Maximizing the Health Benefits of Ontario Garlic on the Farm

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UNIVERSITY OF GUELPH
RIDGETOWN CAMPUS

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Garlic Growers Association of Ontario
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1.0 Executive Summary

Removal of the developing flower stalk (scape) of hard-neck garlic is a common production practice in order to improve bulb size and yield. Scape removal is often delayed in order to allow it grow longer as it can be sold for consumption. Since the presence of the scape has a large impact on garlic productivity, research was initiated to study the impact of delayed scape removal on garlic allicin levels. This was coupled with evaluating changes in allicin levels as garlic was stored in 2 different environments (20°C and 0°C).

Delaying scape removal had no effect on allicin levels of garlic at harvest, or 2 months after harvest. However, after long term (4 month) storage, garlic which had scape removal delayed tended to have lower levels of allicin.

Regardless of storage environment, garlic allicin levels increased an average of 60% after 2 months in storage. Cold (0°C) storage resulted in higher levels of allicin after 2 months when compared to room temperature (20°C) storage; this trend continued when garlic was evaluated after 4 months.

Future research should be directed at further characterizing the impact of storage conditions on garlic quality.

2.0 Detailed Description of Project

2.1 Objectives

The sole objective of this project was:

1. Document garlic allicin levels in response to various garlic production practices, including but not limited to timing of scape removal.

2.2 Project Activities and Output

2.2.1 Project Activities

Trials were established on the Ridgetown College research farm (Brookston clay loam) on 29 November, 2005. Seed cloves (Music strain) are hand planted into trenches 15 cm deep. No seed treatments were applied. Row widths and seed spacings in the row are 70 cm and 10 cm respectively. Weeds were controlled with a spring application of Dacthal (75 WP), followed by cultivation and hand hoeing. Nitrogen was applied twice in the spring at a rate of 35 kg/ha. The plots were not irrigated.

The effect of scape removal timing and storage environment on allicin content were investigated. There were 9 scape removal treatments: the first occurred when scapes first appeared on 09 June, and treatments continued at 3-5 day intervals until harvest on 18 July.
The garlic was hand harvested when 25-50% of the leaf area had died back, and the tops of the cloves began to separate. The tops were removed several inches above the bulbs (except for 1 treatment where the top was left on during curing), and the bulbs were placed in a shaded greenhouse for 2-3 weeks to cure. After curing, the bulbs were counted and weighed, and a 3 bulb sample taken from each scaping treatment for allicin and antioxidant analysis. The remaining bulbs from each scaping treatment were divided, with half being stored at 20°C and the other half at 0°C; both environments were at ambient humidity. Samples from 6 of the 9 scape removal x storage condition treatments were removed on 15 November and again on 15 February and forwarded for allicin and antioxidant analysis.

Analysis for allicin and antioxidants were completed in the lab of Dr. Rong Cao, Food Research Center, Agriculture and Agri-food Canada in Guelph ON. The following methodologies were provided by Dr. Cao:

1. Analysis of Allicin in Garlic
   Three cloves of each garlic sample were cut into slices. An aliquot (4g) was homogenized with a Polytron in 40 mL of distilled water, and extracted for 2 h at ambient temperature. The mixture was then centrifuged at 4000 rpm (3220g) at 22°C and filtered through a 0.45 μ nylon syringe filter. The filtrate was stored at -20 °C before analysis.
   An Agilent Technology 1100 Series HPLC system equipped with a quaternary pump, a degasser, a thermostatic auto-sampler and a diode array detector (DAD) was used for identification and quantification of allicin in the samples. The separation of allicin was carried out in a Phenomenex® Luna C18(2) column (150 X 4.6 mm i.d.; particle size, 5 μ) with a binary mobile phase consisting of methanol (solvent A) and water (solvent B) in a gradient conditions: 25% A at 0 min; 50% A at 10 min; 50% A at 15 min; 70% A at 18 min; 25% A at 20 min. Quantification of allicin was calculated at 18 nm based on the allicin standard curve. The detection limit of the method was <1 ppm.

2. Total Phenolic Contents (TPCs).
   The Folin-Ciocalteu method was modified and used to estimate the total phenolic contents of garlic. Briefly, each extract (0.2 mL) was mixed with 1 mL of the Folin-Ciocalteu reagent and 0.8 mL of 7.5% sodium carbonate solution. The mixture was allowed to stand at room temperature for 30 min and then the absorbance was measured at 765 nm in a Varian Cary 3C spectrophotometer (Varian, Palo Alto, CA, USA). A standard curve was generated with gallic acid ($r^2$=0.9960, concentration range is from 50 to 250 μg/mL), from which TPCs in the various fractions were calculated and expressed as milligrams of gallic acid equivalent (GAE) per 100 gram fresh nut. All samples were tested in duplicate.

   The FRAP assay is based on the reduction of ferric ion (Fe$^{3+}$) to the ferrous form (Fe$^{2+}$) at low pH, which has an intense blue colour (593 nm) when in complex with TPTZ (Fe$^{2+}$/TPTZ). The FRAP reagent was prepared freshly by mixing acetate buffer (300 mM, pH 3.6), 10 mM TPTZ in 40 mL HCl, and 20 mM FeCl$_3$ at 10:1:1 (v:v:v). L-ascorbic acid was prepared at 500 μM in methanol. Ten μL of standard or sample solution was pipetted into the wells of the microplate separately, and then mixed with 300
μL FRAP reagent per well. The plate was kept at 37 °C, and the absorbance was taken at 593 nm immediately after and at 4 min intervals by using a visible-UV microplate kinetics reader (EL 340, Bio-Tek Instruments, Inc., Winooski, VT). All samples were tested in triplicate. The final FRAP value of the samples was calculated on the basis of 500 μM ascorbic acid (equivalent to 1000 μM FRAP values).

The field experiment was established as a 6 x 2 factorial in a randomized complete block design with 4 replications. Factor 1 was timing of scape removal and factor 2 was storage environment. Field plots consisted of a single rows 6.0 m in length, all of which was harvested. All data were subject to analysis of variance using PRM (Ver 7.0, Gylling Data Management, Brookings, SD). No interactions were found between timing of scape removal and storage environment for any of the variables evaluated. Treatment means were separated (P<0.05) using a protected LSD. Means followed by the same letter within a column do not differ significantly.

2.2.2 Project Outputs – Results

2.2.2.1 Timing of Scape Removal

As scape removal was delayed, garlic yields were reduced due to decreases in bulb sizes (Figure 1). This relationship is well documented in garlic, although it varies in intensity from year to year. For the 2006 season, scapes needed to be removed within 14 days of their appearance in order to prevent significant reductions in bulb weight (Figure 1). While there were 9 scape removal timing treatments in the field, samples from only 6 were forwarded for allicin analysis due to cost constraints; these were treatments which had scapes removed 0, 11, 17, 28, and 39 days after they first appeared. One other treatment (39*) was included, which was garlic in which the scape remained on all season and the garlic was cured with the top (including the scape) attached to the bulb. This was done in response to reports in the popular press that garlic storage quality is enhanced if the bulbs are cured with the leaves attached.

At harvest there were no differences in allicin content across all scape removal dates and this trend continued after 2 months of storage (Figure 2). However, allicin levels in all treatments increased by 60% on average after 2 months of storage. (Figure 2). After 4 months of storage allicin levels dropped to near harvest levels again and significant differences in levels were noted; generally, as scape removal was delayed, allicin levels in garlic stored for 4 months was reduced. The only exception to this was the garlic which was cured with its top and scape attached; allicin levels in this garlic was similar to garlic which had scapes removed early in their development (Figure 2).
Figure 1: Garlic yield and bulb weight in response to scape removal timing

Figure 2: Allicin content in response to scape removal timing
2.2.2.2 Storage Environment

Storage environment had an influence on the allicin content of stored bulbs. While increases in allicin were noted after 2 months in both environments, levels were significantly higher when the garlic was stored at 0°C; similarly after 4 months levels of allicin decreased in both storage environments, but were well above initial levels in 0°C and below initial levels in 20°C.

Only water extract (supernatant) was tested for the total phenolic content and antioxidant activity using the FRAP method. Unfortunately, there were no significantly detectable phenolic compounds or antioxidant activity in the water extract of garlic. Extracts prepared by different methods and measurement using different assays should be used in future studies.

Table 3: Garlic allicin content in response to storage environment

<table>
<thead>
<tr>
<th>Harvest</th>
<th>Allicin (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0°C</td>
<td>3</td>
</tr>
<tr>
<td>20°C</td>
<td>2</td>
</tr>
</tbody>
</table>

![Graph showing garlic allicin content in response to storage environment]
3.0 Project Outcomes

3.1 Short Term

This project has provided the following short term outcomes:

a. data to suggest that lower storage temperatures are better at maintaining garlic quality.

b. data to suggest that allicin levels increase after a time of storage

c. timing of scape removal, while having an impact on yield does not appear to influence allicin levels at harvest; however it appears to have an effect on allicin levels after long term storage

3.2 Long Term

Since Ontario (and Canadian) garlic growers only hold a small share of the domestic garlic market, this project helps provide an understanding of the impact of production and storage practices on allicin levels in Ontario garlic. Simply storing garlic for a period of time under the proper conditions may enhance allicin levels and provide a marketing tool for Ontario growers

4.0 Jobs Created

No jobs were created as a direct result of this project, but if the agricultural industry, and garlic industry in particular can be strengthened and expanded, job would be created.

5.0 Reach and Communication

This project targeted the 35 commercial garlic growers in Ontario

6.0 Acknowledgement

The results of this project were presented at the Ontario Garlic Growers Association on 14 April, 2007. Approximately 12 members were in attendance

A research reports will be placed on researcher website http://www.ridgetownc.on.ca/research/research_zandstra.cfm

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