FoodInfo Confidential Report No. 305

Improving the competitive advantage of New Zealand garlic

A report prepared for the

Fresh Vegetable Industry Development Committee of the New Zealand Vegetable and Potato Grower's Federation
New Zealand Garlic Exporters Council

J E Lancaster
September 1998

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J E Lancaster

CONTENTS

1 EXECUTIVE SUMMARY

1. The purpose of this work is to provide information about the levels of allicin in New Zealand garlic.

2. Allicin is the compound in garlic most responsible for its characteristic taste and its health benefits.

3. Levels of allicin were measured in three samples of garlic from Marlborough, New Zealand, and compared with garlic from Australia and China.

4. Allicin levels in Australian garlic were 5.49 mg/g fresh weight compared to 3.15 mg/g fresh weight in Chinese garlic. In New Zealand garlic the levels were between 3.3 and 4.4 mg/g fresh weight.

5. Therefore, New Zealand garlic is higher in allicin than Chinese garlic, but lower than the Australian sample.

6. Possible ways to increase allicin levels in New Zealand garlic include planting a high allicin cultivar, growing the crop under conditions of high temperature, storing bulbs in refrigerated conditions, adding sulfur and enriching soils with selenium. Managing sulfur nutrition to the plant is the best prospect.

2 ALLICIN IN GARLIC

Garlic contains high levels of organic sulfur compounds. These sulfur compounds produce the characteristic flavour of garlic and also have pharmacological activity. There is good medical evidence that garlic has anti-fungal, anti-arteriosclerotic, anti-thrombotic and blood lipid lowering effects.

Garlic flavour is the result of allyl (sometimes confusingly called alliin), methyl and (in refrigerated stored garlic) propenyl cysteine sulfoxides. These compounds do not create flavour by themselves, but when garlic is crushed these compounds generate the thiosulfinates. The thiosulfinates are mainly allylthiosulfinate or **allicin** as well as methyl allyl thiosulfinate and methyl methyl thiosulfinate. The methyl thiosulfinates have a different flavour note from the allicin.

Reserve sulfur compounds called the glutamyl cysteines are also found in garlic. During storage at low temperatures the garlic cloves hydrolyse these glutamyl compounds and propenyl thiosulfinates are formed in crushed garlic.

The aim of this work was to provide information about levels of allicin in New Zealand garlic in order to market New Zealand garlic more effectively. Information is also provided to manage garlic to produce higher levels of allicin.

3 CHECKING THE ACCURACY OF THE AUSTRALIAN ANALYSIS

The compound allicin is unstable over time and therefore, the accuracy of analysis depends on the method used. Analysis by High Performance Liquid Chromatography (HPLC) is the method most recommended in the literature. We compared the German (Iberl et al 1990) and the USA method (Lawson et al. 1991). Both gave similar results.

The Australian method (Sterling & Eagling 1997) uses a slightly different method of sample preparation and a different internal standard to calibrate the peak area of allicin (see correspondence in Appendix II).

The use of an appropriate and accurately calibrated internal standard is the key to the accurate measurement of allicin. We used two methods to calibrate the allicin peak areas:

1. *Synthesis of pure allicin*

Pure allicin was synthesised from diallyl disulfide by the methods used in Iberl et al. and Lawson et al. The synthesised allicin was analysed by HPLC and assessed as 95% pure. Aqueous solutions of allicin were prepared from the pure allicin and the concentration of the allicin determined by spectrophotometry at A=254 nm. An extinction coefficient of 9.33 ml/mg was used (Lawson et al. 1991). A standard curve for allicin by HPLC was determined using these solutions.

2. *Generation of allicin from allyl cysteine sulfoxide (alliin)*

Lawson (1995) has recommended that methods for analysing allicin are standardised by the addition to a garlic extract of known amounts of alliin. The alliin is converted directly to allicin. A calibration curve can be constructed from the HPLC response to these known amounts of generated allicin.

Both of these independent methods gave the same calibration curve for allicin. Therefore, we are confident of the accuracy of the HPLC method used in this work. We correspondend with Ms Sam Sterling, scientist involved in the garlic project underway at the Institute for Horticultural Development, Agriculture Victoria. Sam provided their methods and confirmed that they have recorded levels of allicin in Australian garlic of 50% (95 out of 200) above the 4.5 mg/g fresh weight allicin with a maximum level of 9 mg/g fresh weight.

A sample of powdered garlic and known amounts of allylcysteine sulfoxide was sent to Australia for them to analyse so that we could compare their results with ours. We are awaiting their results.

However, it is my judgement that their results are believable and that high levels of allicin do occur in Australian garlic.

3. *Results of the NZ-Australian method comparison 29/10/98*

The Australian analysis of the powdered garlic was 3.5 mg allicin/g dry weight. This compares favourably with our analysis of 3.4 mg allicin/g dry weight. They were unable to assay the alkyl cysteine sulfoxide sample we sent.

Therefore we conclude that both Australian and New Zealand methods produce the same results. Differences in allicin levels between New Zealand and Australia are result of factors other than the assay techniques.

4 MEASURING ALLICIN LEVELS IN NEW ZEALAND GARLIC

Allicin levels were measured in three boxes of Marlborough Garlic:

1. Gourmet from The Awatere,
2. Piquant from Wairau light soils, and
3. deCastro from Wairau heavy soils.

Allicin levels were also measured in imported garlic from China and Australia.

Levels of other thiosulfinates were measured:

- methyl thiosulfinate (MTh)
- allyl methyl thiosulfinate (AlMTh)
- methyl propenyl thiosulfinate (MPTh)
- allyl propenyl thiosulfinate (AlPTh).

The mean values of these compounds are shown in Table 1.

Table 1: Summary of means of levels of allicin and other thiosulfinates (mg/g fresh weight) in garlic.

Cultivar	MTh	AlMTh	MPTh	Allicin	AlPTh
Gourmet	0.28	0.59	0.11	3.33	1.09
Piquant	0.23	0.78	0.11	3.71	1.05
De Castro	0.29	1.22	0.22	4.46	1.45
Chinese	0.90	1.03	0.38	3.15	2.06
Australian	0.66	2.12	0.33	5.49	1.25
LSD 5% (df = 45)	0.093	0.26	0.058	0.50	0.14

In Figure 1 these values are presented as a histogram.

Allicin levels are highest in the Australian garlic and lowest in the Chinese garlic. De Castro garlic is higher in allicin than Piquant, which is higher than Gourmet. These differences are significant.

Chinese garlic is also higher in three other thiosulfinates (Mth, MPTh and AlPTh) compared to the Australian and New Zealand garlic. Australian garlic is highest in AlMTh.

There were significant differences in levels of allylmethyl thiosulfinate and allylpropenyl thiosulfinate between the New Zealand garlic samples .

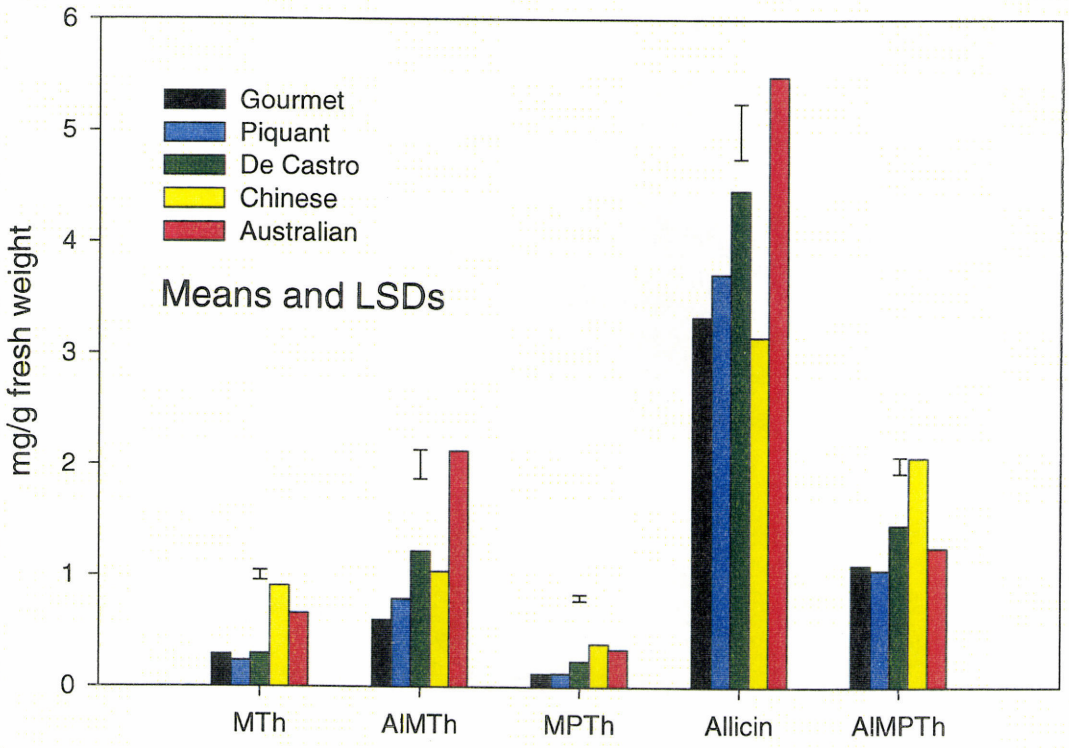

Figure 1: Quantities of allicin and other thiosulfinates in New Zealand, Australian and Chinese garlic cultivars.

The garlic samples can also be analysed for key distinguishing features (called Principal Component Analysis). The result is shown in Figure 2.

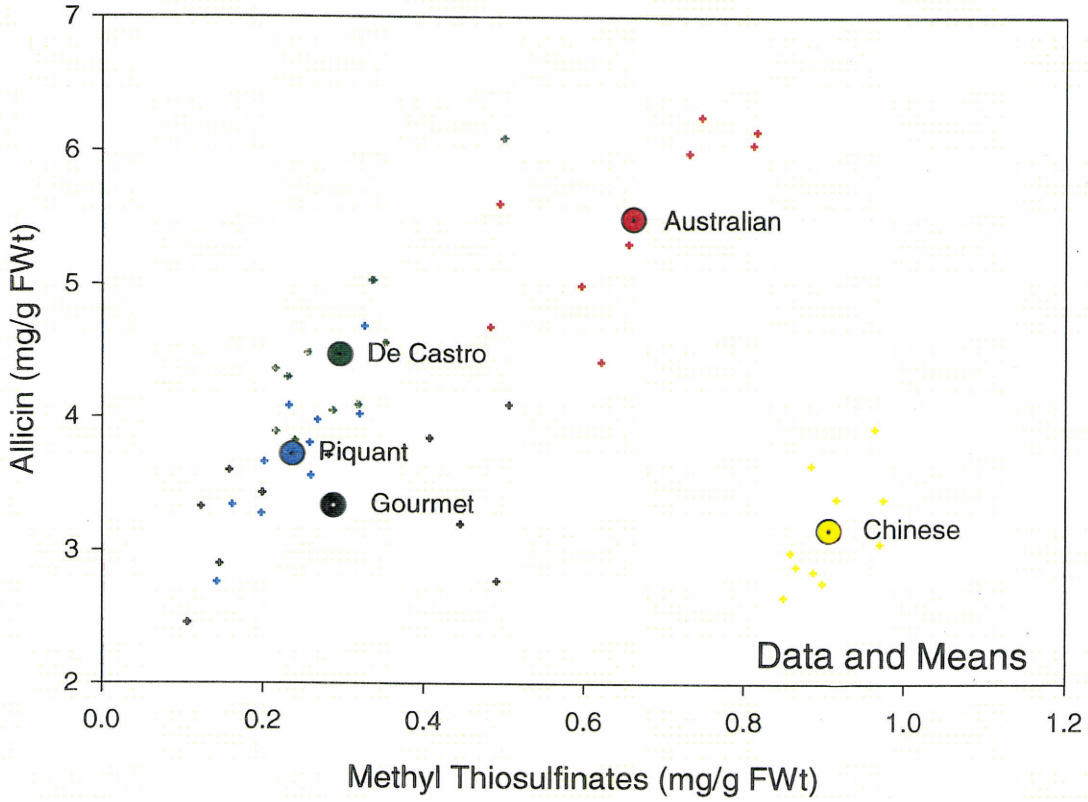

Figure 2: Principal Component Analysis for New Zealand, Australian and Chinese garlic cultivars.

The two features which distinguish the samples most are allicin and methyl thiosulfinates levels.

The New Zealand garlic samples form a cluster of similarity whilst the Australian and Chinese samples are more scattered. The Australian sample is most different in allicin levels and the Chinese garlic is differentiated on both the allicin and the methyl thiosulfinates.

Levels of allicin in garlic bulbs from different countries, using the method of Iberl et al. (1990), are in the range of 3.6-5.3 mg/g fresh weight. Elephant garlic contained 1.2 mg/g fresh weight. Lawson et al. (1991) reported allicin levels of 2.3-4.6 mg/g fresh weight (mean 0.37 ± 0.09, n=6) for USA-grown garlic and 1.28- 6.63 mg/g fresh weight (mean 0.45 ± 0.16, n=16) for 'international' garlic samples (Lawson et al. 1991). However, using the same method Lawson et al (1992) have also reported a very high value of 12.1 mg/g fresh weight for Mexican Colossal garlic.

Australian garlic was reported to contain a maximum level of 9.0 mg/g fresh weight, with more than 50% of samples having more than 4-5 mg/g fresh weight (Sterling and Eagling 1997). Assuming the Australian method of allicin analysis is correct, the results indicate that New Zealand garlic is lower in allicin than its Australian counterpart, but comparable to garlic from other parts of the world.

The existence of garlic with reputed allicin levels of 9 and 12 mg/g fresh weight from two independent groups suggests that it is possible to raise the levels of allicin in garlic.

5 FACTORS AFFECTING LEVELS OF ALLICIN IN NEW ZEALAND GARLIC

5.1 Garlic cultivar

Differences have been reported in the levels of allicin in various sub species and strains of garlic (Table 2).

In general the hard neck garlic types have higher allicin levels than the soft neck types. Printanor is a soft neck type, whilst the Chinese garlic available in New Zealand belongs to the hard neck or rocambole type.

5.2 Climate

There is some evidence that higher temperatures enhance the sulfur flavour compounds in garlic (Block 1992; Lawson 1995). Garlic grown on the hot plains of India (30-32°C) had thiosulfinate levels twice those of garlic grown in the hill regions (22-23°C). However, at the higher temperatures the relative proportions of methyl thiosulfinate to allicin were higher. The levels of reserve thiosulfinate compounds (the glutamyl cysteines) were higher in bulbs grown in the hot dry summer of 1993 in New York State than in the cold wet summer of 1992. Levels were 35% higher for the soft neck strains and 60% higher for the hard neck strains.

These results were based on observations rather than on controlled experiments, but do indicate that higher temperatures favour the production of thiosulfinates.

5.3 Storage

When garlic bulbs are stored at room temperature there is very little change in any of the thiosulfinates and reserve thiosulfinates (propenyl types). However, when bulbs are stored in refrigerated conditions, e.g. 4°C, there is a release of the reserve thiosulfinates. The release is about 5% per week during the first four weeks and up to 50% by three months. These reserve thiosulfinates produce propenyl allyl thiosulfinate, but not allicin.

Information is not available on curing.

Table 2: The main sulfur compounds in 22 strains of crushed garlic.

Variety and strain (state grown, year, number of bulbs)	Bulb weight (g)	Clove weight (g)	Allicin (mg/g)	Other thiosulfinates (mg/g)	Reserve compounds	
					S-Allyl-γ-GC (mg/g)	S-1-Propenyl-γ-GC (mg/g)
Softneck, artichoke						
California Early (CA92-4)	47	2.6	3.2	1.4	2.5	5.1
California Early (UT93-5)	29	2.7	4.6	2.3	-	-
Chinese Sativum (NY93-3)	34	3.3	3.4	1.6	3.6	5.5
Early Red Italian (NY93-3)	29	4.6	3.5	1.2	5.1	4.7
Inchellium (NY93-3)	50	5.4	2.8	0.9	4.0	3.5
Inchellium (NY92-2)	-	-	2.8	0.7	2.0	3.1
Nova Boschaca (NY93-3)	41	5.7	3.5	1.8	2.9	4.0
Oregon Blue (NY93-3)	36	6.9	4.0	1.9	3.2	4.7
Oregon Blue (NY92-2)	-	-	3.2	0.8	3.4	4.3
Softneck, silverskin						
Idaho Silverskin (NY93-3)	40	2.6	4.3	1.8	2.6	6.3
Nichol's Silverskin (NY93-3)	33	3.1	4.0	1.5	4.5	5.5
Nootka Rose (NY93-3)	43	3.2	4.2	1.8	2.7	6.3
Hardneck, rocambole						
Carpathian (NY93-3)	31	5.0	5.4	2.8	2.1	4.2
Carpathian (NY92-3)	-	-	3.5	0.8	1.6	3.3
German Red (NY93-3)	38	6.1	4.9	2.3	2.1	4.0
German Red (NY92-6)	-	-	3.4	1.1	0.9	3.2
German Red (UT93-3)	43	6.7	3.0	1.6	1.2	3.9
Israeli (NY93-3)	41	5.7	5.2	2.2	2.6	4.2
Spanish Roja (NY93-3)	35	11.2	6.7	2.4	3.2	4.8
Hardneck, porcelain						
Brown Rose (NY93-3)	24	3.8	6.1	3.3	6.8	7.8
Georgian Crystal (NY93-3)	35	8.4	4.7	2.9	4.9	5.8
Romanian Red (NY93-3)	28	9.3	7.7	4.1	6.7	7.5
Rosewood (NY93-3)	29	7.0	3.3	2.1	4.7	6.7
Zemo (NY93-3)	31	7.5	5.1	3.3	5.6	6.2
Hardneck, purple stripe						
Brown Tempest (NY93-3)	32	5.2	4.1	3.3	6.0	7.0
Persian Star (NY93-3)	24	2.9	3.5	3.5	4.8	7.5
Red Rezan (NY93-3)	31	3.4	3.1	2.7	4.8	5.9
Yampolski (NY93-3)	29	7.3	4.7	3.7	6.7	7.1

5.4 Sulfur nutrition

In onions the level of flavour compounds is strongly influenced by the level of sulfur supplied to the plant during growth. High sulfur nutrition produces pungent onions. Although information is not available on this effect in garlic it is likely that sulfur enhances garlic flavour because of the similarity in the flavour production pathways in both of these crops.

The sulfur content of onions is about 1 mg S/g fresh weight and garlic about 3 mg S/g fresh weight (Fig. 3). This equates to 1 kg S/tonne onion bulbs and 3 kg S/tonne garlic bulbs.

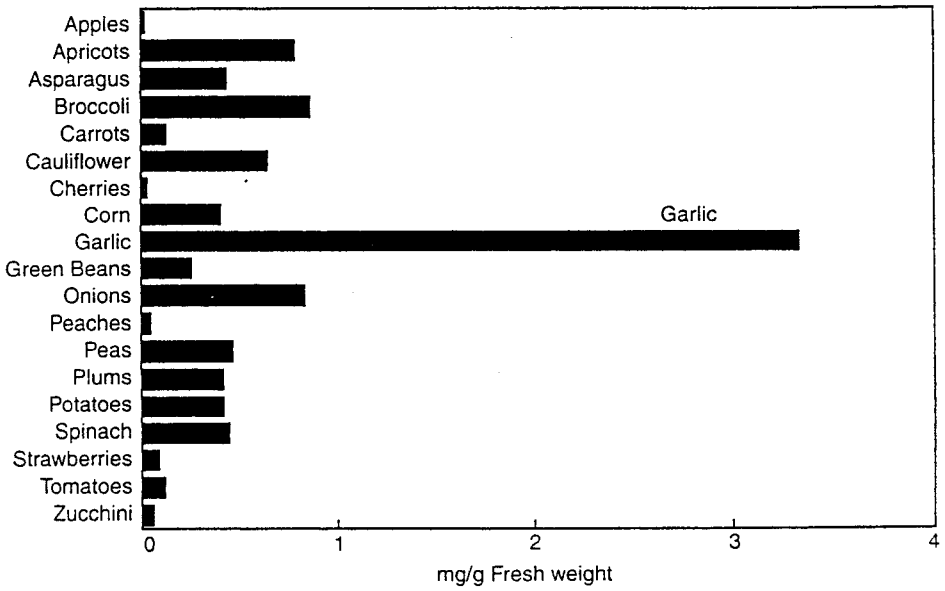

Figure 3: Sulfur content (mg/g fresh weight) of common fruits and vegetables.

Typically, application rates of S for onions have been 35-65 kg/ha on sandy soils and 45-65 kg/ha in organic soils. In New Zealand rates of 114 kg S/ha have been used for pungent onions and 6 kg/ha for mild onions. Assuming a yield of 30 tonne/ha onion bulbs, the rate of 114 kg S equates to about 4 kg S/tonne bulbs, or about 12 kg sulfate/tonne bulbs. Thus the amount of S applied is about four times the bulb content. Applying the same rule of thumb to garlic would mean applying about 120 kg S or 360 kg sulfate/ha for an expected yield of 10 tonne bulbs/ha.

The time of greatest sulfur requirement is at bulb expansion. It is important that the sulfur is applied from pre bulbing to the end of bulb expansion so that maximum formation of sulfur flavour compounds can occur. Since rapid uptake and use of sulfur is necessary, supplying the sulfur in the form of sulfate is most desirable.

5.5 Selenium

Selenium is important in the human diet. Recent thinking is that selenium can function as an antioxidant and as an anti cancer agent.

Selenium is in the same family of elements as sulfur and can substitute for sulfur in the organosulfur compounds of garlic. If garlic is grown in selenium-enriched soils it will take up the selenium and store it as Se methyl selenocysteine and Se methyl selenocysteine sulfoxide. These compounds are very useable forms of selenium for the body. When the garlic is eaten the compounds change to thiosulfinates. Selenium does not appear to be incorporated into allicin even though allicin contains sulfur molecules.

The use of selenium-enriched garlic for pharmaceutical purposes has been patented.

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7 APPENDICES

Appendix I Allicin analytical method

The method used was based on Lawson et al. (1991).

Ten bulbs of each sample were randomly selected. Each bulb was weighed. The 10 bulbs were blended in a Waring blender for 1 minute with 10 ml water added for every 1 g fresh weight. The slurry was left to stand for 10 minutes. One ml was taken and centrifuged at 14 000 rpm for 5 minutes. 600 micro litres of garlic juice was added to 600 micro litres of absolute methanol. The sample was centrifuged at 14 000 rpm for 5 minutes. 1 ml was taken for HPLC

Allicin HPLC

A Waters 'Alliance' 2690 injector/solvent delivery/control system and a Waters 490 UV/visible detector were used. Instrument control and data collection and manipulation were performed using the Waters software package 'Millennium' running on a personal computer. The column was a 220 x 4.6 mm Applied Biosystems 'Brownlee' Aquapore RP-18 fitted with a 18 x 3.5 mm Applied Biosystems 'Brownlee' Aquapore RP-18 guard column. The solvent used was aqueous methanol (50% v/v). Deaeration was achieved by vacuum filtration through a 0.22 µm filter, rapid sparging with helium (100 mL/min for 10 minutes) and constant slow bubbling of helium into capped, vented solvent reservoirs (10 mL/min). Isocratic elution with a flow rate of 1 mL/min was used. Samples (10 µL) were injected onto the column, which was maintained at 30°C using a Waters column heater. Data collection time of 15 minutes was used and eluted components were detected at 254 nm. Using this system, allicin eluted at 5.0 minutes.

Allicin calculation

Pure allicin was prepared by the method of Iberl et al (1990). The allicin was judged to be 95% pure by HPLC. Solutions of allicin were prepared in water and concentration was determined by spectrophotometry using an extinction coefficient of 9.33 ml/mg at 254 nm.

These allicin solutions were used as external C18 HPLC standards.

Allicin	250 µg/ml	1.1x10 6 µvolts
	33 µg/ml	1.13x105 "
	8 µg/ml	2.9x104 "

Because of the instability of allicin solutions a second independent method for determining the response curve of allicin by HPLC was used.

Known amounts of alliin ((+) allyl cysteine sulfoxide) were added to crushed garlic tissue.

The alliin is converted to allicin by alliinase in the garlic in the molar ratio of 2.185 moles alliin to 1 mole of allicin. The HPLC peak area of allicin was determined with and without added alliin.

Allicin	305 µg/ml	12.38 x 105 µvolts
	152 µg/ml	6.44 x 105 µvolts.

Both methods gave the same calibration curve for allicin.

Appendix II Details of Australian method for allicin analysis

FAX

INSTITUTE FOR HORTICULTURAL DEVELOPMENT

Institute for Horticultural Development
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Facsimile 03-9800-3521

To: **Jane Lancaster**
NZ Institute for Crop and Food Research

Fax:	**64 3 325 2074**	From:	Sam Sterling (Scientist, Garlic Project)
Date:	September 1, 1998	Fax:	61-3-9800-3521
Pages:	1	Phone:	61-3-9210-9388

Dear Jane,

Apologies for not getting back to you sooner, I have been on leave etc. This morning I had a meeting with our commercial partner (a pharmaceutical company called Pharmaction Pty. Ltd.), at which I was able to discuss your request for sampling (assaying) method consensus. Being a commercial company, they are not at liberty to divulge all of their sampling protocol, (intellectual property etc.) but like me, they are interested in determining how closely our results compare with those of yours and other lab.s.

Hence they will be happy to assay your sample and I will let you know the result of that over the next week or so. I received your fax detailing the results achieved in your laboratory and it will be quite useful to compare to those recorded in our lab.

Again, sorry about the length of time it has taken but hopefully I will have some results of use to you in the next few days.

Regards,
Sam.
Sam.

NZ Institute for Crop & Food Research Limited
A Crown Research Institute

URGENT/NON-URGENT

To: Sam Sterling

Fax No: 0061398003521

Of: garlic project

FAXED
28 AUG 1998

Subject: Analysis

From: Jane Lancaster

Pages: 1

Date: 28 August 1998

Time: 1:36

Mana Kai Rangahau

Crop & Food Research
Private Bag 4704
CHRISTCHURCH
NEW ZEALAND
Ph 64 3 325 6400
Fax 64 3 325 2074

Dear Sam
Hope you received the garlic powder which I sent and also the allyl cysteine sulfoxide.
We also used it for standardisation and I thought I would send you the results which we got this week.
We added known amounts of alliin to extracts , calculated the expected amount of allicin using the molar ratio of 2.185 and determined the peak area in microvolts/sec.
We found that 0.305 mg/ml allicin gave 12.38x10 0000 peak area and 0.152mg/ml allicin gave 6.44x 10 0000 peak area.
From this we calculated that the powder was 3.4mg allicin/g dry wt.
Have you had a chance to look at this yet and how does that compare with your analysis?

Best wishes

Jane.

Jane Lancaster

12/8/98

Dear Sam,
Sorry I have taken a while to send you this material to standardise our allicin assays.
I enclose about 10 g of garlic powder and also 5 mg of (+) alliin. I also enclose a calibration curve for ethyl paraben and an HPLC trace of the garlic powder. We have synthesised alliicin as per the Lawson method. We used the published extinction coefficient (9.33 in water at 254nm) and a 1cm cell and a spectrophotometer to derive a concentration for the allicin. Using this concentration we generated a standard curve for the allicin on HPLC. The standard curve was then used to determine the concentration of the allicin in the samples.
The equation of the standard curve we used is

$$Y = 163X - 1.85$$

where Y = peak area x 10, 0000 micro volts/sec
and X = concentration of allicin in mg/ml.

Thus a peak area of 15.71039 x 10 0000 equals a concentration of 0.10766 mg/ml allicin.

However we have reservations about this answer because of the instability of the allicin and the difficulty of getting it pure enough .Indications are that our sample is not pure, but we cant see many impurities by HPLC.
I realise that you use paraben as an internal standard but do not know how you relate that to allicin. What is the relationship that you use?
Another way of checking the response factor of allicin in HPLC is to add a known amount of pure alliin to a sample and quantify the amount of extra allicin produced compared to the same sample without added alliin. Since we know the ratio of allicin from alliin (1:2.185) we can relate an experimentally derived peak area to a calculated allicin amount.
I have included the pure alliin from Extrasynthese company for you to use with your method.

The method we use for the dried garlic is via Lawson et al 1991

1. 1 g garlic to 30 mls water. Leave at room temperature(20C) for 10 minutes. Spin in a microfuge. 14000rpm, 5 minutes..
Take 600 microlitres of supernatant add to 600uls MeOH. Spin again as above.
Take 1ml for HPLC.

For the HPLC:
A Waters "Alliance" 2690 injector/solvent delivery/control system and a Waters 490 UV/visible detector were used. Instrument control and data collection and manipulation were performed using the Waters software package "Millennium" running on a personal computer. The column was a 220 x 4.6 mm Applied Biosystems "Brownlee" Aquapore RP-18 fitted with a 18 x 3.5 mm Applied Biosystems "Brownlee" Aquapore RP-18 guard column. The solvent used was aqueous methanol (50% v/v). Deaeration was achieved by vacuum filtration through a 0.22µm filter, rapid sparging with helium (100mL/min for 10 minutes) and constant slow bubbling of helium into capped, vented solvent reservoirs (10mL/min). Isocratic elution with a flow rate of 1 mL/min was used. Samples (10µL) were injected onto the column, which was maintained at 30°C using a Waters column heater. Data collection time of 15 minutes was

FAX

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To: **Jane Lancaster**

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Date: July 24, 1998

Pages: 1

From: Sam Sterling
(Scientist, Garlic Project)

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Phone: 64-3-9210-9388

Dear Jane,

My apologies for not replying to your earlier fax, it was still in my pile of "things to do" which may need to be re-named "things that should have been done by now". It is a busy time!

I too, am keen to standardise our methods, both as a check of the results that I have been achieving, and in order to allow comparisons to be made. My biggest problem at the moment is that I have either re-planted or disposed of all the bulb material that I allicin tested this season. (My current trials are concentrating on four or five varieties under a range of different conditions and treatments and this was the material that I assayed, we are no longer conducting the Australia-wide testing program that we undertook in the first year of the project).

Hence I can proceed in either of two ways: I can buy some garlic at the supermarket and have it tested through our system here and then send the rest over for testing under your system, or I can get you to send over your powder and test that for you. Or we can do both. I have found a fair amount of variation from different bulbs grown under the same conditions, so picking up bulbs from the supermarket which aren't even necessarily from the same paddock/state/country originally may cause problems for us. So perhaps the second option is the best?

If this is your preference, just send the sample to me (at the above address) and I will have it tested for you.

Again, apologies for not acting sooner- action will be much more immediate on receipt of your sample/instructions for how you would like to proceed!

Regards,
Sam.
Sam.

July 23 1998

Dear Sam,
I have not heard back from you and I wondered if I had asked too much of a busy person. Sorry if that is the case.
I would like to standardise my method with yours, though.
A simpler alternative for you would be if I sent over to you some garlic powder, of known alliicin by our method. I would provide the proportions of powder to water etc and the time for mixing before analysis (pretty much as in the Lawson et al method you sent to me).
What I would like would be an alliicin content by the method which you have access to. I would also pay the cost,of the analysis.

Please let me know if this is more acceptable, and a postal address for the sample etc.
Looking forward to hearing from you.

Jane

Jane Lancaster

FAX

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Date:	July 1, 1998	Fax:	61-3-9800-3521
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Dear Jane,

Most of the confusion is my own fault, due to the fact that I asked the Alliums Australia organising committee if I could re-submit my abstract and title to try and clear up any potential confusion. The result of this was that they got the title wrong, and indeed kept part of the old abstract at the end of the new one, making it quite confusing to read!

The actual title of my paper is "Agronomics and allicin yield of Australian garlic" and all figures in the paper are quoted as mg/g allicin frwt.

The findings are that 60% of the varieties we tested (we sampled almost 200 garlic samples from all over Australia) had allicin levels above 4.0 mg/g frwt. This value is significant in that it is the minimum requirement in a crop to make the process of allicin extraction (for pharmaceutical processing) economically viable. In fact, we are now working with 4.5 mg/g allicin frwt. as our preferred minimum level, just due to the large amount of variation we are finding within a crop. We recorded almost 50% (95 out of 200) samples above the 4.5 mg/g allicin (frwt) level.

The highest recorded level during that phase of the testing was 9 mg/g allicin by frwt. (Not alliin as you had noted in your fax). All figures that I presented at the conference, and those printed in the abstract (and indeed the paper when Acta finally comes out) are in mg/g allicin (frwt). As you noted in your last fax, these can be converted to alliin equivalents using Iberl's formula (i.e. multilying the figures by 2.185).

I hope this clears up the confusion. As you can see, I got quite tangled up in the reporting trying to conform to the systems of other authors to allow comparison between the studies, but in the end it was just easier to report in the terms of my actual data (which is mg/g allicin by frwt). There is no real uniformity between other studies that I could conform to! Although most studies use fresh weight rather than dry weight.

Two further publications are currently in the works (still a bit of work to do on those yet), based on our on-going nutrient and physiology studies, but the conference paper is the first publication in this area. We haven't had any feedback from the editors/referees of Acta as to whether any changes etc. are required yet, but once we have something final, I will be happy to send you a copy.

I am currently undertaking some nutrient and physiology studies which have some similarities to the work of yourself and Dr. Randle in onions (but I'm looking at garlic obviously). I wonder whether any of your current or future work (especially if you are looking into garlic) would tie in with any of our work here at IHD? I would be interested in forming ties with you and your group, should you think we could be beneficial to each other. I have so far found it very useful to be in contact with other Alliums researchers, especially as they are in rather short supply here in Australia!

If I can help you with anything else, please let me know.

Regards,

Sam Sterling

July 10 1998

Dear Sam,
Thanks for your fax and for clearing up the confusion about the terminology at the Alliums meeting.
I have been wondering if we could standardise our methods, or rather that I could check that I can measure the same amounts that you do. I was able to get a friend in Sydney to bring me back some Australian garlic or analysis. It was low in Alliicin compared to your figures. This may be because it was one of the low samples or it may be because my method is underestimating.
Would it be possible for you to send me some bulbs for which you have analysed the Alliicin recently or do you have a stock of dried powder of known alliicin as a standard?
In case this is possible I enclose a copy of an import permit. You will need to seal the garlic in 2 plastic bags alongside the permit. You will also need to say on the outside of the parcel that the permit is inside .If you tell me what flight it is on and the air way bill number I can arrange for MAF to collect it at this end. Use the address
J Lancaster
Crop and Food research
Gerald St
Lincoln
Christchurch.
I will pay any expenses upon invoice.
Yes we would be happy to keep in contact and form some ties. We have an Australian who works on garlic transformation coming to visit this month, Wendy Copen from Sydney (or Hawkesbury).
Thank you,

Jane

Jane Lancaster

60% ex 188 >.4% (4mg/g allicin)

FAX highest 0.9%

INSTITUTE FOR HORTICULTURAL DEVELOPMENT

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Vic. 3176

AGRICULTURE VICTORIA

Telephone +61-3-9210-9222
Facsimile +61-3-9800-3521

Alliin% = allicin% x 2.185

20' 30°C

To: Jane Lancaster

Fax:	**0015 64 3 325 2074**	From:	Sam Sterling (Scientist, Garlic Project)
Date:	January 19, 1998	Fax:	+61-3-9800-3521
Pages:	2	Phone:	+61-3-9210-9222

Dear Jane,

In response to your inquiry regarding our method of assaying for alliin/allicin, here are the basics as I understand them. I was unable to contact our chemist this morning directly with your questions, so should anything be unclear, please call me and I will double-check for you!

The method reference is: Iberl, B., Winkler, G., Miller B. and Knobloch, K. 1990. Quantitative determination of allicin and alliin from garlic by HPLC. *Planta Medica 56*: 320-326. The changes we've employed are to cool the autosampler (as per Lawson, L.D., Wood, S.G. and Hughes, B.G. 1991. HPLC analysis of allicin and other thiosulfinates in garlic clove homogenates. *Planta Medica* 57: 263-270) and to use an internal standard of ethyl p-hydroxybenzoate as allowed a shorter run time.

↳ Sigma H2128 100g 42913-90

Here is the relevant method section from my Alliums conference paper anyway-

2.1 Allicin Level Comparison

A total of 200 garlic samples were collected from producers in the Australian States of Victoria, Tasmania, Queensland, New South Wales and South Australia. Samples comprised 3-10 bulbs of an individual variety that were obtained at least 30 days post harvest (ie. fully cured), to avoid rots or disease problems during transport and storage. Once received, samples were stored at 25°C and 65% relative humidity, until tested for allicin content. Information on growing region, soil type and rainfall was provided by the relevant producer for each sample.

HPLC analyses were performed using a Hewlett Packard 1100, with ODS Hypersil column fitted with C18 pre-column. Samples for analysis were prepared from each bulb by removing and peeling 2-3 of the cloves. A sample of 5 +/- 1 g was weighed out and blended with 25 ml of the internal standard using a Barmix coffee grinder. The mixture was then incubated for 20 min at 30°C before being centrifuged for 5 min at 4000 rpm. Two ml of the supernatant solution was added to 8 ml of a 50/50 v/v mixture of Milli-Q water and HPLC grade methanol. The supernatant liquid was then filtered through a 0.45µm HPLC filter into amber HPLC vials for assaying. The autosampler was cooled as the allicin content of a methanol/water mixture has previously been found to be unstable at room temperature (6)*. Results were recorded as % alliin (by fresh weight), and then graphed against regional and climatic factors.

The internal standard used was ethyl p-hydroxybenzoate as this allowed a shorter run time thus reducing the effect of allicin instability, and prepared by adding approximately 500 mg of ethyl p-hydroxybenzoate to 20

19/01/98 Do you measure any allicin, or other thiosulfinates/ vinyl dithins.

Your incubation temp/time is different -

ml of methanol AR and shaking until dissolved. Nine hundred ml of 80°C Milli-Q water was then added and the standard mixture cooled to room temperature, before being diluted to a final volume of 1L with Milli-Q water.

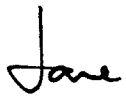
*Ref. number 6 is the one detailed above (Lawson, Wood and Hughes).

In regard to storage, with the method reported above it was difficult to control harvest-to-assay time as samples were supplied by producers (and via the mail in most cases). In my own trials currently being harvested, I will endeavour to cut down this difference in "age" of bulbs. Preliminary post-harvest trials have shown that under our storage regimes of 25 deg.C/65%RH or 0 deg C., there is some fluctuation in alliin levels through time, but that this fluctuation is about a mean (not increasing or decreasing). I will be conducting further storage trials this season.

I hope that I have supplied the information you were looking for. Please feel free to contact me again if you need anything further.

Regards,
Sam Sterling.
Sam Sterling.

Dear Sam,
Thanks for your reply, which was very helpful.
At the Alliums conference in Adelaide last year I went to your presentation. Did you present the values as Alliin? I know that the title indicated this, but in the abstract you also talked about alliicin. I am a little confused about which is used when.
I take it that the levels you found were 60% greater than 4.0mg/g/fr/wt alliin and a highest recorded value of 9.0 mg/g frwt alliin. You also graphed these values against regional and climatic factors.
Do you have any other publications about your garlic work?
Thanks for your time and help.
yours sincerely



Jane Lancaster

FAX

INSTITUTE FOR HORTICULTURAL DEVELOPMENT

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Vic. 3176

Telephone 03-9210-9222
Facsimile 03-9800-3521

To: **Jane Lancaster**
NZ Institute for Crop and Food Research

Fax: **64 3 325 2074**

From: Sam Sterling
(Scientist, Garlic Project)

Date: June 26, 1998

Fax: 03-9800-3521

Pages: 1

Phone: 03-9210-9388

Dear Jane,

In response to your questions:

1) The method followed was largely that of Iberl et al (1990), hence the incubation for 30 min rather than the 5 min. used by Lawson. This was purely a matter of preference by the chemist, who found that a more consistent data set was achieved using the longer incubation time.

2) The internal standard (ethyl p-hydroxybenzoate) allowed a shorter run time than the previously-used standard. This was developed as part of some research currently underway at the Monash Medical Centre in Clayton, Victoria. I don't think it has been published yet, but for more information you could contact the scientist- Dr. Wattanapenpiaboon (Tikki). She spoke at the Alliums Australia conference and is a great source of knowledge.

3) The results are recorded as % allicin, or mg/g allicin by the lab. and then sometimes converted to alliin, depending on which set of data you want to compare your findings to! There doesn't seem to be much uniformity in the reporting of these values around the world, so it's usually dependent on the audience as to whether I use alliin or allicin. The conversion factor used to convert to alliin is 1g alliin = 0.458g allicin (which is the same as alliin = allicin x 2.183).

We graphed alliin against regional and climatic factors as we were interested in determining whether there were any regions or climates that looked superior in terms of alliin production. Standard practice is usually just to report the figures as pretty much stand alone information (most of the work in the past has looked at the compounds in garlic per se and we were looking to tie in any potential agronomic factors/influences on these compounds.)

I hope this helps to answer your questions. Unfortunately, due to the cost of the equipment required, we must outsource our HPLC analyses, which means that we don't have the opportunity to tinker with the methods to optimise for our own purposes. If you have any suggestions/comments on any of these, I would be very interested in them.

Regards,
Sam
Sam Sterling.

NZ Institute for Crop & Food Research Limited
A Crown Research Institute

URGENT/NON-URGENT

To: Sam Sterling (Garlic Project)

Of: Institute Horticultural Development

Fax No: 0061 39800 3521 Date:

From: Jane Lancaster Pages: 2.

Subject: Allicin assays Time:

Crop & Food Research
Private Bag 4704
CHRISTCHURCH
NEW ZEALAND
Ph 64 3 325 6400
Fax 64 3 325 2074

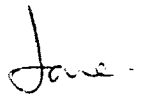
Dear Sam

Thanks for the information you sent me earlier this year about your methods for assaying alliicin in garlic. I have a few questions which I hope you can answer.

1. What was the reason for some of the deviations from the Lawson method eg incubating 20 min 30 C.
2. Why did you use ethyl p hydroxy benzoate as an internal standard. Does it have a similar decay curve to alliicin
3. Did you use the formula recommended by Iberl of
   Alliin% = allicin% x 2.185

In the letter you sent me you said that ' results were recorded as %alliin by fresh weight and then graphed against regional and climatic factors'. Is that standard practise for the work? Did you express your results at the Alliums meeting as alliin % or alliicin%?

Thanks for your help and hope all is well with your work.
Regards



Jane Lancaster

From: Sam Sterling
To: lancasterj@crop.cri.nz
Date: 21 January 1998 12:13am
Subject: HPLC method

Dear Jane,

In response to yesterday's phone conversation, I've been trying to fax you some details of our assay methods for alliin/allicin. Unfortunately the fax doesn't seem to want to go through, so I've looked up your email (and checked that I have the right fax number, and I do!) on the internet. I'll keep trying to send the fax (hoping for a miracle!), but here is the message anyway-

Our method follows Iberl, Winkler, Miller and Knobloch (1990) Planta Med. 56:320-326. The changes we've employed are to cool the autosampler (as per Lawson, Wood and Hughes 1991 Planta Med. 57:263-270) because the methanol/water mixture is unstable at room temp., and to use ethyl p-hydroxybenzoate as an internal standard, as this allows a shorter run time (thus reducing the effect of allicin instability).

In my fax, I have included the relevant section of methods from my Alliums conference paper. If the fax refuses to co-operate, then I'll email this to you also!

I hope I've provided the information you were looking for. If not, please feel free to call me again.

Regards,
Sam Sterling

1 mg/g fw
= 1 / 1000 × 100%

1. Incubate 20mi 30°C
2. Room temp 5'. Lawson
3. 3 ami room temp. Iberl