

40-7-8-.07 Onion Pungency Analysis.

The following standard procedures will be required to analyze and evaluate pungency levels in Vidalia Onions. These procedures must be used when there is the desire to publish such findings and utilize 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 utilized 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 (HCl) 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 to volume (1000 mL) with remaining distilled water, making sure the funnel is washed of any remaining HCl.

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., 0.0001grams). Use a Fisher brand 1 5/8 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 color.

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 weigh boat containing the sodium pyruvate with distilled water and pour into the flask. Then bring the flask to volume (100mL) 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

1.5 mL 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 1.5 mL plastic vials and frozen (-20 to -80 °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 1.5 mL 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 over estimate 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 flavor 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

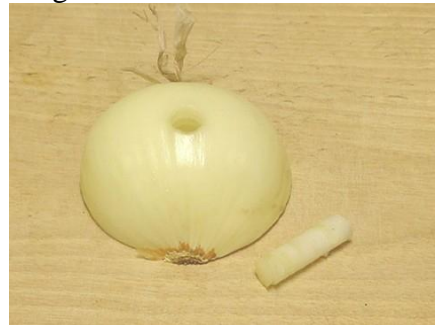


Method B: Obtain tissue cores from each bulb. Tissue cores must be taken just below the equator of the bulb. Whole bulbs or bulbs that have been halved can be used. A cork bore is positioned just below the equator of the bulb and is inserted through the tissue (Figure 3). The tissue core is then pushed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

The use of a spectrophotometer set at 420nm 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° C is required. The water bath must be turned on and allowed to warm for a sufficient time for the water to reach 37 °C (+/- 0.5°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 °C (+/- 0.5 °C) for exactly 10 minutes. This period must be timed with a count-down 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 & 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 °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 analyzed 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 color of the final solution, after the NaOH is added, should be rust colored. The intensity of the color will depend on the amount of pyruvate in the solution. More pyruvate will cause a darker color 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 color to develop. On occasion, bad 2,4 DNPH will cause a bright yellow color to develop.

The pH of the final solution should be close to 12 for the proper rust color 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 spread sheet, 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 flavor characteristics of any onions not tested and have no value in predicting the flavor characteristics of the field or shipment from which they were collected."

Section 2.0: Sample collection procedures

To better inform consumers of the flavor 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 utilized 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 insure 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 onion 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 utilized if pungency values are utilized in the promotion and/or marketing of Vidalia onions.”

Authority O.C.G.A. Secs. 2-14-130 et seq., 2-14-133, 26-2-1 et seq. **History.** Original Rule entitled “Right of Entry” adopted. F. May 30, 1996; eff. Apr. 20, 1996. **Amended:** Rule retitled “Handling Requirements”. F. Dec. 13, 1996; eff. Jan. 2, 1997. **Amended:** F. Sept. 4, 1998; eff. Sept. 24, 1998.

Amended: F. Mar. 4, 2004; eff. Mar. 24, 2004. **Repealed:** New Rule entitled “Onion Pungency Analysis” adopted. F. Aug. 6, 2004; eff. Aug. 26, 2004. **Repealed:** New Rule of same title adopted. F. Oct. 6, 2005; eff. Oct. 26, 2005. **Amended:** F. Aug. 11, 2006; eff. Aug. 31, 2006. **Repealed:** New Rule of same title adopted. F. June 11, 2007; eff. July 1, 2007. **Repealed:** New Rule of same title adopted. F. Sept. 20, 2007; eff. Oct. 10, 2007. **Repealed:** New Rule of same title adopted. F. Mar. 31, 2010; eff. Apr. 20, 2010.