary Data Analysis

5.1 Individual Trained Panel Responses to the 13 Sensory Attributes

5.2 Consumer Classification Analysis

5.3 Consumer Panel Order Analysis

5.4 Principle Component Analysis

Appendix 5.1 Individual Trained Panel Responses to the 13 Sensory Attributes

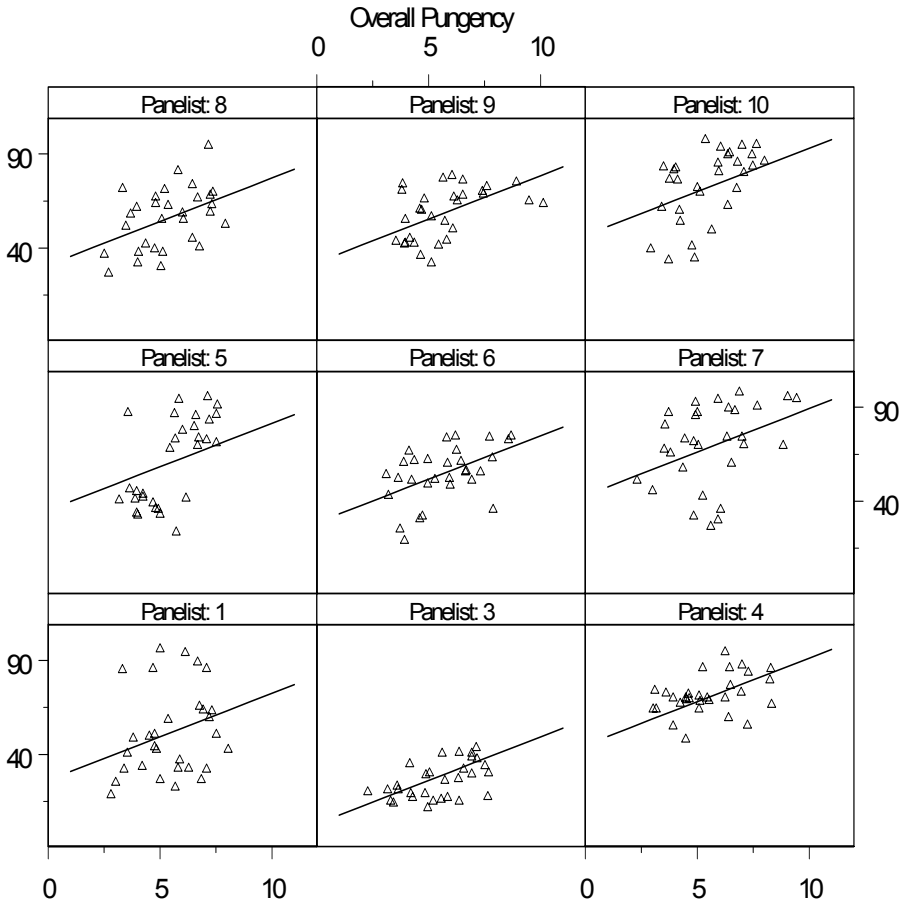
Overall pungency

Source	Model	terms	Gamma	Component	Comp/SE	% C
Panelist	10	10	0.958133	189.553	1.85	0 P
Session.Order	30	30	0.844782E-01	16.7128	1.52	0 P
Panelist.Order	50	50	0.175375	34.6954	2.08	0 P
Panelist.Session	60	60	0.590021E-01	11.6727	0.97	0 P
Variance	270	268	1.00000	197.835	8.92	0 P

Analysis of Variance	NumDF	DenDF	F_inc	Prob
21 mu	1	8.4	143.63	<.001
1 pyruvate	1	170.7	66.46	<.001

		Estimate	Standard Error	T-value	T-prev
1 pyruvate					
	1	4.62450	0.567242	8.15	
21 mu					
	1	32.3580	5.75771	5.62	

Predicted Overall Pungency = 32.36 ± 5.76 + 4.62 ± 0.57 x pyruvate
The variance component for Panellist was large (189.553) as was the component for Panellist.Order (34.695).
Predicted Overall Pungency values for each panellist were obtained from the model and are shown, together with the raw data, in the following graph.

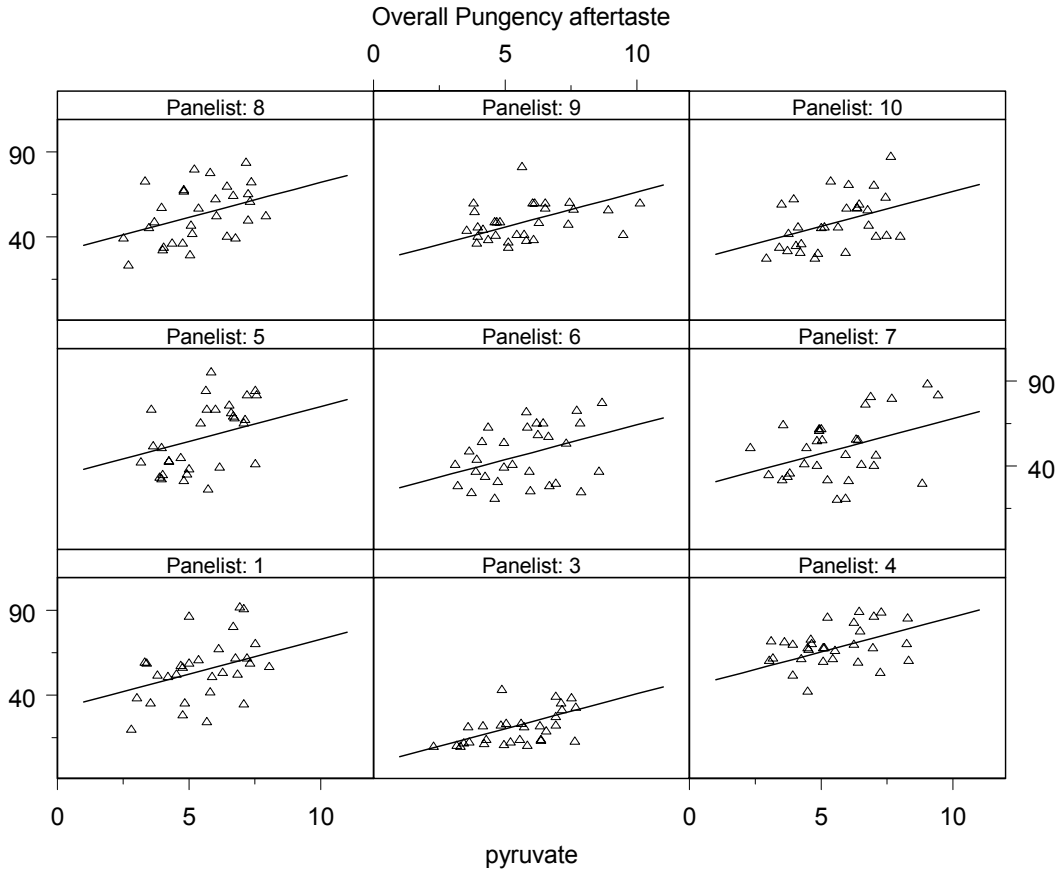


Overall pungency aftertaste

Source	Model	terms	Gamma	Component	Comp/SE	% C
Panelist	10	10	1.07765	156.965	1.86	0 P
Session.Order	30	30	0.167078	24.3356	2.09	0 P
Panelist.Order	50	50	0.341231E-01	4.97018	0.62	0 P
Panelist.Session	60	60	0.223290	32.5232	2.35	0 P
Variance	270	268	1.00000	145.654	9.03	0 P

Analysis of Variance	NumDF	DenDF	F_inc	Prob
21 mu	1	8.7	125.82	<.001
1 pyruvate	1	176.7	71.88	<.001

		Estimate	Standard Error	T-value	T-prev
1 pyruvate					
	1	4.10972	0.484726	8.48	
21 mu					
	1	26.8415	5.16425	5.20	

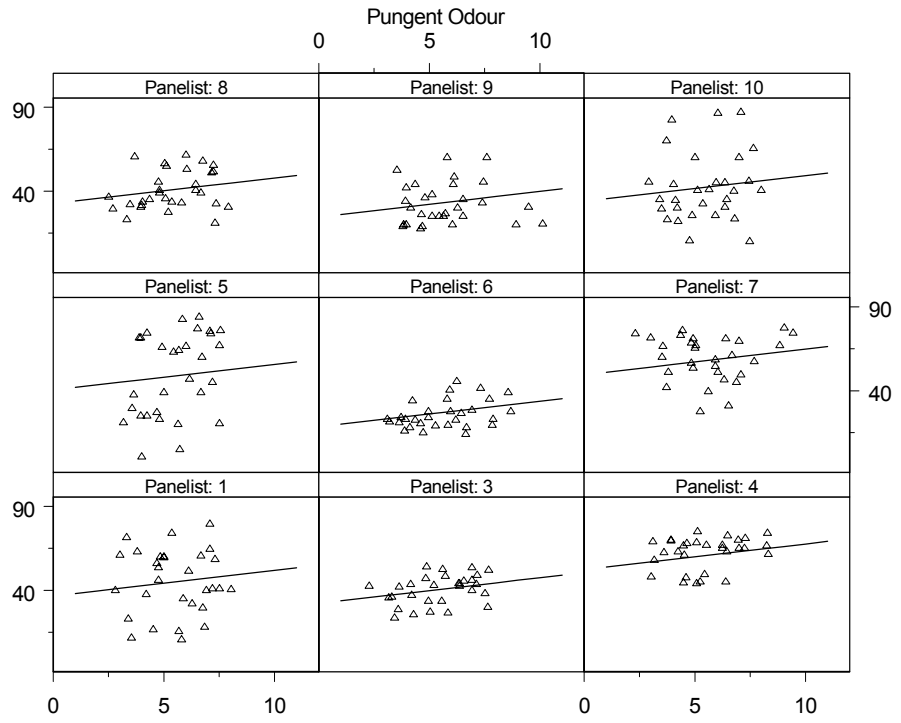


Pungent odour

Source	Model	terms	Gamma	Component	Comp/SE	% C
Panelist	10	10	0.711177	126.513	1.85	0 P
Session.Order	30	30	0.180504	32.1101	2.28	0 P
Panelist.Order	50	50	0.246919E-05	0.439249E-03	0.00	0 B
Panelist.Session	60	60	0.144853	25.7681	1.87	0 P
Variance	270	268	1.00000	177.892	9.74	0 P

Analysis of Variance	NumDF	DenDF	F_inc	Prob
21 mu	1	9.1	119.43	<.001
1 pyruvate	1	194.3	8.22	0.005

		Estimate	Standard Error	T-value	T-prev
1 pyruvate					
	1	1.52928	0.533473	2.87	
21 mu					
	1	35.6269	4.99425	7.13	



Sweetness

The relation between sweetness and pyruvate was not significant.

Source	Model	terms	Gamma	Component	Comp/SE	% C
Panelist	10	10	0.287049	64.1139	1.74	0 P
Session.Order	30	30	0.449834E-06	0.100473E-03	0.00	0 B
Panelist.Order	50	50	0.262618E-01	5.86572	0.54	0 P
Panelist.Session	60	60	0.177501E-01	3.96458	0.35	0 P
Variance	270	268	1.00000	223.356	9.46	0 P

Analysis of Variance	NumDF	DenDF	F_inc	Prob
21 mu	1	8.0	156.71	<.001
1 pyruvate	1	196.2	0.71	0.403

		Estimate	Standard Error	T-value	T-prev
1 pyruvate					
	1	-0.501214	0.596103	-0.84	
21 mu					
	1	38.5161	4.35793	8.84	

Sweet aftertaste

The relation between sweet aftertaste and pyruvate was not significant.

Source	Model	terms	Gamma	Component	Comp/SE	% C
Panelist	10	10	1.08085	80.7399	1.86	0 P
Session.Order	30	30	0.160358E-01	1.19788	0.41	0 P
Panelist.Order	50	50	0.920364E-01	6.87512	1.42	0 P
Panelist.Session	60	60	0.171031	12.7760	2.10	0 P
Variance	270	268	1.00000	74.7000	9.00	0 P

Analysis of Variance	NumDF	DenDF	F_inc	Prob
21 mu	1	8.1	41.63	<.001
1 pyruvate	1	174.7	0.16	0.695 ns

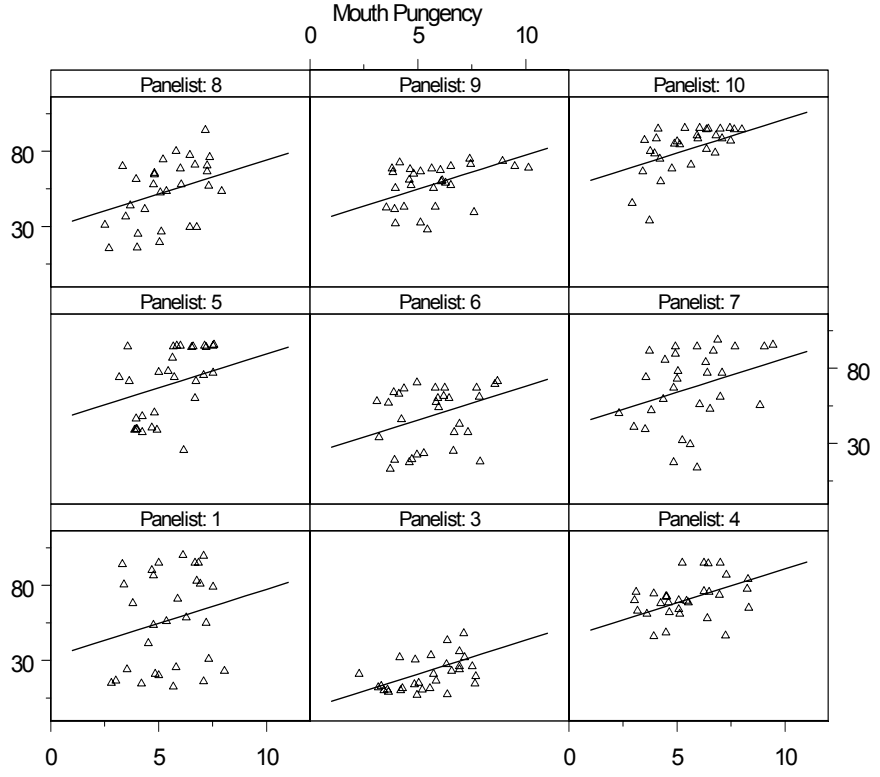
		Estimate	Standard Error	T-value	T-prev
1 pyruvate					
	1	-0.136892	0.347491	-0.39	
21 mu					
	1	20.8263	3.65507	5.70	

Mouth pungency

Source	Model	terms	Gamma	Component	Comp/SE	% C
Panelist	10	10	1.03873	295.888	1.87	0 P
Session.Order	30	30	0.126637	36.0735	1.89	0 P
Panelist.Order	50	50	0.117870	33.5761	1.65	0 P
Panelist.Session	60	60	0.727514E-01	20.7237	1.13	0 P
Variance	270	268	1.00000	284.856	8.95	0 P

Analysis of Variance	NumDF	DenDF	F_inc	Prob
21 mu	1	8.5	94.47	<.001
1 pyruvate	1	172.7	44.26	<.001

	Estimate	Standard Error	T-value	T-prev
1 pyruvate				
1	4.52268	0.679810	6.65	
21 mu				
1	33.5554	7.09632	4.73	

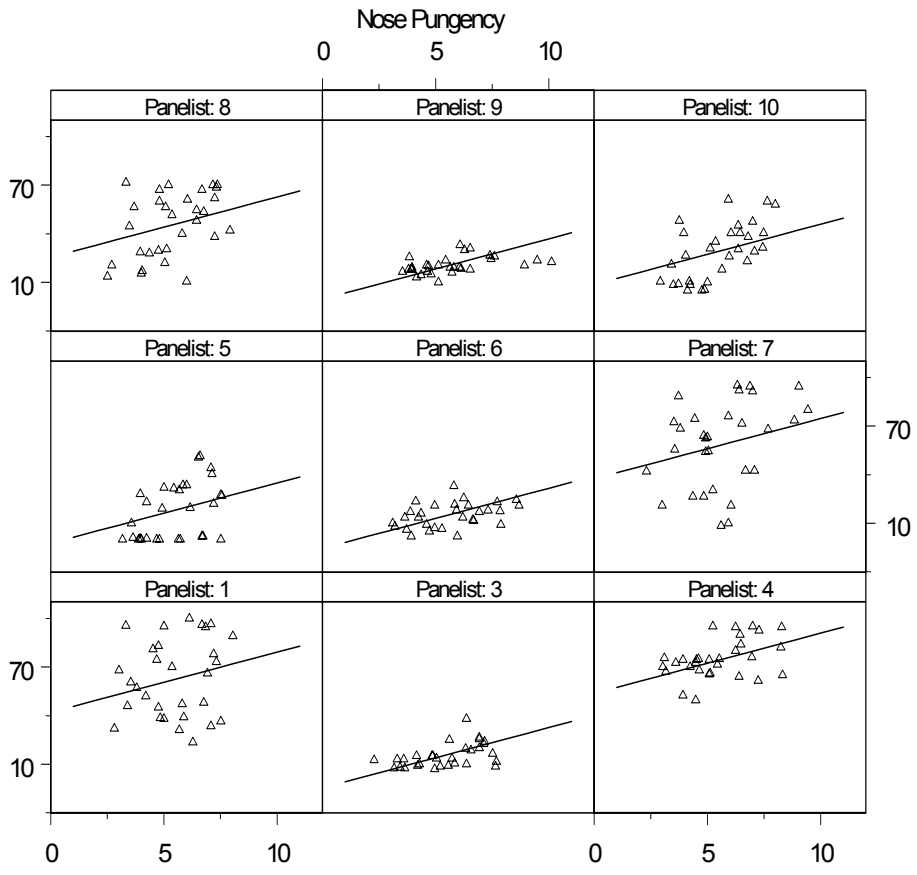


Nose pungency

Source	Model	terms	Gamma	Component	Comp/SE	% C
Panelist	10	10	2.47288	534.818	1.95	0 P
Session.Order	30	30	0.473261E-01	10.2354	1.07	0 P
Panelist.Order	50	50	0.128884	27.8742	1.77	0 P
Panelist.Session	60	60	0.160000E-05	0.346037E-03	0.00	0 B
Variance	270	268	1.00000	216.273	9.96	0 P

Analysis of Variance	NumDF	DenDF	F_inc	Prob
21 mu	1	8.1	23.22	0.001
1 pyruvate	1	209.8	39.59	<.001

		Estimate	Standard Error	T-value	T-prev
1 pyruvate					
	1	3.71757	0.590846	6.29	
21 mu					
	1	17.1590	8.47539	2.02	

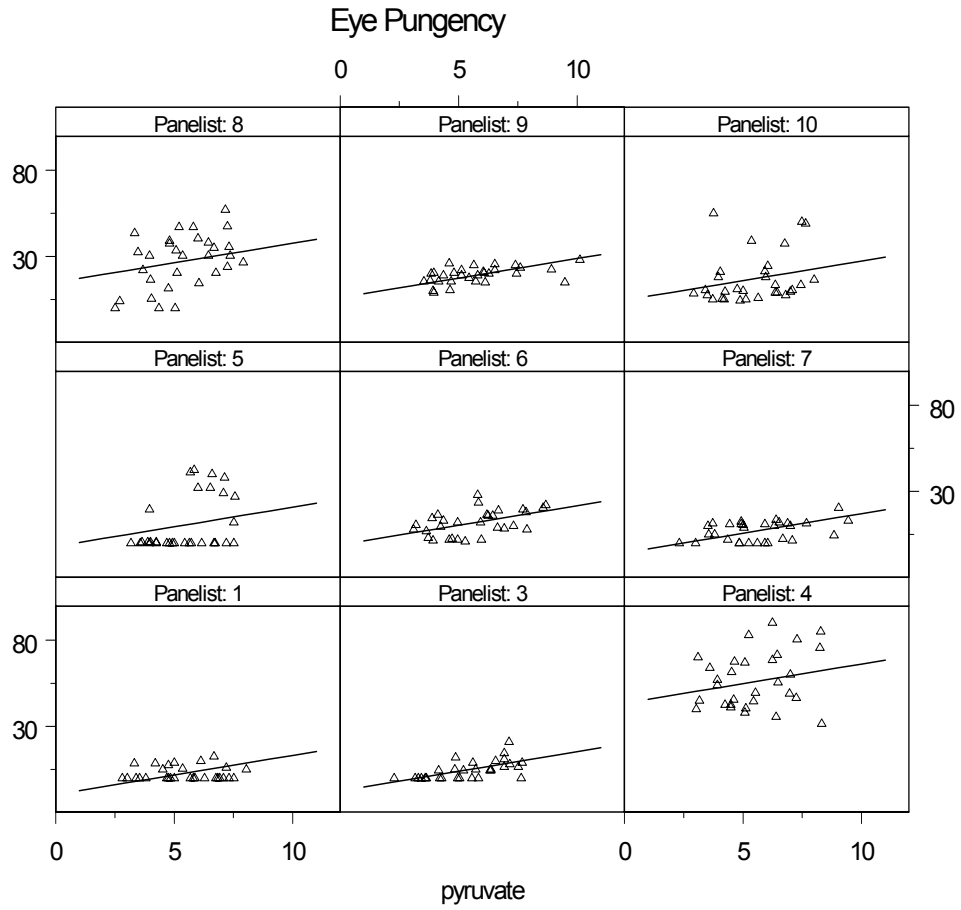


Eye pungency

Source	Model	terms	Gamma	Component	Comp/SE	% C
Panelist	10	10	2.80576	273.187	1.96	0 P
Session.Order	30	30	0.151790E-01	1.47792	0.40	0 P
Panelist.Order	50	50	0.409828E-01	3.99035	0.77	0 P
Panelist.Session	60	60	0.122676	11.9445	1.71	0 P
Variance	270	268	1.00000	97.3664	8.99	0 P

Analysis of Variance	NumDF	DenDF	F_inc	Prob
21 mu	1	8.0	9.76	0.014
1 pyruvate	1	176.3	32.86	<.001

		Estimate	Standard Error	T-value	T-prev
1 pyruvate					
	1	2.26571	0.395220	5.73	
21 mu					
	1	4.89894	5.98644	0.82	



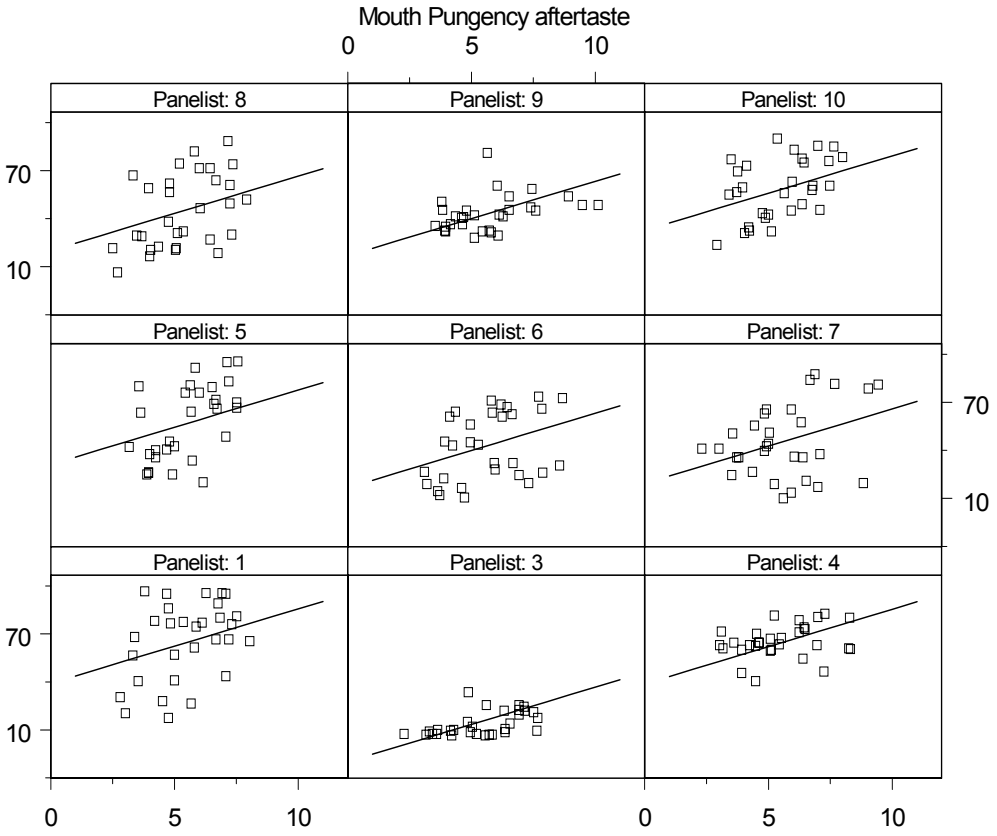
Mouth pungency aftertaste

Source	Model	terms	Gamma	Component	Comp/SE	% C
Panelist	10	10	1.18972	242.762	1.90	0 P
Session.Order	30	30	0.352994	72.0282	2.72	0 P
Panelist.Order	50	50	0.592435E-01	12.0886	0.96	0 P
Panelist.Session	60	60	0.103165	21.0507	1.45	0 P
Variance	270	268	1.00000	204.050	9.01	0 P

Analysis of Variance	NumDF	DenDF	F_inc	Prob
21 mu	1	9.4	76.22	<.001
1 pyruvate	1	175.4	65.67	<.001

Notice: The DenDF values are calculated ignoring fixed/boundary/singular variance parameters using algebraic derivatives.

	Estimate	Standard Error	T-value	T-prev
1 pyruvate				
1	4.65583	0.574524	8.10	
21 mu				
1	22.7331	6.39179	3.56	

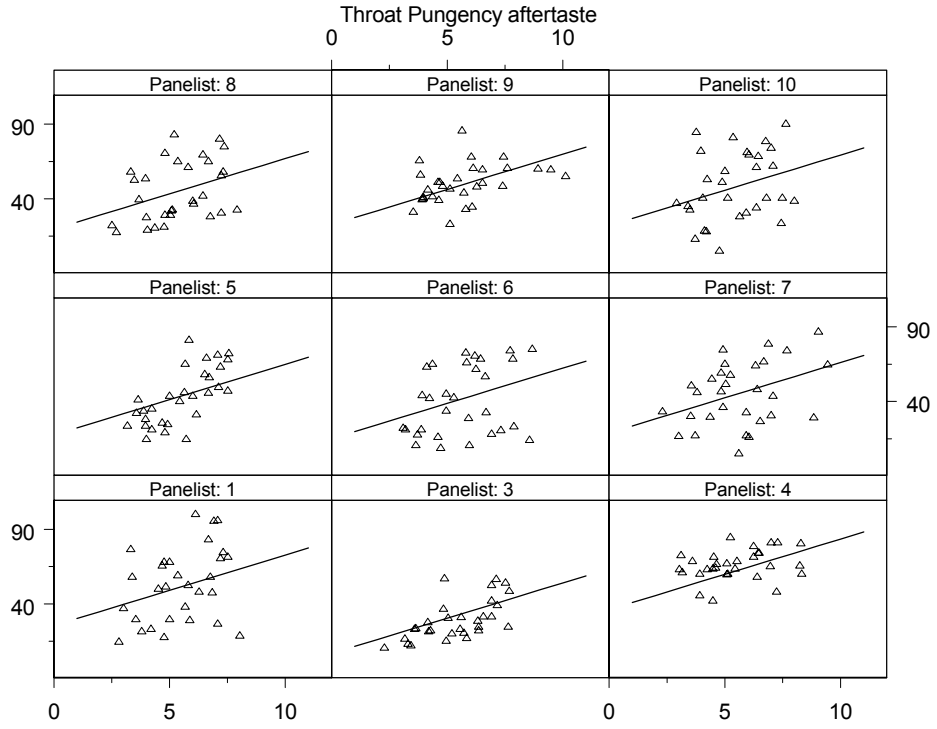


Throat pungency aftertaste

Source	Model	terms	Gamma	Component	Comp/SE	% C
Panelist	10	10	0.337270	79.4210	1.60	0 P
Session.Order	30	30	0.115625	27.2276	1.72	0 P
Panelist.Order	50	50	0.122078	28.7470	1.62	0 P
Panelist.Session	60	60	0.152694	35.9565	1.92	0 P
Variance	270	268	1.00000	235.482	8.99	0 P

Analysis of Variance	NumDF	DenDF	F_inc	Prob
21 mu	1	9.3	182.33	<.001
1 pyruvate	1	172.9	58.38	<.001

	Estimate	Standard Error	T-value	T-prev
1 pyruvate				
1	4.72766	0.618767	7.64	
21 mu				
1	20.4901	4.85648	4.22	



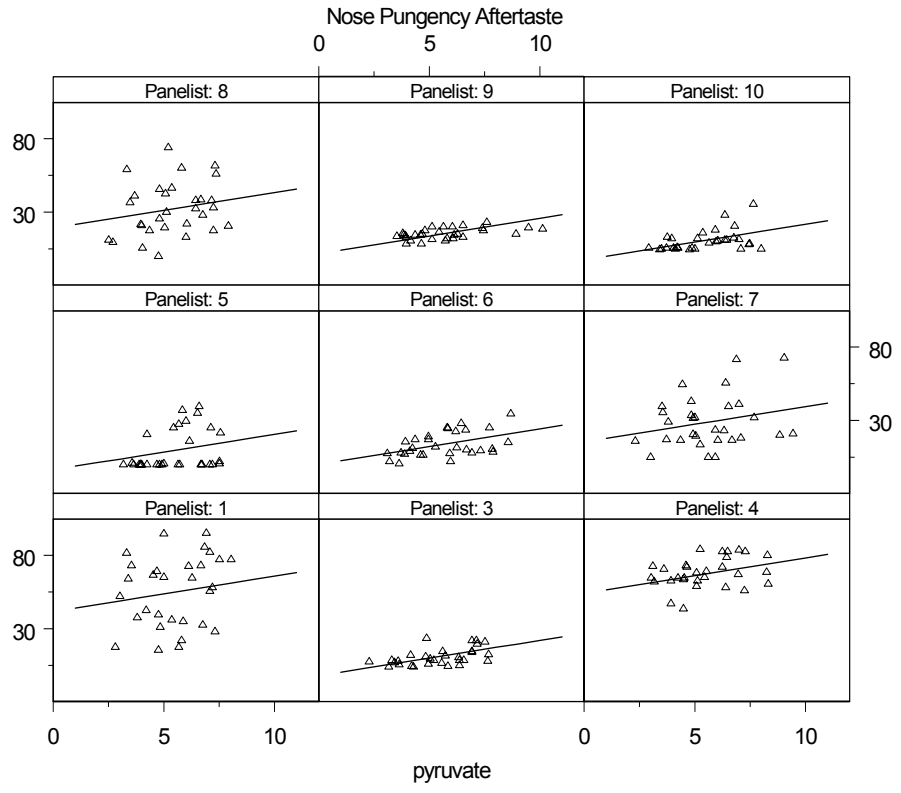
Nose pungency aftertaste

Source	Model	terms	Gamma	Component	Comp/SE	% C
Panelist	10	10	3.01188	454.692	1.96	0 P
Session.Order	30	30	0.445966E-01	6.73256	1.00	0 P
Panelist.Order	50	50	0.723010E-01	10.9150	1.20	0 P
Panelist.Session	60	60	0.541089E-01	8.16860	0.92	0 P
Variance	270	268	1.00000	150.966	8.96	0 P

Analysis of Variance	NumDF	DenDF	F_inc	Prob
21 mu	1	8.1	14.18	0.006
1 pyruvate	1	174.7	23.86	<.001

Notice: The DenDF values are calculated ignoring fixed/boundary/singular variance parameters using algebraic derivatives.

	Estimate	Standard Error	T-value	T-prev
1 pyruvate				
1	2.40791	0.493002	4.88	
21 mu				
1	13.7767	7.68837	1.79	

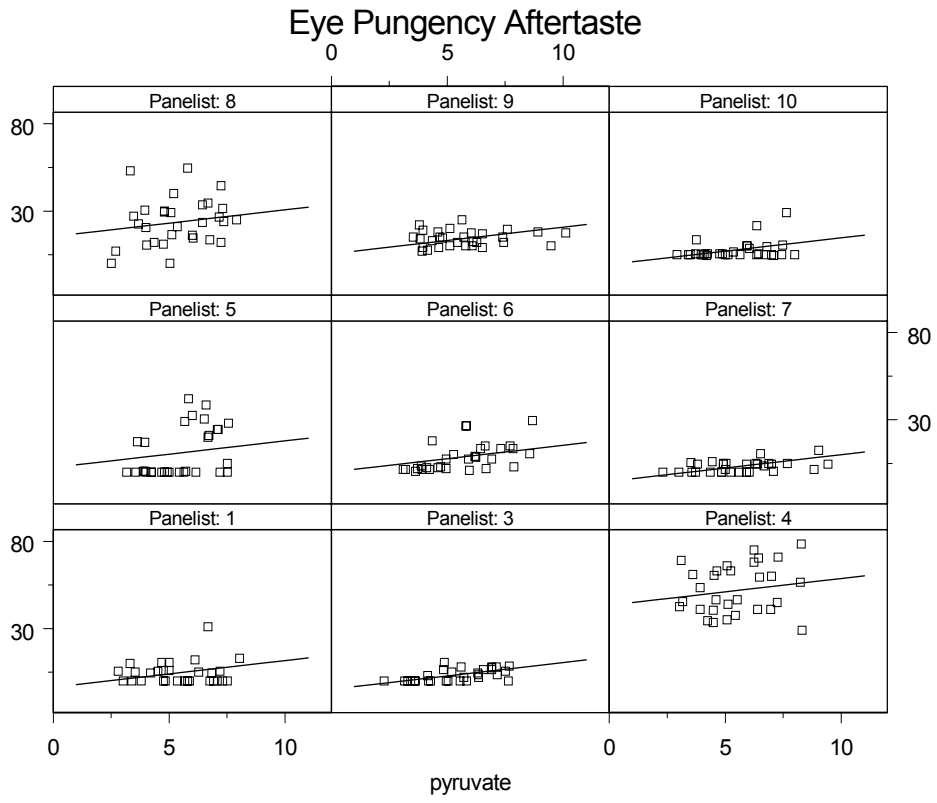


Eye pungency aftertaste

Source	Model	terms	Gamma	Component	Comp/SE	% C
Panelist	10	10	3.84469	244.403	1.96	0 P
Session.Order	30	30	0.160000E-05	0.101710E-03	0.00	0 B
Panelist.Order	50	50	0.953876E-01	6.06369	1.48	0 P
Panelist.Session	60	60	0.192755	12.2532	2.26	0 P
Variance	270	268	1.00000	63.5690	9.46	0 P

Analysis of Variance	NumDF	DenDF	F_inc	Prob
21 mu	1	8.0	7.37	0.026
1 pyruvate	1	190.9	22.79	<.001

		Estimate	Standard Error	T-value	T-prev
1 pyruvate					
	1	1.53008	0.320502	4.77	
21 mu					
	1	5.84843	5.55749	1.05	



Appendix 5.2 Consumer Classification Analysis

Consumer Perception of Mildness – alternative approach

The previous analysis (Section 5.3.4.3) was conducted by assigning 'mildness' as consumer perception of mild and medium, as compared to strong. However analysing mild alone as a distinct flavour (rather than mild plus medium) was done to determine the probability of mildness related to machine pyruvate reading was conducted with the single (mild) data (Table A).

For example an onion of 3.68 $\mu M.mL^{-1}$ pyruvate (95% confidence interval= 3.21, 4.16 $\mu M.mL^{-1}$ pyruvate) will be classed by consumers as mild with probability 0.6.

Probability	Predicted pyruvate	Lower 95% confidence limit	Upper 95% confidence limit
0.3	5.72	5.36	6.09
0.4	5.00	4.67	5.34
0.5	4.34	3.96	4.72
0.6	3.68	3.21	4.16
0.7	2.96	2.35	3.58
0.8	2.09	1.28	2.89

Table A Predicted pyruvate level for given probabilities of an onion being perceived as mild (mild only – Not medium)

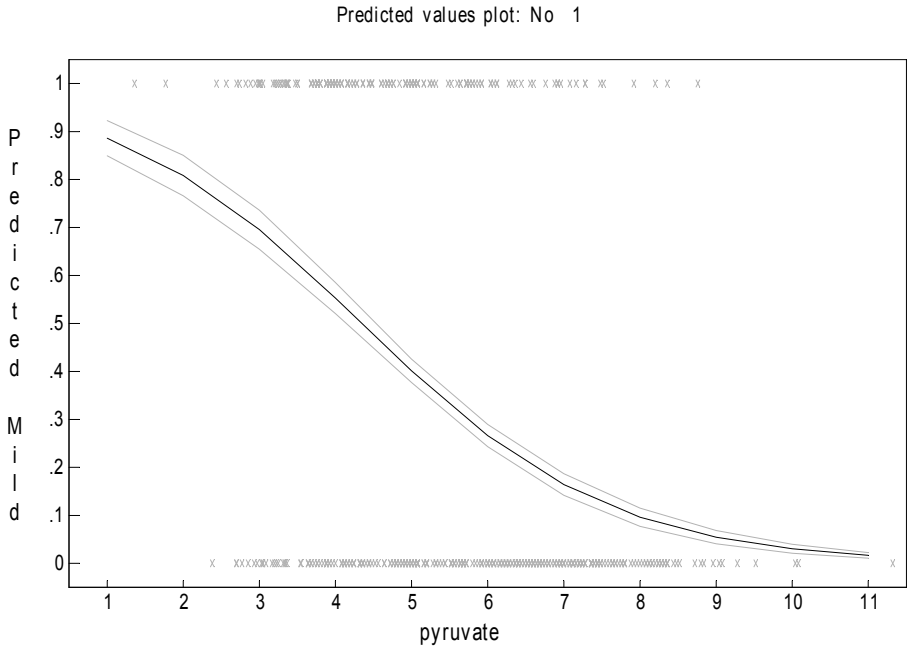


Figure B Relation between the probability of an onion being perceived as mild and pyruvate reading ($\mu M.mL^{-1}$ pyruvate)

- **Pyruvate Category**

Determination of the proportion of onions classified as mild in each pyruvate category

The relation between the proportion of onions classified as mild and pyruvate category was examined using a generalised linear model in a similar manner to the previous analysis. However this time pyruvate category was used instead of pyruvate reading. The proportions of the onions considered mild from this analysis is summarised in Table C.

In the first pyruvate category (< 4 $\mu M.mL^{-1}$ pyruvate) the proportion of onions being classified by consumers as 'mild' was 0.63. Whilst in the second pyruvate category (4-5 $\mu M.mL^{-1}$ pyruvate) the proportion considered mild was 0.49. These two proportions were not significantly different from each other.
However in the higher pyruvate category (4 and 5 = 6-7 and >7 $\mu M.mL^{-1}$ pyruvate) the proportions of onions considered mild were 0.17 and 0.10 respectively.

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	Proportion considered mild	SE
1	(< 4 μM)	0.63 a	0.051
2	(4 – 5 μM)	0.49 ab	0.053
3	(5 – 6 μM)	0.40 b	0.053
4	(6 – 7 μM)	0.17 c	0.040
5	(> 7 μM)	0.10 c	0.032

Table C Predicted proportion of onions in each pyruvate category meeting consumer expectations of being mild (as distinct to medium and / or strong).
SE is the standard error of the predicted proportion

Probability of Mildness related to machine pyruvate reading

Classification 1

Onion classification was then re-assigned into 2 classes: Mild and Stronger.
The Medium and Strong flavour responses were combined to make one level.

We now have a binary variable "P" such that:

P=1 when consumers classify an onion sample as Mild
P=0 when consumers classify an onion sample as Stronger (Medium or Strong)

Let p=Pr(P=1) ie the probability that an onion is classified as mild.

The following model is proposed:

Log(p/(1-p)) = a + b*pyruvate + error

A generalised linear model (GLM) with binomial error distribution and logit link function was used to relate the probability of "mildness" to machine pyruvate reading.

The GLM directive in GenStat returns regression coefficients for "a" and "b"

a = 2.67 (0.39)
b = -0.61 (0.07)

The predicted pyruvate value, and a 95% confidence interval, corresponding to a particular probability of an onion being mild can be calculated by inverting the regression equation.

Probability	Predicted pyruvate	Lower 95% confidence limit	Upper 95% confidence limit
0.3	5.72	5.36	6.09
0.4	5.00	4.67	5.34
0.5	4.34	3.96	4.72
0.6	3.68	3.21	4.16
0.7	2.96	2.35	3.58
0.8	2.09	1.28	2.89

Classification 1 - continued

```
summary(class.glm)

Call: glm(formula = cbind(Class, fail) ~ pyruvate, family = binomial, data =
    DAT)
Deviance Residuals:
       Min          1Q      Median       3Q      Max
 -1.713216 -0.8806306 -0.5585617 1.069238 2.356524

Coefficients:
                Value Std. Error   t value
(Intercept)  2.6670876 0.39242920  6.796354
   pyruvate -0.6140889 0.07437749 -8.256381

(Dispersion Parameter for Binomial family taken to be 1 )

    Null Deviance: 652.2588 on 499 degrees of freedom

Residual Deviance: 565.8217 on 498 degrees of freedom

Number of Fisher Scoring Iterations: 4

Correlation of Coefficients:
         (Intercept)
pyruvate -0.965683


             Dose        SE
p = 0.3: 5.7229261 0.1825636
p = 0.4: 5.0034329 0.1663456
p = 0.5: 4.3431619 0.1893208
p = 0.6: 3.6828909 0.2383372
p = 0.7: 2.9633978 0.3069366
p = 0.8: 2.0856804 0.4005081
p = 0.9: 0.7651384 0.5500777

> upper.class.ld <- class.ld + 2 * (attr(class.ld, "SE"))
> lower.class.ld <- class.ld - 2 * (attr(class.ld, "SE"))
> cbind(lower.class.ld, class.ld, upper.class.ld)
         lower.class.ld  class.ld upper.class.ld

p = 0.3:       5.357799 5.7229261       6.088053
p = 0.4:       4.670742 5.0034329       5.336124
p = 0.5:       3.964520 4.3431619       4.721804
p = 0.6:       3.206217 3.6828909       4.159565
p = 0.7:       2.349525 2.9633978       3.577271
p = 0.8:       1.284664 2.0856804       2.886697
p = 0.9:      -0.335017 0.7651384       1.865294
```

Probability of Mildness related to machine pyruvate reading

Classification 2

Onion classification was then re-assigned into 2 classes: Milder and Strong.

The Mild and Medium flavour responses were combined to make one level.

We now have a binary variable "P" such that:

P=1 when consumers classify an onion sample as Mild or Medium
P=0 when consumers classify an onion sample as Strong

Let p=Pr(P=1) ie the probability that an onion is classified as mild.

The following model is proposed:

Log(p/(1-p)) = a + b*pyruvate + error

The GLM directive in GenStat returns regression coefficients for "a" and "b"

a = 4.10 (0.45)
b = -0.54 (0.07)

The pyruvate value corresponding to a particular probability of an onion being mild can be calculated by inverting the regression equation.

Probability	Predicted pyruvate	Lower 95% confidence limit	Upper 95% confidence limit
0.3	9.21	8.27	10.15
0.4	8.39	7.64	9.13
0.5	7.63	7.04	8.22
0.6	6.88	6.42	7.34
0.7	6.06	5.65	6.46

Classification 2 - continued

```
Call: glm(formula = cbind(Class2, fail) ~ pyruvate, family = binomial, data =
   DAT)
Deviance Residuals:
       Min         1Q     Median         3Q      Max
 -2.400776 -0.9950342 0.5361805 0.7784236 2.173483

Coefficients:
                Value Std. Error   t value
(Intercept)  4.1040808 0.44873298  9.145931
   pyruvate -0.5377639 0.07215116 -7.453296

(Dispersion Parameter for Binomial family taken to be 1 )

    Null Deviance: 584.6023 on 498 degrees of freedom

Residual Deviance: 518.1321 on 497 degrees of freedom

Number of Fisher Scoring Iterations: 4

Correlation of Coefficients:
         (Intercept)
pyruvate -0.9706182
> # get the inverse predictions + s.e
library("MASS")
> class.ld <- dose.p(class.glm, p = seq(0.3, 0.9, by = 0.1))
> #class.ld <- dose.p(class.glm, p=.3)
print(class.ld)
             Dose        SE
p = 0.3: 9.207346 0.4704175
p = 0.4: 8.385735 0.3737026
p = 0.5: 7.631751 0.2934611
p = 0.6: 6.877768 0.2303309
p = 0.7: 6.056157 0.2008046
p = 0.8: 5.053865 0.2402103
p = 0.9: 3.545898 0.3898578
> upper.class.ld <- class.ld + 2 * (attr(class.ld, "SE"))
> lower.class.ld <- class.ld - 2 * (attr(class.ld, "SE"))
> cbind(lower.class.ld, class.ld, upper.class.ld)
         lower.class.ld class.ld upper.class.ld
p = 0.3:       8.266511 9.207346      10.148181
p = 0.4:       7.638330 8.385735       9.133140
p = 0.5:       7.044829 7.631751       8.218674
p = 0.6:       6.417106 6.877768       7.338430
p = 0.7:       5.654548 6.056157       6.457766
p = 0.8:       4.573444 5.053865       5.534286
p = 0.9:       2.766183 3.545898       4.325614
```

Appendix 5.3 Consumer Panel Order Analysis

Consumer results
Actual Pyruvate Reading used in analysis

100 consumers each tasted the 5 pyruvate levels (1,2,3,4,5) in a set order. 20 5x5 Latin square designs were used to balance the order of tasting, however the design was not followed explicitly and is unbalanced as a result. The actual pyruvate reading of each onion was used in this analysis.

Flavour intensity
This was defined as the intensity of the onion flavour from 'low' to 'high' as measured on a continuous scale from 0 to 100.

Mixed model analysis of ***Flavour Intensity***
The machine pyruvate reading was used in this analysis and Panellist was fitted as a random effect using ASReml.
There was a significant effect of Pyruvate reading on *Flavour Intensity* responses for consumers. They gave higher flavour intensity scores to onions with higher pyruvate readings.
Preliminary analysis showed that the order of presentation of the onions to consumers is important. A contrast which compared the first presented onion to the rest (called FIRST) was included the analysis. Another contrast (called FOURTH) which compared the onion presented in 4th place to the rest was also included. Both contrasts were significant. Consumers tended to score the *flavour intensity* of the onion sample presented first significantly higher than subsequent onions. There is also some evidence that the consumers' response to increasing pyruvate was flatter for onions presented first, than to onions presented later.
Also, onions presented to consumers in 4th place were scored significantly lower than other samples.

Source	Model	terms	Gamma	Component	Comp/SE	% C
Panellist		106	106	0.192881	79.3692	3.34	0 P
Variance		499	494	1.00000	411.492	14.06	0 P

Analysis of Variance	NumDF	DenDF_con	F_inc	F_con	M	P_con
32 mu	1	484.1	1563.36	13.03	.	<.001
7 pyruvate	1	404.3	110.62	108.68	A	<.001
30 FIRST	1	395.3	27.00	18.50	A	<.001
31 FOURTH	1	395.3	8.45	8.45	A	0.004
33 pyr.FIR	1	482.8	5.06	5.06	B	0.026

		Estimate	Standard Error	T-value	T-prev
33 pyr.FIR	1	-3.26966	1.45344	-2.25	
31 FOURTH	1	-6.75153	2.34422	-2.88	
30 FIRST	1	28.1843	8.38230	3.36	
7 pyruvate	1	6.49602	0.634340	10.24	
32 mu	1	13.7624	3.81190	3.61	

The final fitted model was:-

y= mu + pyruvate + FIRST + pyruvate.FIRST + FOURTH + *panellist* + *error*

where y=flavour intensity and x=pyruvate reading

y=13.76 ± 3.81 + 6.50 ±0.63 * pyruvate + 28.18 ±8.38* FIRST -3.27±1.45 * pyruvate x FIRST + -6.75 ±2.34* FOURTH

If a sample is tasted first the equation to describe the *flavour intensity* response of consumers is:

y= 13.76 + 28.18 + (6.50 -3.27) * pyruvate

y = 41.94 + 3.23 * pyruvate

If a sample is tasted 2nd, 3rd or 5th, the relevant equation is:

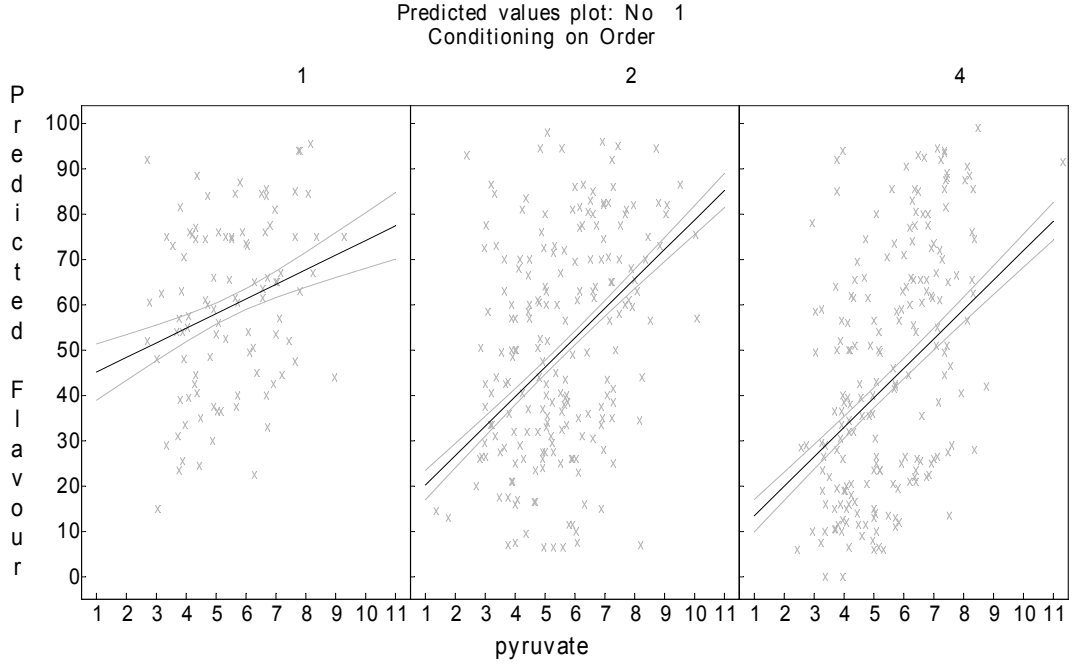
y=13.76 + 6.50 * pyruvate

And the equation for a sample presented 4th is:

y= 13.76 – 6.75 + 6.50 x pyruvate

y= 7.01 + 6.50 * pyruvate

The plot below shows the predicted values and raw data for *Flavour Intensity* for the 3 different situations.



Liking

After tasting an onion sample consumers were asked:
"Considering the flavour intensity, how much do you like or dislike this onion?"
It was also a continuous measure on a scale of 0-100 with 'Dislike extremely'=0 and 'Like extremely'=100.

The analysis of *Liking* was conducted in a similar manner to the *Flavour Intensity* analysis although there was no significant order effect for *Liking*.
Consumers preferred onions with low pyruvate levels.
The slope of the relation between Consumer *Liking* and Pyruvate reading was negative.

The fitted model was

y= mu + pyruvate + *panellist* + *error*

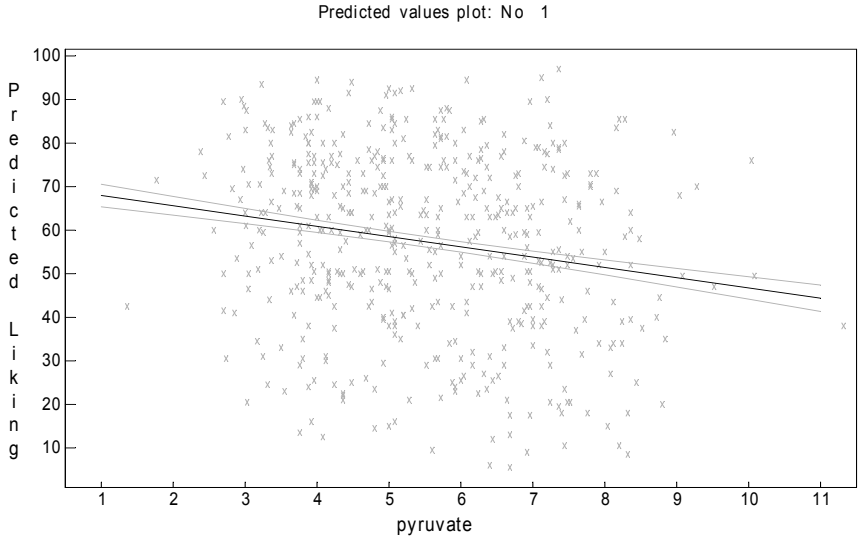
This equation describes the response of consumer *liking* to changing pyruvate.

y=70.33 ±3.04 -2.36±0.51 * pyruvate

Source	Model terms		Gamma	Component	Comp/SE	% C
Panellist	106	106	0.209626	71.4318	3.49	0 P
Variance	499	497	1.00000	340.758	14.11	0 P

Analysis of Variance	NumDF	DenDF_con	F_inc	F_con	M	P_con
32 mu	1	492.3	2349.17	534.05	.	<.001
7 pyruvate	1	406.7	21.60	21.60	A	<.001

		Estimate	Standard Error	T-value	T-prev
7 pyruvate					
	1	-2.35974	0.507778	-4.65	
32 mu					
	1	70.3256	3.04314	23.11	



Appendix 5.4 Principle Component Analysis

To compare similarities and differences between the complex set of trained panel modalities, means and variances were calculated for each modality and principal component analysis (PCA) was conducted on the data.

Chatfield and Collins (1991) suggest that when conducting Principal Component Analysis "if one variable has a much larger variance than all the other variables, then this variable will dominate the first principle component of the covariance matrix whatever the correlation structure, whereas if the variables are scaled to have unit variance, then the first principal component will be quite different in kind. Because of this, there is, generally thought to be little point in carrying out a PCA unless the variables have 'roughly similar' variances". The conventional way of solving the scaling problem is to analyse the correlation matrix rather than the covariance matrix, so that "all variables are scaled to have unit variance and so in some sense have equal importance" (Beverley Orchard, NSW DPI pers comm. 1998). Therefore the following analysis is based on the correlation matrix rather than the covariance since the variation for each modality was different (Table 1 = below). For example the variance for the pyruvate data us 2.35, whilst for the pungent odour data, the variance was 349.59. This necessitated the use of a correlation matrix for each modality = used in Main Trained Panel Analysis - Section 5).

Modality	Mean	Variance
pyruvate	5.52	2.36
Pungent.odour	44.07	349.59
Sweetness	35.75	289.73
Mouth.pungency	58.53	687.17
Throat.pungency	54.92	557.29
Nose.pungency	37.69	759.09
Eye.pungency	17.41	368.51
Overall.pungency	57.89	481.91
Sweet.aftertaste	20.07	166.87
Mouth.pungency.aftertaste	48.44	578.40
Throat.pungency.aftertaste	46.60	449.89
Nose.pungency.aftertaste	27.07	592.21
Eye.pungency.aftertaste	14.30	303.80
Overall.pungency.aftertaste	49.54	386.20

Table 1 Mean and variance of each trained panel sensory attribute

Appendix 6 Media Releases and Related Articles

6.1 'Onions Australia Newsletter'
December 2005

6.2 'Sweet Science, Serious Business'
John Golding and Trevor Twigden
Onions Australia. Volume 22. November 2005, page 11 – 14

6.3 'Onion Pungency Testing and Consumer Classification'
John Golding, Lorraine Spohr, Richard Meyer and
Patrick O'Riordan
The Australian Onion Industry Conference
Brisbane Convention Centre. 10 – 12 May 2006.

6.4 Newspaper and Radio Interviews

Appendix 6.1

'Onions Australia Newsletter' – December 2005

NSW DPI can now measure onion pyruvate content at the Wagga Wagga Agricultural Institute. The price for chemical analysis is dependent on the volume of samples to be analysed with the costs reducing with the number of samples to be analysed. The current price for pyruvate testing of a single sample (ten bulb sample) is $33.50. For 11-30 samples the cost is $28.55 per sample and for greater than 30 samples the cost is $25.30 per sample. The onion bulbs are sampled and crushed in a pneumatic press. The juice is rapidly analysed for pyruvate concentration using an adoption of the "Schwimmer & Weston" method. The addition of soluble solids content (SSC%) to the analysis incurs another small cost. The NSW DPI laboratory is accredited with the National Associations of Testing Authorities (NATA) which means all systems and results are quality assured from the national laboratory testing authority ensuring the reproducibility, quality and rigour of the results. The price (including GST) for the number of samples (as at December 2005) for both pyruvate and SSC(%) is outlined in the table below:

Number of Samples	Pyruvate only	Pyruvate + SSC(%)
1-10	$33.50	$38.55
11-30	$28.55	$32.85
>30	$25.30	$29.10

The laboratory will be in the position to receive samples for analysis in the second half of January 2006.

For more information, please contact
Richard Meyer
NSW DPI
Wagga Wagga Agricultural Institute
Pine Gully Road
Wagga Wagga NSW 2650

contact phone 02 6938 1945
e-mail richard.meyer@dpi.nsw.gov.au

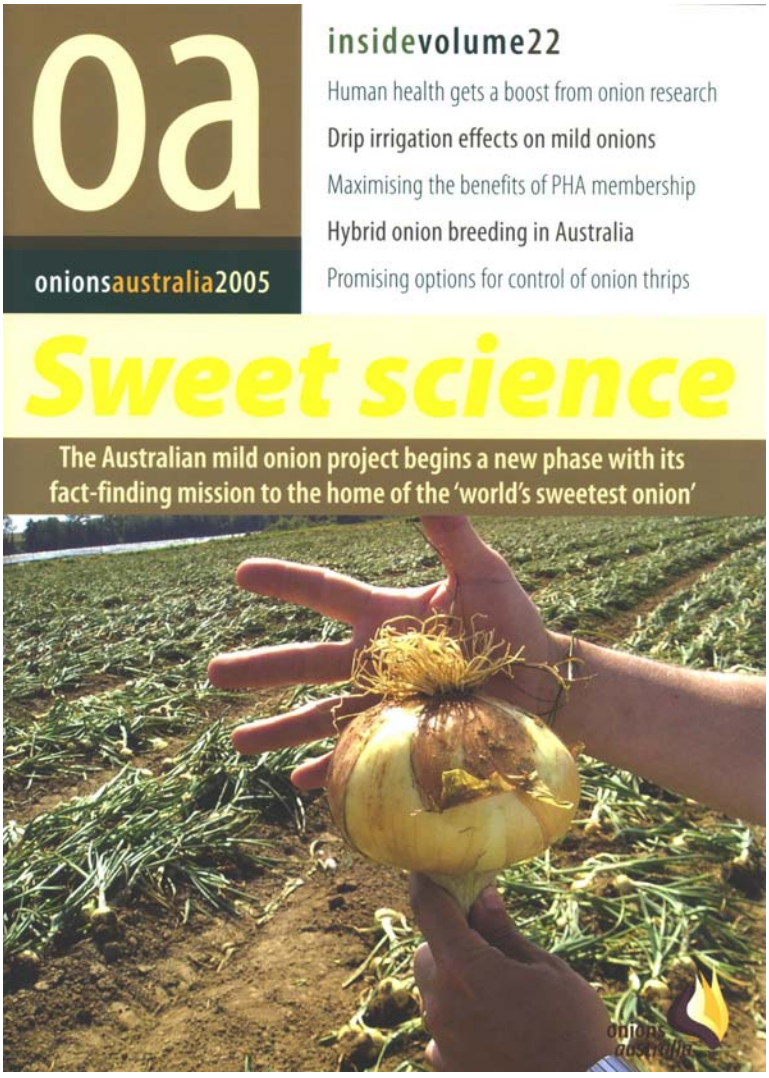
9 December 2005

Appendix 6.2

'Onions Australia' Volume 22
November 2005, page 11 – 14

'Sweet Science, Serious Business'

John Golding and Trevor Twigden



t Vidalia onions near Lyons, GA. Onions
ved to air 'cure' before hand harvesting.

Sweet science, serious business

OA visits Georgia to find out the secrets behind the success of the Vidalia onion

In May 2005, **Trevor Twigden** (chair, onion industry advisory committee) and **John Golding** (NSW Department of Primary Industries) travelled to Georgia in the US to visit a commercial mild onion pungency testing program and meet with Professor Bill Randle at the University of Georgia. This visit was the first stage of a project funded by Onions Australia and Horticulture Australia Ltd that aims to develop a local pungency testing and assessment facility. The discussions and visits with industry and Bill Randle were invaluable to the directions and progress toward a profitable mild onion industry in Australia.

Vidalia onions in Georgia

The Vidalia onion is a Georgia-grown, yellow Granex hybrid. It is mild to taste and has a characteristic flat top and bottom. The mild flavour of the onion is due to the combination of onion genetics, soils and climate in the 20-county production area in southern Georgia.

The Vidalia onion industry is highly regulated. In 1989, the Vidalia onion growers in Georgia united to help form a Federal Marketing Order which established the Vidalia Onion Committee and extended the definition of a 'Vidalia' onion to the federal level. This provided a way for growers to jointly fund research and promotion programs.

To ensure continued quality and protection of the Vidalia onion, Georgia's Department of Agriculture created and implemented the Vidalia Onion Quality Control Inspection Service. This compulsory service protects the 130 registered growers who plant about 6,000 ha of Vidalia onions each year.

There are about 15 seed varieties of onions approved by the Georgia Agricultural Commission for planting as Vidalia onions. To be approved, the yellow Granex varieties need to survive two consecutive years of experimental testing, be subjected to chemical analysis and be taste tested by a panel of trained experts.

Harvesting

The standard practices in Vidalia onion harvesting include undercutting the onions and allowing them to cure (air dry) for two to three days; clipping the tops and roots; bagging in

Above: standard harvesting practice includes undercutting and air drying. Above right: Trevor Twigden with Dr Davey Kopsell and Professor Bill Randle in the field.

burlap sacks; transporting to a warehouse; drying; grading; bagging or boxing; and shipping. The delicate nature of the onions and the apparent abundance of cheap migrant labour means most of the undercut Vidalia onions are harvested by hand.

The remainder are lifted using TopAir or similar harvesters. They are then forced-air dried prior to grading and packing. Generally recognised Vidalia onion sizes are small (starting at 25 mm), medium, large and jumbo (up to 150 mm).

Storage and marketing

Vidalia onions are harvested from late April through mid-June; retailers usually have fresh Vidalia onions available through mid-July. However, Vidalia onions can be stored for several months using controlled atmosphere (CA) storage to prolong the marketing season well into November and December.

They can be stored for several months in an atmosphere of 5% CO_2 and 3% O_2 with the air temperature maintained at approximately 0.5°C with 70% humidity. However, CA storage is being used less often due to counter-seasonal imports of fresh mild onions from South America (eg Peru).

About 70 percent of the Vidalia crop is distributed through grocery stores as a specialty item. The remaining 30 percent is distributed through roadside stands and mail order businesses and as an added-value product.

What makes Vidalia onions different?

Pungency is responsible for the hot flavour when eating raw onions. Mild onions such as Vidalia onions have low levels of pungency and are eaten raw in salads etc. The Vidalia onion industry in the state of Georgia is worth around $A100 million each year. To ensure American consumers have complete confidence in Vidalia onions, the state of Georgia has mandated standards for Vidalia onions. The current testing of pungency in Georgia is based on measuring pyruvate (a chemical compound in onions). If Vidalia onions are measured to have a pungency less than 3 $\mu M.mL^{-1}$ pyruvate, then they can be marketed as Certified Extra Sweet. If the pungency is between 3 and 5 $\mu M.mL^{-1}$ pyruvate, then the onions can be marketed as Certified Sweet. If the onions are above 5 $\mu M.mL^{-1}$ pyruvate, then the onions

Mechanically harvested onions are forced-air dried in cotton trailers before grading.

Onions are individually machine-stickered for box packs.

cannot be marketed as Vidalia onions, as they are considered too pungent.

In addition to pyruvate, onions are now being tested for another flavour compound called Lacrimatory Factor (LF). This experimental test may refine the process of identifying some onions which do not taste mild to consumers, even though their pyruvate levels might be low enough to classify them as mild.

The pyruvate testing methodology developed at UGA has now been mandated by the Georgia Department of Agriculture if pungency levels are used in the promotion and marketing of Vidalia onions.

Certification

A commercial testing laboratory in southern Georgia (National Onion Labs Inc.) has commercialised the technology developed by the University of Georgia. They provide a commercial pungency testing service for growers. This allows growers to stamp their produce Certified Sweet or Certified Extra Sweet if the test returns the appropriate pungency levels. Stickers indicating the level of sweetness are applied to the grower's or packer's branded cartons prior to shipment to the retailer.

Two 10-bulb samples are collected per acre in order to allow for field variability, which may be due to soil type, pH or nutrient level variations across a patch.

The samples are collected after undercutting, with results available within two days. The test results are valid for one month, because pungency can vary with storage.

At the time of collection, GPS readings are taken. This allows the pungency results to be mapped by location within the patch. Any areas returning above standards can then be excised from the certification. This greatly increases the reliability of certification, which then ensures greater certainty that consumers will be happy with the taste of their purchase.

In addition to bulb samples collected, one soil sample per acre is also collected, with location also being recorded by GPS. This allows field maps of pH, various nutrients and nutrient ratios to be drawn. These can then be overlaid with pungency maps so that relationships can be identified be-

Onions ready for repacking outside a controlled atmosphere room.

Vidalia onion pungency testing preparation at National Onion Labs Inc., Collins, GA

tween soil types, nutrient level and pungency.

In this way, National Onion Labs has been able to establish what effect various soil factors have on pungency. This information is then used to calculate treatments that can be applied before planting and during crop growth to increase both yield and the proportion of a field that achieves certification, with resultant improvement in economic returns.

National Onion Labs has now extended its certification service to other US states (eg Texas, Washington, California) and to Central and South American countries (eg Mexico, Peru) that are shipping off-season produce into the US.

The Australian situation

The Australian mild onion industry lacks a reliable cost-effective test for pungency. To guarantee to consumers that their mild onions will not taste pungent, the development of a rapid and cost-effective method for the assessment of onion pungency is critical. This is essential to underpin the sustained development of the mild onion sector.

A research and development project was established to develop this test and to calibrate this pungency assessment method to the Australian palate using comprehensive taste panel comparisons.

The project is funded by grower levies through Onions Australia and Horticulture Australia Ltd. The project is being coordinated through NSW Department of Primary Industries. The pungency testing is being conducted through NSW DPI at the Wagga Wagga Agricultural Institute by Richard Meyer, who helped in building the onion press. This project is also using the practical expertise of Food Science Australia at North Ryde to calibrate the pungency assessment to the Australian palate using comprehensive taste panel comparisons.

Conclusions

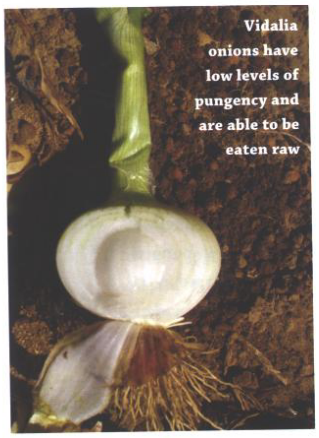
This visit to Georgia was invaluable to this Horticulture Australia project and the Australian industry. Salient lessons on pungency testing and industry application for the procedure need to be carefully adapted to the Australian industry. The outcomes of the visit will fast-track the development and reliability of the pungency test and assist the development of a mild onion industry in Australia.

Acknowledgements

We would like to acknowledge and thank Onions Australia and Horticulture Australia. This project is being funded by onion levies, facilitated by HAL in partnership with Onions Australia. The Australian government provides matched funding for all HAL's research and development activities. (Onion pungency testing and consumer calibration, VN04016).

We would also like to sincerely thank Professor Bill Randle from the Department of Horticulture at the University of Georgia. We also appreciate the time and discussions with National Onion Labs Inc.

Some of the information here comes from the Vidalia Onion Committee website at <www.vidaliaonion.org>.



Vidalia onions have low levels of pungency and are able to be eaten raw

Appendix 6.3

The Australian Vegetable Industry Conference – A New Vision
10 – 12 May 2006

Brisbane Convention Centre

onions australia

HAL
Know-how for Horticulture™

Paper Presentation:
9:50 - 10:20am Thursday 11 May 2006

Mild Onion Industry Panel Forum:
11:00 – 12:00noon Thursday 11 May 2006

Paper presented for proceedings

'Onion Pungency Testing and Consumer Classification'

John Golding[1], Lorraine Spohr[1], Richard Meyer[2] and Patrick O'Riordan[3]

[1] Gosford Horticultural Institute, NSW Department of Primary Industries
[2] Wagga Wagga Agricultural Institute, NSW Department of Primary Industries
[3] Food Quality (Measurement & Perception), Food Science Australia, Sydney

NSW DEPARTMENT OF PRIMARY INDUSTRIES

Onion pungency testing and consumer classification

John Golding[1], Lorraine Spohr[1], Richard Meyer[2] and Patrick O'Riordan[3]

[1] Gosford Horticultural Institute, [2] Wagga Wagga Agricultural Institute, NSW Department of Primary Industries; [3] Food Quality (Measurement & Perception), Food Science Australia, Sydney

Abstract

The Australian mild onion industry lacks a reliable cost-effective test for pungency. To guarantee that mild onions are not pungent, the development of a rapid and cost effective method for the assessment of onion pungency (pyruvate) is critical for industry. An onion press was constructed at NSW DPI at the Wagga Wagga Agricultural Institute and was used in measuring onion pyruvate levels using the modified 'Schwimmer and Weston' method. This NATA accredited laboratory at NSW DPI measured over 1,500 onions for pyruvate and soluble solids content (SSC%) which were used to calibrate this to the Australian palate utilizing comprehensive taste panel comparisons. These trained and consumer panel assessments of raw onions were conducted at Food Science Australia in Sydney. The results show that trained panel could reliably and accurately perceive differences in pyruvate levels (pungency) between some different classes of onions based on their pyruvate level. Similarly the 100 untrained consumers could not detect differences in pungency between onions with the lower levels of pyruvate, but were able to reliably tell these onions from the higher levels of pyruvate. Conversely, the degree of consumer 'liking' of the different onions classes varied with perceived pungency. As expected, onions with the lower levels of pyruvate (less than 6 $\mu M.mL^{-1}$ pyruvate) were equally 'likable', with the more pungent onions equally 'un-likable'. The results will provide industry with a tool to consider the establishment of the mild onion industry in Australia. This project was funded by onion levies, facilitated by HAL in partnership with Onions Australia (HA project VN 04016).

Introduction

Mild onions are not pungent (hot) and are generally eaten raw in salads, sandwiches etc. However to guarantee that mild onions are not pungent, the development of a rapid and cost effective method for the assessment of onion pungency is critical for the Australian Onion Industry. Pyruvate is a chemical compound in onions that is associated with the pungent taste in onions. The aim of this project was to:

- Develop a reliable and reproducible pyruvate pungency test utilizing the modified "Schwimmer & Weston" method
- Calibrate the "Schwimmer and Weston" method against the Australian palate utilizing extensive taste panel comparisons
- Construct an onion juice press, establishing a recognized testing facility that will enable rapid and cost effective sampling of onion pungency

This was a collaborative project with NSW Department of Primary Industries (DPI) at Gosford Horticultural Institute with the pungency testing being conducted through NSW DPI Diagnostic and Analytical Services at the Wagga Wagga Agricultural Institute. This project also utilized the extensive practical expertise of Food Science Australia in Sydney to calibrate the pungency assessment to the Australian palate utilizing comprehensive taste panel comparisons.

Materials and Methods

Onions were sourced from Queensland and New South Wales. Onion variety was not a factor in classification, as all onions were measured for pyruvate and classified according to pyruvate category:

Classification of pyruvate groups

1 < 4 $\mu M.mL^{-1}$ pyruvate
2 4 – 5 $\mu M.mL^{-1}$ pyruvate
3 5 – 6 $\mu M.mL^{-1}$ pyruvate
4 6 – 7 $\mu M.mL^{-1}$ pyruvate
5 > 7 $\mu M.mL^{-1}$ pyruvate

Each onion was cut in half. One half of the onion was crushed in the onion press, juice collected and pyruvate, lachrymatory factor (LF) and soluble solids content (SSC %) measured at the NSW DPI Onion Testing Laboratory at Wagga Wagga Agricultural Institute. The other half of the onion was refrigerated and sent to Food Science Australia for sensory assessments.

Sensory Assessments

Trained panel Ten specialist trained taste assessors were trained to assess onion pungency at Food Science Australia. Onions from the five pyruvate levels were assessed by the trained assessors in six different sessions. Assessors were trained to assess the following 13 pungency attributes: *'Eye Pungency', 'Eye Pungency aftertaste', 'Mouth Pungency', 'Mouth Pungency aftertaste', 'Nose Pungency', 'Nose Pungency aftertaste', 'Overall Pungency', 'Overall Pungency aftertaste', 'Pungent Odour', 'Sweet aftertaste', 'Sweetness', 'Throat Pungency*' and *'Throat Pungency aftertaste'*.

Consumer panel 100 onion consumers each tasted the five onion pyruvate levels in a set order. A design of 20 5x5 Latin Square designs were used to balance the order of tasting. Consumers were asked to assess; '*flavour intensity*', '*liking*' of each sample on continuous scales, and how they would classify the onion (*mild, medium or strong*). A series of consumer opinion questions was also asked of each panellist.

Results and Discussion

Onion Press and Pyruvate Analysis

The onion press was constructed from plans adapted from the University of Georgia. The press was locally constructed and commissioned at NSW DPI Wagga Wagga. The pneumatic pressing of the onion immediately releases the juice from the flesh under normal room temperatures. The juice was then used to measure pyruvate levels using a spectrophotometric procedure. The Onion Testing Laboratory at NSW Department of Primary Industries Wagga Wagga Agricultural Institute adapted the ("Schwimmer and Weston" method for measuring pyruvate in onion juice. Lachrymatory factor (LF) and soluble solids content (SSC %) were also measured on the same juice sample. The Onion Testing Laboratory at NSW Department of Primary Industries Wagga Wagga Agricultural Institute is now accepting commercial samples for pyruvate analysis.

Sensory Calibration

Trained Panel

The trained panel assessed each onion for 13 different pungency attributes. The results of '*Overall Pungency*' is presented in Table 1 and shows that the trained panel can not distinguish between onions in pyruvate category 1 and 2 (< 4 and 4-5 $\mu M.mL^{-1}$ pyruvate). However the trained panel could reliably detect those onions in pyruvate category 3 (5-6 $\mu M.mL^{-1}$ pyruvate) and these were different again significantly different those onions greater than 6 $\mu M.mL^{-1}$ pyruvate. (Table 1)

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	Predicted Value
1	(< 4 μM)	50.6 a
2	(4 – 5 μM)	50.4 a
3	(5 – 6 μM)	55.7 b
4	(6 – 7 μM)	64.5 c
5	(> 7 μM)	68.2 c

Table 1. Overall Pungency of onions as assessed by trained panel (*n* = 9). Overall Pungency was defined as the overall pungency of the onion flavour from 'low' to 'high' as measured on a continuous scale from 0 to 100. (*Least Significant Difference* = 4.5)

Consumer panel

There was a significant effect of pyruvate category on '*Flavour Intensity*' and '*Liking*' responses for consumers. Consumers could not detect any significant differences in flavour intensity between the three lowest pyruvate categories (less than 6 $\mu M.mL^{-1}$ pyruvate). The flavour intensity of onions in pyruvate category 4 was significantly higher than the first three categories. Pyruvate category 5 (greater than 7 $\mu M.mL^{-1}$ pyruvate) had the highest flavour intensity. There was also a significant effect of pyruvate level on consumer '*liking*'. Consumers assigned the highest onion '*liking*' responses for pyruvate categories 1, 2 and 3 (less than 6 $\mu M.mL^{-1}$ pyruvate) and these were not significantly different from each other. However onions from pyruvate categories 4 and 5 (greater than 6 $\mu M.mL^{-1}$ pyruvate) received significantly lower '*liking*' responses (i.e. liked the least) and were not different from each other.

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	'Flavour intensity'	'Liking'
1	(< 4 μM)	40.5 a	61.4a
2	(4 – 5 μM)	42.9 a	59.9 a
3	(5 – 6 μM)	44.1 a	59.5 a
4	(6 – 7 μM)	58.6 b	54.0 b
5	(> 7 μM)	65.4 c	51.6 b

Table 2 'Flavour intensity' and 'Liking' of onions as assessed by consumer panel (*n* = 100). Flavour intensity was defined as the intensity of the onion flavour from 'low' to 'high' as measured on a continuous scale from 0 to 100. (*Least Significant Difference* = 5.6). 'Liking' was defined as the intensity of the liking from 'Dislike extremely' to 'Like extremely'. It was assessed on a continuous measure on a scale of 0-100. (*Least Significant Difference* = 5.1)

After assessing the onions for flavour and liking, the consumers were asked "in a commercial situation (e.g. supermarket), do you think this onion should be labelled as *mild, medium or strong"*. The results are presented in Table 3 and show that consumers can accurately associate 'Flavour Classification' and pyruvate level. Analysis showed there was a significant association ($p < 0.001$) between the two factors, i.e. low onion flavour classifications are associated with low pyruvate levels and *visa versa*.

Pyruvate Category	Pyruvate Level ($\mu M.mL^{-1}$ pyruvate)	Onion Flavour		
		1 Mild	2 Medium	3 Strong
1	(< 4 μM)	63	25	12
2	(4 – 5 μM)	49	39	12
3	(5 – 6 μM)	40	42	17
4	(6 – 7 μM)	17	41	42
5	(> 7 μM)	10	37	53

Table 3 Classification of onions as assessed by the consumer panel (*n* = 100). Panellists were asked to classify each onion into a flavour category.

Flavour Classification

To relate the consumer perception of '*mildness*' to the objective pyruvate reading, the onion classification was then re-assigned into two classes: either Mild (i.e. = 'Mild' + 'Medium') or Strong. The Mild and Medium flavour responses (Table 3) were combined to make one classification. An analysis to determine the probability of Mildness related to machine pyruvate reading was conducted with the combined data. The pyruvate value corresponding to a particular probability of an onion being mild was calculated and is summarized in Table 4. For example an onion of pyruvate level of 6.06 $\mu M.mL^{-1}$ pyruvate will be classed by consumers as mild with probability 0.7. More examples are shown in the Table 4.

Probability	Predicted pyruvate ($\mu M.mL^{-1}$ pyruvate)
0.3	9.21
0.4	8.39
0.5	7.63
0.6	6.88
0.7	6.06
0.8	5.05
0.9	3.55

Table 4 Probability of the onion with the pyruvate level being perceived as mild.

Conclusions

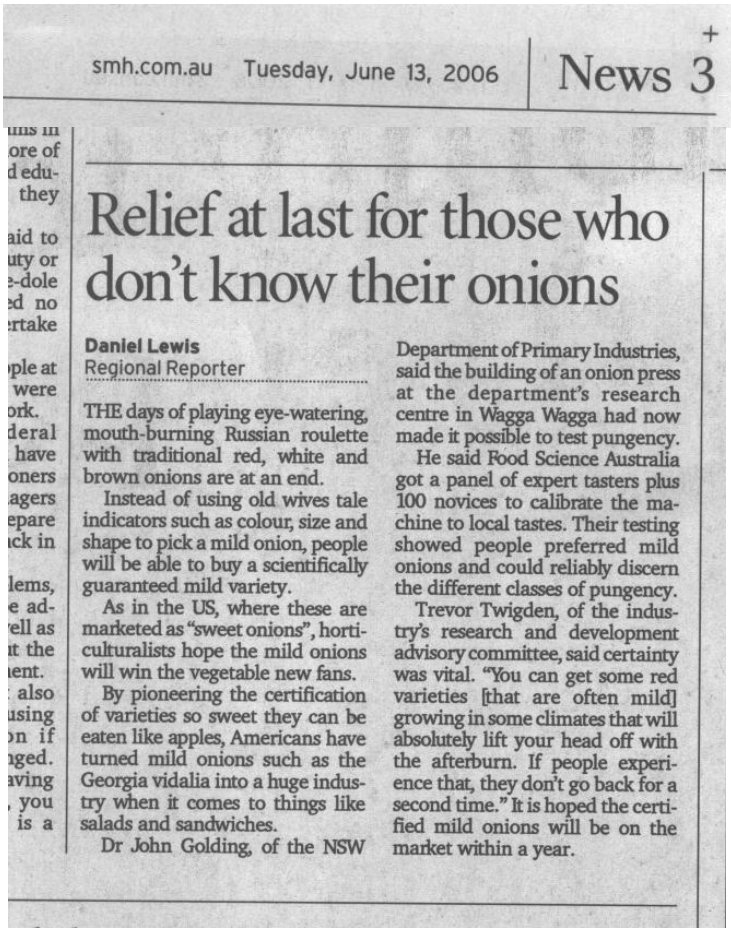
An onion press was constructed to pneumatically crush the onion sample to obtain juice for the pyruvate testing. Pungency testing was established at the Onion Testing Laboratory at NSW Department of Primary Industries Wagga Wagga Agricultural Institute. Calibration with the Australian palate show that both the trained and consumer panel could reliably and accurately distinguish between onions of different pyruvate levels. Furthermore, consumers could reliably and accurately classify onions into different categories. This information will provide the Australian Onion industry with the opportunity to develop the mild onion in Australia using a consumer driven approach.

Acknowledgements

This project has been funded by onion levies, facilitated by HAL in partnership with Onions Australia. The Australian Government provides matched funding for all HAL's research and development activities. ['Onion Pungency Testing and Consumer Calibration' (VN 04016)]. We also like to thank Trevor Twigden for sharing his vast knowledge and experience in guiding this project. We would also like to sincerely thank National Onion Labs Inc., Professor Bill Randle and students at the University of Georgia for their time, invaluable expertise, discussions and organisation. We also thank the contributions of Dr. Neil Coombes (Wagga Wagga Agricultural Institute) and Steve Morris (Wollongbar Agricultural Institute), NSW Department of Primary Industries for help with experimental design and statistical analysis. Nick Mendham (Enza Zaden Australia) and Alan Duff (Queensland Department of Primary Industries and Fisheries) for supply of onions for trial. Simon Drum (Horticulture Australia) and the Onion Research and Development Committee for valuable suggestions and guidance.

Appendix 6.4

<u>Newspapers</u>
Sydney Morning Herald. page 3
Tuesday 13 June 2006



smh.com.au Tuesday, June 13, 2006 | News 3

Relief at last for those who don't know their onions

Daniel Lewis
Regional Reporter

THE days of playing eye-watering, mouth-burning Russian roulette with traditional red, white and brown onions are at an end.

Instead of using old wives tale indicators such as colour, size and shape to pick a mild onion, people will be able to buy a scientifically guaranteed mild variety.

As in the US, where these are marketed as "sweet onions", horticulturalists hope the mild onions will win the vegetable new fans.

By pioneering the certification of varieties so sweet they can be eaten like apples, Americans have turned mild onions such as the Georgia vidalia into a huge industry when it comes to things like salads and sandwiches.

Dr John Golding, of the NSW Department of Primary Industries, said the building of an onion press at the department's research centre in Wagga Wagga had now made it possible to test pungency.

He said Food Science Australia got a panel of expert tasters plus 100 novices to calibrate the machine to local tastes. Their testing showed people preferred mild onions and could reliably discern the different classes of pungency.

Trevor Twigden, of the industry's research and development advisory committee, said certainty was vital. "You can get some red varieties [that are often mild] growing in some climates that will absolutely lift your head off with the afterburn. If people experience that, they don't go back for a second time." It is hoped the certified mild onions will be on the market within a year.

Wagga Wagga Daily Advertiser

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