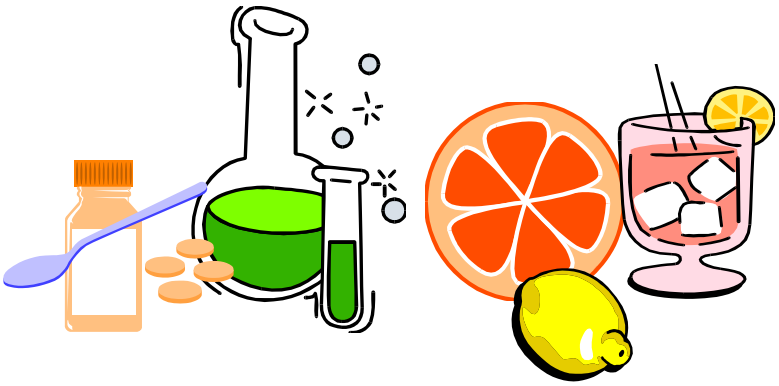
FMC FoodTech

Laboratory Manual

# PROCEDURES FOR ANALYSIS OF CITRUS PRODUCTS



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# Preface To the Fourth Edition

This fourth edition includes updates in the following chapters: Chapter IV-Juice Quality Analysis, Chapter V-Pulp Analysis and Chapter VI-Oil Analysis of Fruit and By Products. Chapter IV is updates to the Limonin and Hesperidn methods. Chapter V is an update to the Defects (FMC Method) section. Chapter VI is an update to the Whole Fruit Available Oil section.

We want to thank those who sent in their comments and suggestions to help improve the manual. The comments and suggestions have been integrated into this edition. We hope to have continuous supports from all of you in the future. Please direct your inputs to my attention.

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# Note From The Editor

These procedures are intended for our laboratory analysts as well as those in the citrus processing industry as part of our committed technical support and service to our customers. The analytical methods provide the basic quality analyses of citrus products including juice, pulp, and cold-pressed oils. While there are numerous books containing laboratory protocols for citrus products, for our customers we hope this document will minimize, if not eliminate, the trouble of searching and organizing the needed information.

This second edition has retained the essential methods of the original version and has included a few additional methods. The analytical methods described follow the principles of the original standard methods, e.g., AOAC and USDA, and the research papers cited in each method. Methods referenced to research papers are often adopted in our laboratory with modification. Users are encouraged to consult the original papers. The methods presented here are by no means complete. The selection of procedures is based on consideration of analysts who have limited access to modern instrumentation and have to prepare all reagents in-house as well as those with state of the art instrumentation and commercial pre-mixed reagents.

One goal of the revised edition is to present analytical methods in a clear and concise form. The procedures have been rewritten in a new format in an attempt to make it easier to follow the analytical instructions. There are some adaptations of terminology for better global communication. All values are presented in both the metric and the U.S. system of measures and weights when necessary.

The editor wants to thank Dr. Denny Nelson for his extensive review of the manuscript and Dr. Jose Flores, Dr. Salvador Garcia, Carol Ratcliff, Armando Morales, Melinda Watkins, Kevin Gaffney, Charles Blakely, Terry Curtis, and Linda Beasley for their support and comments.

Every effort was made to ensure that the information presented meets the stated intent. Despite our combined efforts, some errors or omissions may have occurred. Users of this book are encouraged to submit suggestions on how to improve the procedure and notification of any errors for future updating. Please direct your inputs to my attention. We would appreciate your help.

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# Abbreviation and Unit

| | | |
|---|---|---|
| ″ | = | inch(s) |
| AC | = | acid correction factor for °Brix |
| ATC | = | automatic temperature compensation |
| °Brix | = | degrees Brix, % |
| $°Brix_C$ | = | degrees Brix, %, corrected for acid |
| CA | = | citric acid |
| cP | = | centipoise ($10^{-3}$ Poise or 1 mPa·S) |
| °C | = | degrees Celsius (Centigrade) |
| °F | = | degrees Fahrenheit |
| EOA | = | Essential Oil Association |
| FCC | = | Food Chemical Codex |
| FDA | = | Food and Drug Administration |
| g | = | gram(s) |
| *g* | = | gravity, centrifuge force |
| GC | = | gas chromatography |
| GPL | = | gram(s) citric acid per liter |
| h | = | hour(s) |
| HPLC | = | high pressure (performance) liquid chromatography |
| kg | = | kilogram(s) |
| l | = | liter(s) |
| lb | = | pound(s) |
| LC | = | liquid chromatography |
| M | = | Molar |
| ml | = | milliliter(s) |
| min | = | minute(s) |
| mm | = | millimeter(s) |
| mPa·S | = | miliPascal per second |
| MT | = | metric ton(s) |
| MW | = | molecular weight |
| nm | = | nanometer ($10^{-9}$ m), formerly mμ |
| ppm | = | parts per million |
| rpm | = | rotations per minute |
| s | = | second(s) |
| SSL | = | soluble solids level |
| ST | = | short ton(s) |
| TC | = | temperature correction factor |
| μg | = | microgram(s) ($10^{-6}$ g) |
| USDA | = | United States Department of Agriculture |
| USP | = | United States Pharmacopoeia |
| UV | = | Ultraviolet |

# Guides to Table, Figure, and In-text Reference

**Table III – 2A**

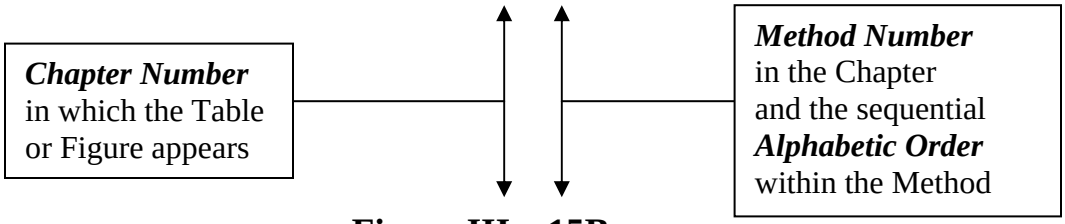
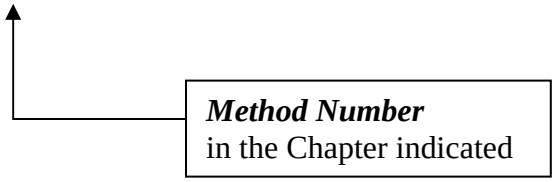



**Figure III – 15B**

**See Chapter III, 15**



# Chapter I. Sample Preparation and Handling

## 1. Whole Fruit

1. Collect fruit samples that are representative (i.e., including all loads, locations, sizes). Generally, exclude defective fruit that are decayed, rotten, and/or unwholesome (slight exterior decomposition, spongy, splits, punctured, or seed germinating).
2. For fruit used for testing involving different extractor setups, fruit must be sorted to the sizes for the particular extractor settings.
3. For fruit used for extractions of juice, pulp, and oil, the fruit must be randomized. A simple procedure for fruit randomization is to distribute fruit individually into each replicate sample of all treatments in a circulative manner.
4. Handle fruit sample with care and avoid physical and temperature abuses.
5. Make prompt analysis to avoid chemical and physical changes due to respiration, evaporation, fermentation, etc.

## 2. Fruit Juice

1. Always keep juice under refrigeration until analyzed. If analysis is delayed beyond a few hours, the sample should be frozen.
2. Thaw frozen single-strength and concentrate juice in sealed containers in a water bath (<25°C or 77°F). Make sure to avoid water getting into the sample. Concentrates of low °Brix contain more water and will require more time to thaw. Five gallon containers of 58°Brix concentrate take about 8 to 12 h to thaw at 21°C (70°F).
3. Analysis should take place right after thawing and warming.
4. Make sure all juice is at the proper strength (°Brix) before conducting analysis or record the °Brix.
5. For single-strength juices, no adjustment in strength is necessary. Concentrate needs be reconstituted to a proper strength (normally the minimum °Brix corrected for acids required for USDA grade or common industrial practice)(see Table III – 1 and discussion below).
6. Juice reconstituted from concentrate must be deaerated by vacuum to remove air bubbles incorporated during reconstitution before color evaluation and measurement of °Brix if hydrometer is used.
7. Make sure all juices are homogeneous by thoroughly shaking, stirring, and/or inverting before taking samples for analysis.

8. When reconstituting juice for flavor evaluation, use distilled water. Water with residual chlorine level of higher than 0.1 ppm can cause detrimental off-flavor and water with a alkalinity above 0 ppm as calcium carbonate may cause destruction of flavorful esters and acids.

# 3. Pulp and Other Solid Materials

1. Collect representative samples. For pulp samples, wait until operation conditions of the finishers or separators are stabilized and exclude the initial and final discharge.
2. Samples must be analyzed promptly to avoid enzymatic degradation and other deterioration.
3. Ensure sample homogeneity by mixing materials at all sampling steps.
4. Analyses such as percent oil require homogenization of the sample.

# 4. Oil Emulsions

1. Collect representative samples. Make sure the materials are thoroughly mixed before collecting.
2. Sampling should be conducted while the solutions are being stirred by hand or with a magnetic stirrer.
3. If oil-bearing samples are for Scott oil test and cannot be analyzed promptly, store the sample in glass bottles under refrigeration and sealed conditions after mixing with an equal weight of isopropanol.

# 5. Finished Oil

1. Collect representative samples. Make sure the materials are thoroughly mixed before collecting, especially from large containers such as drums.
2. When collecting samples, use amber glass bottles and fill the bottles to minimize air space and thus potential deterioration due to oxidation.
3. Samples should be stored under sealed conditions, away from light, and best at refrigeration conditions

# Chapter II. Fruit Character Analysis

## 1. Fruit Size and Shape

Collect a representative fruit sample of 20 fruit from the bulk sample.
Measure each fruit's longitudinal length (major diameter) and width (minor diameter, the average of the largest and smallest widths if fruit are not symmetrical).
Weigh total fruit weight and divide by 20 to get average fruit weight.
Fruit size is expressed either as average weight in gram per fruit and/or fruit number per 90-lb/40.8 kg box or per ton (metric, short, or long), based on the average weight per fruit.
Fruit shape is expressed by the ratio of width to length.

## 2. Peel Thickness

1. Use the same fruit sample from fruit size measurement.
2. Cut each fruit into halves along the equator.
3. Measure the peel thickness (distance from the outside edge to the inner edge of the white albedo tissue) at the thickest and thinnest positions and record the average.

## 3. Seed Number

1. Use the fruit sample used for fruit size and peel thickness measurements.
2. Slice each fruit along the equator into two halves with a knife.
3. Pick out and count the seeds from the segments using the tip of the knife. Separated count may be recorded for undeveloped seeds that are small enough to pass through the strainer tube's holes (diameters of 1.0 mm/0.04″ to 2.3 mm/0.062″).

# Chapter III. Juice Reconstitution

## 1. °Brix$_C$ Guidelines for Juice Reconstitution

1. The acid-corrected °Brix (°Brix$_C$) values of juice reconstituted from concentrate depend on requirements of the product and research and development formulations. Minimum requirements for some citrus juice are listed in Table III – 1.

2. It is a common practice to follow the USDA minimum standards for °Brix$_C$ when reconstituting juice for general laboratory analysis.

Table III – 1A. Minimum acid-corrected °Brix ($°Brix_C$) for USDA grades of orange, grapefruit, and tangerine juice products.

| Juice Type | Minimum $°Brix_C$ | |
|---|---|---|
| | Grade A (unsw/sw*) | Grade B (unsw/sw) |
| Orange Juice | | |
| Orange juice from concentrate | 11.8 | |
| Reconstituted frozen concentrated orange juice | 11.8 | |
| Reconstituted canned concentrated orange juice | 11.8 | |
| Reconstituted reduced acid orange juice | 11.8 | |
| Concentrated orange juice for manufacturing | 11.8 | |
| Dehydrated orange juice | 11.8 | |
| Pasteurized orange juice | 11.0 | 10.5 |
| Canned orange juice | 10.5/10.5 | 10/10.5 |
| Grapefruit Juice | | |
| Grapefruit juice | 9.0/11.5 | |
| Grapefruit juice from concentrate | 10.0/11.5 | |
| Reconstituted frozen concentrated grapefruit juice | 10.6 | |
| Reconstituted concentrated grapefruit juice for manufacturing | 10.5 | |
| Reconstituted dehydrated grapefruit juice | 10.0/11.5 | |
| Grapefruit and Orange Blend | | |
| single-strength | 10.0/11.5 | 9.5/11.5 |
| Reconstituted | 11.0/12.5 | |
| Tangerine Juice | | |
| Concentrated tangerine juice for manufacturing | 10.6 | |
| Canned tangerine juice | 10.5/12.5 | |

* unsw/sw stands for unsweetened and sweetened.

** For color determination, reconstitute juice to $°Brix_C$ on product label.

Table III – 1B. Acid-corrected (°$Brix_C$) or °Brix and acid level for lemon and lime juice products.

| USDA | °$Brix_C$ | | Acid (%, w/v) | |
|---|---|---|---|---|
| Lemon | | | | |
| Canned lemon juice | | | Grade A<br>5 – 7 | Grade C<br>4.5 – 7.5 |
| Frozen concentrate for lemonade | Grade A<br>≥ 10.5 | Grade B<br>≥ 10.5 | Grade A<br>0.7 ≤ | Grade B<br>0.7 ≤ |
| Lime | | | | |
| Frozen concentrate for limeade | Grade A<br>≥ 10.5 | Grade B<br>≥ 10.5 | Grade A<br>0.7 ≤ | Grade B<br>0.7 ≤ |
| FDA | °Brix | | Acid (%, w/w) | |
| Lemon | | | | |
| Lemon juice from concentrate<br>Or reconstituted lemon juice | ≥ 6 | | ≥ 4.5 | |

* For lemon juice, there is no grade B.

# 2. °Brix Reading of Reconstituted Juice

The °Brix reading on a refractometer for a juice to be reconstituted equals the value of the desired acid-corrected °Brix subtracted of the acid contribution and temperature effect.

1. The values of °Brix for most reconstitution are listed in Table III – 2A.
2. The values of °Brix can be calculated using the following procedure:

Calculate the total titratable acidity (% Acid) of the reconstituted juice based on the reconstituted juice's °Brix and Brix/Acid ratio (the later is the same as the concentrate's):

$$\%\ \text{Acid (w/w)} = \frac{\text{°Brix of Reconstituted Juice}}{\text{Brix/Acid Ratio of Reconsituted Juice or Concentrate}}$$

Calculate the reading of °Brix on refractometer:

- For refractometer without ATC

$$\text{°Brix} = \text{°Brix}_C \text{ of Reconstituted Juice} - \text{AC} - \text{TC}$$

- For refractometer with ATC

$$\text{°Brix} = \text{°Brix}_C \text{ of Reconstituted Juice} - \text{AC}$$

where the acid correction factor (AC) is either looked up from Table III – 2B or calculated as:

- For most citrus juices

$$AC = 0.012 + 0.193\ (\%\ Acid) - 0.0004\ (\%\ Acid)^2$$

- For frozen concentrate for lemonade

$$AC = (-0.027) + 0.125\ (\%Acid)$$

and the temperature correction factor (TC) is either looked up from Table III – 2C or calculated based on sample temperature (T) as:

$$TC = (°Brix)^2(+1.425\times10^{-4} - 8.605\times10^{-6}\ T + 7.138\text{-}8\ T^2)$$

$$+ (°Brix)(-2.009\times10^{-2} + 1.378\times10^{-3}\ T - 1.857\times10^{-5}\ T^2)$$

$$+ (-7.788\times10^{-1} + 1.700\times10^{-2}\ T + 1.100\times10^{-3}\ T^2)$$

The temperature of the juice to be reconstituted should be the same as that of concentrate and water kept at the same temperature.

Table III – 2A. The corresponding °Brix for acid-corrected °Brix (°$Brix_C$) at different percent acid levels (w/w) (both are temperature corrected).

| °$Brix_C$ | % Acid | °Brix | °$Brix_C$ | % Acid | °Brix | °$Brix_C$ | % Acid | °Brix |
|---|---|---|---|---|---|---|---|---|
| 9.0 | 0.50 | 8.89 | 10.5 | 0.50 | 10.39 | 11.8 | 0.50 | 11.69 |
| 9.0 | 0.60 | 8.87 | 10.5 | 0.60 | 10.37 | 11.8 | 0.60 | 11.67 |
| 9.0 | 0.70 | 8.85 | 10.5 | 0.70 | 10.35 | 11.8 | 0.70 | 11.65 |
| 9.0 | 0.80 | 8.83 | 10.5 | 0.80 | 10.33 | 11.8 | 0.80 | 11.63 |
| 9.0 | 0.90 | 8.81 | 10.5 | 0.90 | 10.31 | 11.8 | 0.90 | 11.61 |
| 9.0 | 1.00 | 8.80 | 10.5 | 1.00 | 10.30 | 11.8 | 1.00 | 11.60 |
| 9.0 | 1.10 | 8.78 | 10.5 | 1.10 | 10.28 | 11.8 | 1.10 | 11.58 |
| 9.0 | 1.20 | 8.76 | | | | 11.8 | 1.20 | 11.56 |
| 9.0 | 1.30 | 8.74 | 10.6 | 0.70 | 10.45 | 11.8 | 1.30 | 11.54 |
| 9.0 | 1.40 | 8.72 | 10.6 | 0.80 | 10.43 | 11.8 | 1.40 | 11.52 |
| 9.0 | 1.50 | 8.70 | 10.6 | 0.90 | 10.41 | | | |
| 9.0 | 1.60 | 8.68 | 10.6 | 1.00 | 10.40 | 12.5 | 0.70 | 12.35 |
| | | | 10.6 | 1.10 | 10.38 | 12.5 | 0.80 | 12.33 |
| 9.5 | 0.50 | 9.39 | 10.6 | 1.20 | 10.36 | 12.5 | 0.90 | 12.31 |
| 9.5 | 0.60 | 9.37 | 10.6 | 1.30 | 10.34 | 12.5 | 1.00 | 12.30 |
| 9.5 | 0.70 | 9.35 | 10.6 | 1.40 | 10.32 | 12.5 | 1.10 | 12.28 |
| 9.5 | 0.80 | 9.33 | 10.6 | 1.50 | 10.30 | 12.5 | 1.20 | 12.26 |
| 9.5 | 0.90 | 9.31 | | | | 12.5 | 1.30 | 12.24 |
| 9.5 | 1.00 | 9.30 | 11.0 | 0.50 | 10.89 | 12.5 | 1.40 | 12.22 |
| 9.5 | 1.10 | 9.28 | 11.0 | 0.60 | 10.87 | | | |
| | | | 11.0 | 0.70 | 10.85 | | | |
| 10.0 | 0.50 | 9.89 | 11.0 | 0.80 | 10.83 | | | |
| 10.0 | 0.60 | 9.87 | 11.0 | 0.90 | 10.81 | | | |
| 10.0 | 0.70 | 9.85 | 11.0 | 1.00 | 10.80 | | | |
| 10.0 | 0.80 | 9.83 | 11.0 | 1.10 | 10.78 | | | |
| 10.0 | 0.90 | 9.81 | 11.0 | 1.20 | 10.76 | | | |
| 10.0 | 1.00 | 9.80 | 11.0 | 1.30 | 10.74 | | | |

* Acid correction factors for various percent Acid are calculated using equation for most citrus juices.

Table III – 2B. Acid corrections (AC) to be added to temperature-compensated °Brix readings from refractometer.

| % Acid | AC | % Acid | AC | % Acid | AC | % Acid | AC |
|---|---|---|---|---|---|---|---|
| 0.0 | 0.00 | 6.0 | 1.15 | 12.0 | 2.27 | 18.0 | 3.35 |
| 0.2 | 0.04 | 6.2 | 1.19 | 12.2 | 2.31 | 18.2 | 3.38 |
| 0.4 | 0.08 | 6.4 | 1.23 | 12.4 | 2.36 | 18.4 | 3.42 |
| 0.6 | 0.12 | 6.6 | 1.27 | 12.6 | 2.39 | 18.6 | 3.46 |
| 0.8 | 0.16 | 6.8 | 1.30 | 12.8 | 2.42 | 18.8 | 3.49 |
| 1.0 | 0.20 | 7.0 | 1.34 | 13.0 | 2.46 | 19.0 | 3.52 |
| 1.2 | 0.24 | 7.2 | 1.38 | 13.2 | 2.50 | 19.2 | 3.56 |
| 1.4 | 0.28 | 7.4 | 1.42 | 13.4 | 2.54 | 19.4 | 3.59 |
| 1.6 | 0.32 | 7.6 | 1.46 | 13.6 | 2.57 | 19.6 | 3.63 |
| 1.8 | 0.36 | 7.8 | 1.50 | 13.8 | 2.61 | 19.8 | 3.68 |
| 2.0 | 0.39 | 8.0 | 1.54 | 14.0 | 2.64 | 20.0 | 3.70 |
| 2.2 | 0.43 | 8.2 | 1.58 | 14.2 | 2.69 | 20.2 | 3.73 |
| 2.4 | 0.47 | 8.4 | 1.62 | 14.4 | 2.72 | 20.4 | 3.77 |
| 2.6 | 0.51 | 8.6 | 1.66 | 14.6 | 2.75 | 20.6 | 3.80 |
| 2.8 | 0.54 | 8.8 | 1.69 | 14.8 | 2.78 | 20.8 | 3.84 |
| 3.0 | 0.58 | 9.0 | 1.72 | 15.0 | 2.81 | 21.0 | 3.88 |
| 3.2 | 0.62 | 9.2 | 1.76 | 15.2 | 2.85 | 21.2 | 3.91 |
| 3.4 | 0.66 | 9.4 | 1.80 | 15.4 | 2.89 | 21.4 | 3.95 |
| 3.6 | 0.70 | 9.6 | 1.83 | 15.6 | 2.93 | 21.6 | 3.99 |
| 3.8 | 0.72 | 9.8 | 1.87 | 15.8 | 2.97 | 21.8 | 4.02 |
| 4.0 | 0.78 | 10.0 | 1.91 | 16.0 | 3.00 | 22.0 | 4.05 |
| 4.2 | 0.81 | 10.2 | 1.95 | 16.2 | 3.03 | 22.2 | 4.09 |
| 4.4 | 0.85 | 10.4 | 1.99 | 16.4 | 3.06 | 22.4 | 4.13 |
| 4.6 | 0.89 | 10.6 | 2.03 | 16.6 | 3.09 | 22.6 | 4.17 |
| 4.8 | 0.93 | 10.8 | 20.6 | 16.8 | 3.13 | 22.8 | 4.20 |
| 5.0 | 0.97 | 11.0 | 2.10 | 17.0 | 3.17 | 23.0 | 4.24 |
| 5.2 | 1.01 | 11.2 | 2.14 | 17.2 | 3.21 | 23.2 | 4.27 |
| 5.4 | 1.04 | 11.4 | 2.18 | 17.4 | 3.24 | 23.4 | 4.30 |
| 5.6 | 1.07 | 11.6 | 2.21 | 17.6 | 3.27 | 23.6 | 4.34 |
| 5.8 | 1.11 | 11.8 | 2.24 | 17.8 | 3.31 | 23.8 | 4.38 |

* Based on citric acid content of citrus juices or other acid-containing sugar solutions.

** For % Acid values between the list numbers, use the average of the nearest lower and higher correction values.

Table III – 2C. Temperature corrections for °Brix readings of percent sucrose in sugar solutions by either Abbe or immersion refractometer at temperature other than 20°C (68°F).

| Temp. | | Percent Sucrose | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| °C | °F | 0 | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 60 | 65 |
| Subtract from Percent Sucrose | | | | | | | | | | | | | | | |
| 10 | 50.0 | .50 | .54 | .58 | .61 | .64 | .66 | .68 | .70 | .72 | .73 | .74 | .75 | .76 | .78 |
| 11 | 51.8 | .46 | .49 | .53 | .55 | .58 | .60 | .62 | .64 | .65 | .66 | .67 | .68 | .69 | .70 |
| 12 | 53.6 | .42 | .45 | .48 | .50 | .52 | .54 | .56 | .57 | .58 | .59 | .60 | .61 | .61 | .63 |
| 13 | 55.4 | .37 | .40 | .42 | .44 | .46 | .48 | .49 | .50 | .51 | .52 | .53 | .54 | .54 | .55 |
| 14 | 57.2 | .33 | .35 | .37 | .39 | .40 | .41 | .42 | .43 | .44 | .45 | .45 | .46 | .46 | .47 |
| 15 | 59.0 | .27 | .29 | .31 | .33 | .34 | .34 | .35 | .36 | .37 | .37 | .38 | .39 | .39 | .40 |
| 16 | 60.8 | .22 | .24 | .25 | .27 | .27 | .28 | .28 | .29 | .30 | .30 | .3 | .31 | .31 | .32 |
| 17 | 62.6 | .17 | .18 | .19 | .20 | .21 | .21 | .21 | .22 | .22 | .23 | .23 | .23 | .23 | .24 |
| 18 | 64.4 | .12 | .13 | .13 | .14 | .14 | .14 | .14 | .15 | .15 | .15 | .15 | .16 | .16 | .16 |
| 19 | 66.2 | .06 | .06 | .06 | .10 | .07 | .07 | .07 | .08 | .08 | .08 | .08 | .08 | .08 | .08 |
| Add to Percent Sucrose | | | | | | | | | | | | | | | |
| 21 | 69.8 | .06 | .07 | .07 | .07 | .07 | .08 | .08 | .08 | .08 | .08 | .08 | .08 | .08 | .08 |
| 22 | 71.6 | .13 | .13 | .14 | .14 | .15 | .15 | .15 | .15 | .15 | .16 | .16 | .16 | .16 | .16 |
| 23 | 73.4 | .19 | .20 | .21 | .22 | .22 | .23 | .23 | .23 | .23 | .24 | .24 | .24 | .24 | .24 |
| 24 | 75.2 | .26 | .27 | .28 | .29 | .30 | .30 | .31 | .31 | .31 | .31 | .31 | .32 | .32 | .32 |
| 25 | 77.0 | .33 | .35 | .35 | .37 | .38 | .38 | .39 | .40 | .40 | .40 | .40 | .40 | .40 | .40 |
| 26 | 78.8 | .40 | .42 | .43 | .44 | .45 | .46 | .48 | .48 | .48 | .48 | .48 | .48 | .48 | .48 |
| 27 | 80.6 | .48 | .50 | .52 | .53 | .54 | .55 | .55 | .56 | .56 | .56 | .56 | .56 | .56 | .56 |
| 28 | 82.4 | .56 | .57 | .60 | .61 | .62 | .63 | .63 | .64 | .64 | .64 | .64 | .64 | .64 | .64 |
| 29 | 84.2 | .64 | .66 | .68 | .69 | .71 | .72 | .72 | .73 | .73 | .73 | .73 | .73 | .73 | .73 |
| 30 | 86.0 | .72 | .74 | .77 | .78 | .79 | .80 | .80 | .81 | .81 | .81 | .81 | .81 | .81 | .81 |

Source: Official Methods of Analysis. 1970. 11th Edition, Association of Official Analytical Chemists, Washington DC, 47.015.

# 3. Reconstitute a Predetermined Volume of Juice

1. Decide the volume of juice to be reconstituted.
2. Look up the soluble solids level (SSL) in both concentrate and reconstituted juice at the specified °Brix values from Table III – 3 or calculate from the following formula:

$$\text{Soluble Solid Level} = \frac{°\text{Brix}_C}{100} \times \text{Density}$$

$$= \frac{°\text{Brix}_C}{100} \times 0.524484\, e^{\frac{(°\text{Brix}_C + 330.872)^2}{170435}} \text{ (g/ml)}$$

$$= \frac{°\text{Brix}_C}{100} \times 4.37691\, e^{\frac{(°\text{Brix}_C + 330.872)^2}{170435}} \text{ (lb/gal)}$$

3. Calculate the quantities of concentrate (either in volume or weight) and distilled water needed for the required reconstituted juice volume:

$$\text{Volume of Concentrate} = \frac{(\text{Volume of Reconstituted})(\text{SSL of Reconstituted})}{(\text{SSL of Concentrate})}$$

$$\text{Weight of Concentrate} = (\text{Volume of Concentrate})(\text{Density of Concentrate})$$

$$\text{Volume of Water} = (\text{Volume of Reconstituted}) - (\text{Volume of Concentrate})$$

4. Determine the °Brix on refractometer (see Chapter III, 2).
5. Measure the desired quantity of concentrate, either in volume or weight.

6. Add the majority of the water.
7. Thoroughly mix the solution and monitor its Brix reading using refractometer when adding the last small portion of water. The calculation, thought based on sucrose solutions at 20°C (68°F) (Table III – 3), is generally accurate enough for industrial purpose.
8. Example:

   To reconstitute 1000 ml orange juice of 11.8°$Brix_C$ from a concentrate of 41.8°$Brix_C$ and 14.5 Brix/Acid ratio at 24°C.

   Since the soluble solids levels are 0.49521 g/ml for 41.8°$Brix_C$ concentrate (density of 1.18471 g/ml) and 0.12326 g/ml for 11.8°$Brix_C$ juice, as in Table III – 3. The quantities of concentrate and water needed for reconstitution are:

$$\text{Volume of Concentrate} = \frac{(\text{Volume of Reconstituted Juice})(\text{SSL of Reconstituted Juice})}{(\text{SSL of Concentrate})}$$

$$= \frac{(1000\ \text{ml})(0.12326\ \text{g/ml})}{(0.49521\ \text{g/ml})}$$

$$= 249\ \text{ml}$$

or

Weight of Concentrate = (Volume of Concentrate)(Density of Concentrate)

= (249 ml)(1.18471 g/ml)

= 295 g

and

Volume of Water = (Volume of Reconstituted) – (Volume of Concentrate)

= (1000 ml) – (249 ml)

= 751 ml

The volume ratio of water to concentrate in this example is 3.016 (= 751 ml ÷ 249 ml).

The °Brix reading of the reconstituted juice can be calculated as shown below (see also Table III – 2A):

Since

$$\%\,\text{Acid (w/w)} = \frac{°\text{Brix}_C \text{ of Reconstituted Juice}}{\text{Brix/Acid Ratio of Reconsituted Juice}}$$

$$= \frac{11.8}{14.5}$$

$$= 0.81$$

and the acid correction is 0.16 for 0.81% of total titratable acidity from Table III – 2B and temperature correction is +0.28 for juice at 24°C from Table III – 2C.

Therefore, for juice of 11.8°$\text{Brix}_C$,

°Brix reading of refractometer with ATC

= °$\text{Brix}_C$ of Reconstitute Juice – Acid Correction

= 11.8 – 0.16

= 11.64

°Brix reading of refractometer without ATC

= °$\text{Brix}_C$ of Reconstitute Juice – Acid Correction – Temperature Correction

= 11.8 – 0.16 – (+0.28)

= 11.36

Table III – 3. Relationship of °Brix, density in air, specific gravity in air, and solids weight of sucrose solutions at 20°C (68°F).

| °Brix$_{Sucrose}$ (%, w/w) | Apparent Density (solution weight per unit solution volume) | | Apparent Specific Gravity (g/ml) | Soluble Solids Level (solids weight per unit solution volume) | |
|---|---|---|---|---|---|
| | (g/ml) | (lb/gal) | | (g/ml) | (lb/gal) |
| 9.0 | 1.03297 | 8.621 | 1.03590 | 0.09297 | 0.776 |
| 9.5 | 1.03503 | 8.638 | 1.03796 | 0.09833 | 0.821 |
| 10.0 | 1.03709 | 8.655 | 1.04003 | 0.10371 | 0.865 |
| 10.5 | 1.03916 | 8.672 | 1.04210 | 0.10911 | 0.911 |
| 10.6 | 1.03957 | 8.676 | 1.04252 | 0.11019 | 0.920 |
| 11.0 | 1.04124 | 8.690 | 1.04419 | 0.11454 | 0.956 |
| 11.5 | 1.04332 | 8.707 | 1.04628 | 0.11998 | 1.001 |
| 11.6 | 1.04374 | 8.710 | 1.04670 | 0.12107 | 1.010 |
| 11.7 | 1.04416 | 8.714 | 1.04712 | 0.12217 | 1.020 |
| 11.8 | 1.04457 | 8.717 | 1.04754 | 0.12326 | 1.029 |
| 11.9 | 1.04499 | 8.721 | 1.04795 | 0.12435 | 1.038 |
| 12.0 | 1.04541 | 8.724 | 1.04837 | 0.12545 | 1.047 |
| 12.5 | 1.04751 | 8.742 | 1.05048 | 0.13094 | 1.093 |
| 41.8 | 1.18471 | 9.887 | 1.18806 | 0.49521 | 4.133 |
| 42.0 | 1.18574 | 9.896 | 1.18911 | 0.49801 | 4.156 |
| 43.0 | 1.19149 | 9.939 | 1.19434 | 0.51211 | 4.274 |
| 43.4 | 1.19306 | 9.957 | 1.19644 | 0.51779 | 4.321 |
| 44.0 | 1.19622 | 9.983 | 1.19961 | 0.52634 | 4.392 |
| 45.0 | 1.20151 | 10.027 | 1.20492 | 0.54068 | 4.512 |
| 50.0 | 1.22854 | 10.253 | 1.23202 | 0.61427 | 5.126 |
| 55.0 | 1.25651 | 10.486 | 1.26007 | 0.69108 | 5.767 |
| 58.0 | 1.27375 | 10.630 | 1.27736 | 0.73878 | 6.165 |
| 60.0 | 1.28544 | 10.727 | 1.28908 | 0.77126 | 6.436 |
| 65.0 | 1.31532 | 10.977 | 1.31905 | 0.85496 | 7.135 |
| 66.0 | 1.32141 | 11.028 | 1.32516 | 0.87213 | 7.278 |
| 66.5 | 1.32447 | 11.053 | 1.32823 | 0.88077 | 7.350 |

* For values for additional °Brix levels, consult the 'Tables of Brix, apparent specific gravity, apparent density, weight, and pounds solids of sucrose solutions' by C.S. Chen, 1983, Proc. Fla. State Hort. Soc., 96: 313–315.

# 4. Reconstitute Juice from Concentrate of a Known Volume

1. Calculate, for a given volume of concentrate, the required volume of distilled water needed:

$$\text{Volume of Water} = (\text{Volume of Concentrate}) \times \frac{(\text{SSL of Concentrate}) - (\text{SSL of Reconstituted})}{(\text{SSL of Reconstituted})}$$

2. Determine the Brix reading on refractometer (see Chapter III, 2).
3. Measure the required quantity of water.
4. Add the majority of water into the concentrate.
5. Thoroughly mix and monitor its °Brix reading with refractometer when adding the last small portion of water. The calculation, thought based on sucrose solution at for 20°C (68°F), is generally accurate enough for industrial purpose.
6. Example:

   To reconstitute juice of 11.8°$Brix_C$ from 1 gallon of concentrate at 41.8°$Brix_C$ and 14.5 Brix/Acid ratio at 24°C.

   a). Calculate the reading of refractometer as shown in the previous example.
   b). Since the soluble solids levels are 4.133 lb/gal for 41.8°$Brix_C$ and 1.029 lb/gal for 11.8°$Brix_C$ juice, as in Table III – 3. The volume of water need for reconstitution should be:

$$\text{Volume of Water (gal)} = (\text{Volume of Concentrate}) \times \frac{(\text{SSL of Concentrate}) - (\text{SSL of Reconstituted})}{(\text{SSL of Reconstituted})}$$

$$= (1\ \text{gal}) \times \frac{(4.1333\ \text{lb/gal}) - (1.029\ \text{lb/gal})}{(1.029\ \text{lb/gal})}$$

$$= 3.016\ \text{(gal)}$$

The volume ratio of water to concentrate in this example is 3.016 (= 3.016 gal ÷ 1 gal).

# Chapter IV. Juice Quality Analysis

## 1. Total Soluble Solids by Refractometer

I. Apparatus

Refractometer with degrees Brix scale and ATC

II. Chemicals

None

III. Reagents

None

IV. Procedure

1. Bring single-strength or reconstituted juice samples to ambient temperature and mix thoroughly.
2. Measure sample temperature if refractometer has no automatic temperature compensation.
3. Clean the prisms of the refractometer before each reading with distilled water and soft tissue or nonabrasive materials.
4. Apply an aliquot of sample (~3 drops) to the refractometer prism, avoiding bubbles and large pulp particles.
5. If sample temperature differs from the refractometer's, allow time for adjustment.
6. Cover the sample with the fogged glass and position the light beam to shine through the fogged glass.
7. Adjust the shadow to the cross hairs.
8. Read the °Brix.

V. Calculations

Total soluble solids of citrus juice is expressed in degrees Brix, in equivalent of sucrose solution at 20°C (68°F), after acid correction (Table III – 2B) and temperature correction (Table III – 2C). Acid correction and temperature correction can also be calculated from the %Acid and temperature of juice (see Chapter III, 2).

For refractometer with ATC:

$$°Brix_C = \text{Refractometer } °Brix + \text{Acid Correction}$$

For refractometer without ATC:

$$°Brix_C = \text{Refractometer } °Brix + \text{Acid Correction} + \text{Temperature Correction}$$

The weight of the soluble solids in juice is calculated using the following formula:

$$\text{Soluble Solids (kg)} = \text{Juice Weight (kg)} \times \frac{°Brix_C}{100}$$

or

$$\text{Soluble Solids (lb)} = \text{Juice Weight (lb)} \times \frac{°Brix_C}{100}$$

## VI. Reference

Official Methods of Analysis. 1999. 16th Edition, 5th Reversion, AOAC International, Gaithersburg, MD, method 983.17.

Citrus Handbook. 1998. Agricultural Marketing Service, USDA. Washington, D.C.

Kimball, D.A. 1999. Citrus processing – A complete guide. 2nd Edition. Aspen Publishers, Inc., MD.

# 2. Total Titratable Acidity (Industry Method)

I. Apparatus

25 or 50 ml Buret with 0.1 ml graduation and Teflon® stopcock
Magnetic stirrer and Teflon® coated stirring bar
250 ml glass flask or beaker

II. Chemicals

Isopropanol ($C_3H_8O$)
Phenolphthalein ($C_{20}H_{14}O_4$)
Sodium hydroxide (NaOH)
Potassium biphthalate ($KHC_8H_4O_4$)

III. Reagents

A. Sodium hydroxide solution (0.3125 N): Dissolve 125.0 g of NaOH in 10 liters of $CO_2$-free water (boil water for 20 min and cool with soda-lime protection or bubble water with nitrogen gas for 12 h).

To standardize the solution, accurately weigh enough dried (2 h at 120°C) $KHC_8H_4O_4$ to titrate about 40 ml NaOH solution into a 300-ml flask that has been swept free of $CO_2$ and contains 50 ml of $CO_2$-free water. Once dissolved, titrate with the NaOH solution to pH 8.6, taking precautions to exclude $CO_2$.

$$\text{Normality of NaOH solution} = \frac{(\text{g } KHC_8H_4O_4) \times 1000}{(\text{ml NaOH})} \times 204.229$$

B. Dye solution (1%): Dissolve 1 g of phenolphthalein in 100 ml 50% isopropanol and then add just enough NaOH to neutralize the solution to a faint pink color.

IV. Procedure

1. Thoroughly mix the juice or concentrate sample.
2. Measure analysis sample into 250-ml glass flasks or beakers (see table on next page).
3. Add 100 ml of distilled water and mix.
4. Add 5 to 10 drops of phenolphthalein solution and mix thoroughly.
5. Titrate with NaOH solution until solution shows a faint discernible pink color that persists for ~25 seconds (end point pH 8.2).

| Sample Type | Analysis Sample Size (g or ml) |
|---|---|
| Blank | – |
| Orange or grapefruit single-strength juice | 25 |
| Orange or grapefruit concentrate | 10 |
| Lemon or lime single-strength juice | 5 |
| Lemon or lime concentrate | 2 |

6. If analysis samples are measured in volume instead of weight, the sample specific weight must be determined or estimated. Specific gravity for juice of known °Brix can be obtained from Table III – 3, values in which are based on sucrose solutions, or can be determined with deaerated juice with a pycnometer (see Chapter VII, 3).

V. Calculations

1. The total titratable acidity is expressed as anhydrous citric acid on a weight basis. Due to its three carboxyl groups, one mole of citric acids (MW 192.12) can react with three moles of $OH^-$, therefore 1 mole of NaOH equals 64.04 g citric acid (= 192.12 ÷ 3) and the milliequivalent of citric acid is 0.064:

$$\%\,\text{Acid (w/w)} = \frac{\left(\frac{\text{Net ml Titrant}}{1000\ \text{ml/l}}\right)(\text{N Titrant})\left(\frac{64.04\ \text{g Citric Acid}}{1\ \text{mole OH}^-}\right)}{(\text{Sample Weight})} \times 100$$

$$= \frac{(\text{Net ml Titrant})(\text{N Titrant})(0.064)}{(\text{Sample Weight})} \times 100$$

$$= \frac{(\text{Net ml Titrant})(\text{N Titrant})}{(\text{g Sample})} \times 6.4$$

or

$$= \frac{(\text{Net ml Titrant})(\text{N Titrant})}{(\text{ml Sample})(\text{Sample Specific Gravity, g/ml})} \times 6.4$$

Where (Net ml Titrant) = (ml Titrant for Sample) – (ml Titrant for Blank)

2. The %Acid for accurately weighed sample titrated with 0.3125 N NaOH as titrant is calculated as:

- 25 g of orange juice

$$\%\ \text{Acid (w/w)} = \frac{(\text{Net ml NaOH})(\text{N NaOH})}{(\text{Sample Weight})} \times 6.4$$

$$= \frac{(\text{Net ml NaOH})(0.3125\ \text{N})}{(25\ \text{g})} \times 6.4$$

$$= (\text{Net ml NaOH}) \times 0.084$$

- 5 g of lemon or lime single-strength juices

$$\%\ \text{Acid (w/w)} = (\text{Net ml NaOH}) \times 0.4$$

- 5 g of orange juice concentrate

$$\%\ \text{Acid (w/w)} = (\text{Net ml NaOH}) \times 0.4$$

- 2 ml of lemon or lime concentrates

$$\%\ \text{Acid (w/v)} = (\text{Net ml NaOH})$$

3. The parameter of concentration for lemon and lime concentrates is the weight of acid as anhydrous citric acid per unit volume, or gram citric acid per liter (GPL). GPL is calculated from the concentrate's % Acid:

$$GPL = (\frac{\%\ \text{Acid, w/v}}{100})(1000\ \text{ml})$$

$$= (\%\ \text{Acid, w/v}) \times 10$$

or

$$GPL = (\frac{\%\ \text{Acid, w/w}}{100})(\text{Specific Gravity, g/ml})(1000\ \text{ml})$$

$$= (\%\ \text{Acid, w/w})(\text{Specific Gravity, g/ml}) \times 10$$

VI. Reference

Citrus Handbook. 1998. Agricultural Marketing Service, USDA. Washington, D.C.

Official Methods of Analysis. 1999. 16th Edition, 5th Reversion, AOAC International, Gaithersburg, MD, method 936.16.

Table IV – 2. Equivalents of total titratable acid (% Acid) per volume of 0.3125 N NaOH as titrant on orange juice sample of 25 ml.

| ml of NaOH | % Acid | ml of NaOH | % Acid | ml of NaOH | % Acid | ml of NaOH | % Acid | ml of NaOH | % Acid |
|---|---|---|---|---|---|---|---|---|---|
| 1.0 | 0.064 | 7.6 | 0.592 | 11.7 | 0.920 | 15.8 | 1.248 | 19.9 | 1.576 |
| 2.0 | 0.144 | 7.7 | 0.600 | 11.8 | 0.928 | 15.9 | 1.256 | 20.0 | 1.584 |
| 3.0 | 0.224 | 7.8 | 0.608 | 11.9 | 0.936 | 16.0 | 1.264 | 20.1 | 1.592 |
| 3.5 | 0.264 | 7.9 | 0.616 | 12.0 | 0.944 | 16.1 | 1.272 | 20.2 | 1.600 |
| 3.7 | 0.280 | 8.0 | 0.624 | 12.1 | 0.952 | 16.2 | 1.280 | 20.3 | 1.608 |
| 4.0 | 0.304 | 8.1 | 0.632 | 12.2 | 0.960 | 16.3 | 1.288 | 20.4 | 1.616 |
| 4.1 | 0.312 | 8.2 | 0.640 | 12.3 | 0.968 | 16.4 | 1.296 | 20.5 | 1.624 |
| 4.2 | 0.320 | 8.3 | 0.648 | 12.4 | 0.976 | 16.5 | 1.304 | 20.6 | 1.632 |
| 4.3 | 0.328 | 8.4 | 0.656 | 12.5 | 0.984 | 16.6 | 1.312 | 20.7 | 1.640 |
| 4.4 | 0.336 | 8.5 | 0.664 | 12.6 | 0.992 | 16.7 | 1.320 | 20.8 | 1.648 |
| 4.5 | 0.344 | 8.6 | 0.672 | 12.7 | 1.000 | 16.8 | 1.328 | 20.9 | 1.656 |
| 4.6 | 0.352 | 8.7 | 0.680 | 12.8 | 1.008 | 16.9 | 1.336 | 21.0 | 1.664 |
| 4.7 | 0.360 | 8.8 | 0.688 | 12.9 | 1.016 | 17.0 | 1.344 | 21.1 | 1.672 |
| 4.8 | 0.368 | 8.9 | 0.696 | 13.0 | 1.024 | 17.1 | 1.352 | 21.2 | 1.680 |
| 4.9 | 0.376 | 9.0 | 0.704 | 13.1 | 1.032 | 17.2 | 1.360 | 21.3 | 1.688 |
| 5.0 | 0.384 | 9.1 | 0.712 | 13.2 | 1.040 | 17.3 | 1.368 | 21.4 | 1.696 |
| 5.1 | 0.392 | 9.2 | 0.720 | 13.3 | 1.048 | 17.4 | 1.376 | 21.5 | 1.704 |
| 5.2 | 0.400 | 9.3 | 0.728 | 13.4 | 1.056 | 17.5 | 1.384 | 21.6 | 1.712 |
| 5.3 | 0.408 | 9.4 | 0.736 | 13.5 | 1.064 | 17.6 | 1.392 | 21.7 | 1.720 |
| 5.4 | 0.416 | 9.5 | 0.744 | 13.6 | 1.072 | 17.7 | 1.400 | 21.8 | 1.728 |
| 5.5 | 0.424 | 9.6 | 0.752 | 13.7 | 1.080 | 17.8 | 1.408 | 21.9 | 1.736 |
| 5.6 | 0.432 | 9.7 | 0.760 | 13.8 | 1.088 | 17.9 | 1.416 | 22.0 | 1.744 |
| 5.7 | 0.440 | 9.8 | 0.768 | 13.9 | 1.096 | 18.0 | 1.424 | 22.1 | 1.752 |
| 5.8 | 0.448 | 9.9 | 0.776 | 14.0 | 1.104 | 18.1 | 1.432 | 22.2 | 1.760 |
| 5.9 | 0.456 | 10.0 | 0.784 | 14.1 | 1.112 | 18.2 | 1.440 | 22.3 | 1.768 |
| 6.0 | 0.464 | 10.1 | 0.792 | 14.2 | 1.120 | 18.3 | 1.448 | 22.4 | 1.776 |
| 6.1 | 0.472 | 10.2 | 0.800 | 14.3 | 1.128 | 18.4 | 1.456 | 22.5 | 1.784 |
| 6.2 | 0.480 | 10.3 | 0.808 | 14.4 | 1.136 | 18.5 | 1.464 | 22.6 | 1.792 |
| 6.3 | 0.488 | 10.4 | 0.816 | 14.5 | 1.144 | 18.6 | 1.472 | 22.7 | 1.800 |
| 6.4 | 0.496 | 10.5 | 0.824 | 14.6 | 1.152 | 18.7 | 1.480 | 22.8 | 1.808 |
| 6.5 | 0.504 | 10.6 | 0.832 | 14.7 | 1.160 | 18.8 | 1.488 | 22.9 | 1.816 |
| 6.6 | 0.512 | 10.7 | 0.840 | 14.8 | 1.168 | 18.9 | 1.496 | 23.0 | 1.824 |
| 6.7 | 0.520 | 10.8 | 0.848 | 14.9 | 1.176 | 19.0 | 1.504 | 23.1 | 1.832 |
| 6.8 | 0.528 | 10.9 | 0.856 | 15.0 | 1.184 | 19.1 | 1.512 | 23.2 | 1.840 |
| 6.9 | 0.536 | 11.0 | 0.864 | 15.1 | 1.192 | 19.2 | 1.520 | 23.3 | 1.848 |
| 7.0 | 0.544 | 11.1 | 0.872 | 15.2 | 1.200 | 19.3 | 1.528 | 23.4 | 1.856 |
| 7.1 | 0.552 | 11.2 | 0.880 | 15.3 | 1.208 | 19.4 | 1.536 | 23.5 | 1.864 |
| 7.2 | 0.560 | 11.3 | 0.888 | 15.4 | 1.216 | 19.5 | 1.544 | 23.6 | 1.872 |
| 7.3 | 0.568 | 11.4 | 0.896 | 15.5 | 1.224 | 19.6 | 1.552 | 23.7 | 1.880 |
| 7.4 | 0.576 | 11.5 | 0.904 | 15.6 | 1.232 | 19.7 | 1.560 | 23.8 | 1.888 |
| 7.5 | 0.584 | 11.6 | 0.912 | 15.7 | 1.240 | 19.8 | 1.568 | 23.9 | 1.896 |

# 3. Total Titratable Acidity (AOAC Method)

I. Apparatus

25 or 50 ml Buret with 0.1 ml graduation and Teflon® stopcock
Magnetic stirrer and Teflon® coated stirring bar
250 ml glass flask or beaker

II. Chemicals

Isopropanol ($C_3H_8O$)
Phenolphthalein ($C_{20}H_{14}O_4$)
Sodium hydroxide (NaOH)

III. Reagents

A. Dye solution (1%): Dissolve 1 g of phenolphthalein in 100 ml 50% isopropanol and then add just enough NaOH to neutralize the solution to a faint pink color.
B. Sodium hydroxide solution (0.100 N): Dissolve 40.0 g of NaOH in 10 liters of $CO_2$-free water. For standardization, see Chapter IV, 2.

IV. Procedure

1. Thoroughly mix the juices or concentrates before taking analysis samples.
2. Measure analysis sample into 250 ml glass flasks or beakers according to the following:

| Sample Type | Sample Size (g) |
|---|---|
| Blank | – |
| Orange or grapefruit single-strength juice | 10 |
| Orange or grapefruit concentrate | 5 |
| Lemon or lime single-strength juice | 5 |
| Lemon or lime concentrate | 5 |

3. Add ~250 ml of distilled water.
4. Add 0.75 ml (0.3 ml per 100 ml solution) of phenolphthalein solution and mix thoroughly.
5. Titrate with 0.1 N NaOH solution until solution shows a faintest discernible pink color persisting for 30 seconds.

## V. Calculations

1. The total titratable acidity is expressed as anhydrous citric acid on a weight basis. Due to its three carboxyl groups, one mole of citric acids (MW 192.12) can react with three moles of $OH^-$, therefore 1 mole of NaOH equals 64.04 g citric acid (= 192.12 ÷ 3) and the milliequivalent of citric acid is 0.064:

$$\%\ \text{Acid (w/w)} = \frac{(\frac{\text{Net ml Titrant}}{1000\ \text{l/ml}})(\text{N Titrant})(\frac{64.04\ \text{g CA}}{1\ \text{mole OH}^-})}{(\text{Sample Weight})} \times 100$$

$$= \frac{(\text{Net ml Titrant})(\text{N Titrant})(0.064)}{(\text{Sample Weight})} \times 100$$

$$= \frac{(\text{Net ml Titrant})(\text{N Titrant})}{(\text{g Sample})} \times 6.4$$

where (Net ml Titrant) = (ml Titrant for Sample) – (ml Titrant for Blank)

2. % Acid for accurately weighed sample For titration using 0.100 N NaOH as titrant and the sample quantity is

   - 10 g juice

$$\%\ \text{Acid (w/w)} = (\text{Net ml NaOH}) \times 0.064$$

   - 5 g juice or concentrate

$$\%\ \text{Acid (w/w)} = (\text{Net ml NaOH}) \times 0.128$$

## VI. Reference

Official Methods of Analysis. 1999. 16th Edition, 5th Reversion, AOAC International, Gaithersburg, MD, method 942.15.

# 4. Brix / Acid Ratio

1. The Brix / acid ratio is obtained by dividing the total soluble solids (°Brix corrected for acids and temperature) by the total titratable acid (% Acid, w/w) at 20°C (68°F).

$$\text{Brix / Acid Ratio} = \frac{°\text{Brix}_C}{\%\ \text{Acid (w/w)}}$$

2. Reference

Citrus Handbook. 1998. Agricultural Marketing Service, USDA. Washington, D.C.

# 5. pH

I. Apparatus

pH meter with ± 0.1 accuracy with ATC
Magnetic stirrer and Teflon® coated stirring bar
100 ml glass beaker

II. Chemicals

(only for making pH standards)
Potassium phosphate, monobasic ($KH_2PO_4$)
Sodium phosphate, dibasic ($Na_2HPO_4$)
Sodium bicarbonate ($NaHCO_2$)
Sodium carbonate ($Na_2CO_3$)
Potassium biphthalate ($KHC_8H_4O_4$)

III. Reagents

A. Carbon dioxide free water: Boil distilled water for 20 min and cool under a $CO_2$-free condition.
B. pH 4.0 Standard solution (0.0496 M): Dissolve 10.120 g of $KHC_8H_4O_4$ in $CO_2$-free water and make up to 1000 ml.
C. pH 7.0 Standard solution (0.2 M): Mix 500 ml of 0.2 M $KH_2PO_4$ (previously dried at 110-130°C for 2 h, 27.232 g/1000 ml) and 295.4 ml of standardized 0.2 M NaOH (see Chapter IV, 2).
D. pH 10.0 Standard solution (0.0249 M): Dissolve 2.092 g of $NaHCO_2$ (no heating) and 2.640 g of $NaCO_3$ (previously dried at 110-130°C for 2 h) in $CO_2$-free water and make up to 1000 ml.

IV. Procedure

1. Maintain sample temperature near 20°C (68°F), especially if the pH meter has no ATC.
2. Calibrate pH meter with standard buffer solutions of pH 7.0 and pH 4.0 according to pH meter manufacturer's procedure.
3. Place sample in a 100 ml beaker and immerse electrodes. Use sufficient sample so that the tips of the electrodes are covered.
4. Read pH to the nearest 0.05 after reading stabilizes.
5. Remove electrodes from sample, rinse with distilled water, and blot with paper tissue.
6. After using, repeat step 5 and store probe in a pH 7.0 buffer or follow manufacturer's instruction.

V. Calculations

For pH meter equipped with ATC, the pH values observed are used directly. Make temperature correction for readings from pH meter without ATC.

VI. Reference

Official Methods of Analysis. 1999. 16th Edition, 5th Reversion, AOAC International, Gaithersburg, MD, method 964.24 and 936.16.

# 6. Color by Hunterlab Colorimeter

I. Apparatus

Hunterlab Model D-45 citrus colorimeter with constant voltage regulator
USDA orange juice color standard tube No. 4
Vacuum pump or aspirator
25 × 200 mm Glass test tube

II. Chemicals

None

III. Reagents

None

IV. Procedure

1. Bring juice sample to 27 ± 1°C(80 ± 2°F).
2. Deaerate samples under vacuum (at least 3 min).
3. Turn Hunterlab colorimeter to "on" position for at least 10 min before making measurements (instrument is maintained on standby).
4. Calibrate colorimeter's Citrus Red (CR) and Citrus Yellow (CY) by:

    - Inserting the number coded standard tube into the tube holder at indexed position.
    - Turn the CR/CY switch to CR position.
    - Turn the reading dial to the USDA certified CR value for the standard tube.
    - Centralize the meter needle using the CR adjusting.
    - Turn the CR/CY switch to CY position.
    - Turn the reading dial to the USDA certified CY value for the standard tube.
    - Centralize the meter needle using the CR adjusting.

5. Remove the standard tube.
6. Leave the CR/CY switch on CY position.
7. Fill a test tube with sample and insert into the tube holder.
8. Turn reading dial until the meter needle is centralized.
9. Read the CY value.
10. Turn the CR/CY switch to CR position.
11. Turn reading dial until the meter needle is centralized.
12. Read CR value.

V. Calculations

The CR and CY values are used to calculate the juice color number based on the following formulation:

$$\text{Color Number} = 22.510 + 0.165\,(CR) + 0.111\,(CY)$$

The calculated color number is then used to determine the USDA color score according to Table IV – 6.

VI. Reference

Citrus Handbook. 1998. Agricultural Marketing Service, USDA. Washington, D.C.

Table IV – 6. Conversion of color number to USDA color score for orange juice.

| Color Number | Color Score | | | |
|---|---|---|---|---|
| | FCOJ | CCOJ | COJ & OFM | OJFC & POJ |
| 32.5 – 33.4 | 33 | 35 | 35 | 33 |
| 33.5 – 34.4 | 34 | 36 | 36 | 34 |
| 34.5 – 35.4 | 35 | 36 | 36 | – |
| 34.5 – 34.9 | – | 37 | – | 35 |
| 35.0 – 36.4 | – | 37 | – | 36 |
| 35.5 – 36.4 | 36 | 37 | 37 | – |
| 36.5 – 37.4 | 37 | 38 | 38 | 37 |
| 37.5 – 38.4 | 38 | 38 | 38 | 38 |
| 38.5 – 39.4 | 39 | 39 | 39 | 39 |
| 39.5 – 40.4 | 40 | 40 | 40 | 40 |

* FCOJ, frozen concentrate orange juice; CCOJ, canned concentrated orange juice; COJ, canned orange juice; COJFM, concentrate orange juice for manufacturing; OJFC, orange juice from concentrate; POJ, pasteurized orange juice.

For other colorimeters approved by USDA, the color numbers are calculated after converting the information into CR and CY.

# 7. Color by Macbeth Spectrophotometer

I. Apparatus

Macbeth Color-Eye 3100 spectrophotometer with color calibration plate and a computer with Optiview® Lite color quality control software
Vacuum pump or aspirator
25 × 200 mm Glass test tube

II. Chemicals

None

III. Reagents

None

IV. Procedure

1. Bring juice sample to 27 ± 1°C (80 ± 2°F).
2. De-aerate samples under vacuum (at least 3 min).
3. Turn on the computer and also CE 3100 if it has been turned off.
4. Open program Optiview.
5. If system is ready (make sure Plunger is up, SCE and SAV indicators on the CE 3100 front panel are lit), insert sample tube in the tube holder, hit F4 key or with mouse hit the measure trail, then hit Return to activate measurement.
6. If CE 3100 has been turned off, the system will prompt for calibration. Daily calibration is recommended. Calibration is done by:

   - Remove the tube holder from CE 3100 and install the thin calibration plate.
   - Place a clean calibration tile with the smooth surface facing the instrument, held by hinged holder.
   - Low plunger (from top cover).
   - Follow calibration instruction on computer.
   - Raise plunger, remove thin calibration plate, and reinstall tube holder.

V. Calculations

None

## VI. Reference

Macbeth CE 3100 User Manual.

# 8. Viscosity by Viscometer Using Low Centipoise Adapter

I. Apparatus

Viscometer of low viscosity measurement capacity (with a low centipoise adapter sample cup)

Water bath with temperature control

II. Chemicals

None

III. Reagents

None

IV. Procedure

1. Bring the single-strength or reconstituted juice to 30°C (86°F).
2. Level viscometer.
3. Attach extension link to the shaft and then low-centipoise spindle to the extension link.
4. Set rotation speed and spindle setting according to manufacturer's instructions. The following are examples for two viscometers.

| Model | Measurement Maximum (cP) | Rotation Speed (rpm) | Spindle Setting | Sample Volume (ml) |
|---|---|---|---|---|
| Brookfield® LV DV-I | 10 | 60 | – | 16 |
| | 20 | 30 | | |
| | 50 | 12 | | |
| Cole-Parmer® 98936-00/05 | 10 | 60 | 4 | 18 |
| | 20 | 30 | | |
| | 50 | 12 | | |

5. Measure required quantity of sample and pour into the sample adapter cup.
6. Slide the sample tube up over the spindle with care to avoid trapping any air in the sample fluid and prevent hitting the spindle against the container and consequently damaging shaft alignment.
7. Engage the pin on the bracket into the slot on the sample tube collar.
8. Fix sample tube by pushing in the thumbscrew and thread into tube collar.

9. Turn on the viscometer and allow at least one full rotation.
10. Record reading.

V. Calculations

Brookfield® LV DV-I viscometer with 12 rpm setting:

$$\text{Viscosity (cP)} = (\text{Reading} - \text{Windage Correction}) \times \text{Factor}$$

$$= (\text{Reading} - 0.04) \times 0.5$$

Cole-Parmer® 98936-00/05 viscometer with 60 rpm and 4 spindle settings:

$$\text{Viscosity (cP)} = \text{Reading} \times \text{Factor} - \text{Windage Correction}$$

$$= \text{Reading} \times 0.001 - 0.04$$

| Measurement Maximum (cP) | 10 | 20 | 50 | 100 | 200 | 400 | 1000 | 2000 |
|---|---|---|---|---|---|---|---|---|
| Brookfield® LV DV-I viscometer | | | | | | | | |
| Speed Setting (rpm) | 60 | 30 | 12 | 6 | 3 | 1.5 | 0.6 | 0.3 |
| Factor | 0.1 | 0.2 | 0.5 | 1 | 2 | 4 | 10 | 20 |
| Windage Correction | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 |
| Cole-Parmer® 98936-00/05 | | | | | | | | |
| Speed Setting (rpm) | 60 | 30 | 12 | 6 | 3 | 1.5 | 0.6 | 0.3 |
| Spindle Setting | 4 | 4 | 4 | 1 | 1 | 1 | 1 | 1 |
| Factor | 0.001 | 0.001 | 0.001 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Windage Correction | 0.04 | 0.02 | – | – | – | – | – | – |

## VI. Reference

FMC Technologies, Inc., FMC FoodTech, Citrus Systems.

Brookfield® viscometer user manual.

Cole-Parmer® rotational viscometer user manual.

# 9. Viscosity by Viscometer Using Standard Spindle

I. Apparatus

Viscometer with standard spindles
600 ml Glass beaker or 6 ounce can
Water bath with temperature control

II. Chemicals

None

III. Reagents

None

IV. Procedure

1. Bring the juice concentrate to 30°C (86°F).
2. Select the spindle size and rotation speed combination so that the true viscosity will be within 60 to 80% of the maximum measurement range. Select a combination with a larger spindle among the acceptable ones.

| Brookfield® LV DV-I or Cole-Parmer® 98936-00/05 | | | | |
|---|---|---|---|---|
| Speed | Viscosity Range (cP) for Spindle Number | | | |
| | 1 or L1 | 2 or L2 | 3 or L3 | 4 or L4 |
| 60 | 10 – 90 | 50 – 450 | 200 – 1,800 | 1,000 – 9,000 |
| 30 | 20 – 180 | 100 – 900 | 400 – 3,600 | 2,000 – 18,000 |
| 12 | 50 – 450 | 250 – 2,250 | 1,000 – 9,000 | 5,000 – 45,000 |
| 6 | 100 – 900 | 500 – 4,500 | 2,000 – 18,000 | 10,000 – 90,000 |
| 3 | 200 – 1,800 | 1,000 – 9,000 | 4,000 – 36,000 | 10,000 – 90,000 |

3. Level viscometer and attach the spindle to the viscometer.
4. Fill a 6-ounce can with concentrate (recommend using a 600 ml beaker).
5. Slowly insert spindle into sample until the concentrate level is at the immersion groove cut in the spindle's shaft. Care should be taken to avoid hitting the spindle against the container, as this could damage shaft alignment.
6. Turn on the viscometer and allow at least one full rotation.
7. Record reading.

## V. Calculations

Brookfield® LV DV-I viscometer:

$$\text{Viscosity (cP)} = \text{Reading} \times \text{Factor}$$

| Speed (rpm) | Factor for Spindle Number | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| 3 | 20 | 100 | 400 | 2000000 |
| 6 | 10 | 50 | 200 | 1000000 |
| 12 | 5 | 25 | 100 | 500 |
| 30 | 2 | 10 | 40 | 200 |
| 60 | 1 | 5 | 20 | 100 |

Cole-Parmer® 98936-00/05 viscometer:

$$\text{Viscosity (cP)} = \text{Reading}$$

## VI. Reference

FMC Technologies, Inc., FMC FoodTech, Citrus Systems.

Brookfield® viscometer user manual.

Cole-Parmer® rotational viscometer user manual.

# 10. Recoverable Oil (Scott Method)

I. Apparatus

Electric heater with recessed refractory top, 500 – 700 watts
Still with 500 ml flat-bottom distillation flask with 24/40 neck; 200 mm Graham condenser with 28/15 receiving socket and drip tip; connecting bulb (Iowa state type 90 × 35 O.D.) (see Figure IV – 10)
Hot glove or pad
Magnetic stirrer and Teflon® coated stirring bar
10 ml Buret with 0.1 ml division

II. Chemicals

Potassium bromide (KBr)
Potassium bromate ($KBrO_3$)
Isopropanol ($C_3H_8O$)
Arsenious oxide ($As_2O_3$)
Sulfuric acid ($H_2SO_4$)
Methyl orange ($C_{14}H_{14}N_3O_3SNa$)
Hydrochloric acid (HCl)

III. Reagents

A. Potassium bromide-bromate solution (PBB, ~0.1 N): Dissolve 2.8 g of $KBrO_3$ and 12 g of KBr in distilled water and make up to 1000 ml.

To standardize the PBB solution, titrate it with a mixture of 40 ml standard $As_2O_3$ solution and 10 ml diluted HCl solution (1:3, v/v with distilled water) with 3 drops of methyl orange based on the formula:

$$\text{Normality of PBB} = \frac{(\text{ml } As_2O_3)(\text{N } As_2O_3)}{(\text{ml PPB})}$$

$$= \frac{(40\text{ ml})(0.1\text{ N})}{(\text{ml PPB})}$$

$$= \frac{4}{(\text{ml PPB})}$$

Based on the actual normality of the PBB stock solution, make proper dilution with distilled water to obtain 0.0247 N solution for titration.

$$\text{PBB (ml to make 1000 ml 0.0247 N Solution)} = \frac{(0.0247\ \text{N})(1000\ \text{ml})}{(\text{N PPB})}$$

Arsenious oxide standard (0.100 N) is prepared as: Dry ~6 g of $As_2O_3$ for 1 h at 105°C (221°F), immediately accurately weigh 4.950 g and dissolve in 1 N NaOH (50 ml/5 g $As_2O_3$) in flask or beaker by heating on a steam bath, add the same volume of 1 N $H_2SO_4$ to neutralize the solution, and transfer to a 1000 ml volumetric flask, rinse the beaker repeatedly with distilled water to assure complete transfer and then make up to the 1 liter mark.

B. Methyl orange solution (0.1%): Dissolve 0.1 g of methyl orange in 100 ml distilled water.
C. Dye solution: In a fume hood or a well-ventilated area, slowly add 1 part of HCl to 2 parts of distilled water. To 1000 ml of acid solution, add 5 ml of 0.1% methyl orange solution and mix.

IV. Procedure

1. To a 500-ml distillation flask, add 25 ml of isopropanol, 25 ml of $H_2O$ and 25 ml of sample.
2. Turn on the heater and run cold water through the condenser from bottom to top.
3. Place a 150-ml beaker under the condenser flow out.
4. Attach the flask to the connecting trap of the condenser and rest on the turned-on heater.
5. Wait for distillation completion that is indicated by water condensation inside the connecting tubes or stop of solvent reflux. Time is about 3 to 3.5 min and distillate volume exceeds 30 ml.
6. Add 10 ml of the dye solution into the beaker.
7. Titrate the distillate in the beaker with the 0.0247 N $KBrO_3$-KBr solution to the disappearance of the dye color.
8. Record the amount of titrant used.
9. Determine reagent blank by titrating 3 mixtures of 25 ml of isopropanol and 10 ml of dye-HCl solution without refilling the buret. Divide total titrant volume used by 3 to get the average blank value.

V. Calculations

Since 1 mole of *d*-limonene reacts with 2 moles of $Br_2$ or 4 moles of Br (bromine), 1 ml of 0.0247 N $KBrO_3$-KBr titrant equals 0.001 ml or 0.00084 g of *d*-limonene and equals 0.004% oil by volume for a 25-ml sample.

$$\%\,\text{Oil (v/v)} = \frac{\text{Volume of Oil in Sample}}{\text{Volume of Sample}} \times 100$$

$$= \frac{\frac{(\text{ml Titrant})}{(1000\ \text{ml/l})}(\text{N Tritrant})(\frac{1}{4})(\text{MW of Limonene})(\frac{1}{\text{Oil Specific Gravity, g/ml}})}{(\text{Volume of Sample})} \times 100$$

$$= \frac{\frac{(\text{ml Titrant})}{(1000\ \text{ml/l})}(0.0247\ \text{N})(\frac{1}{4})(136.23\ \text{g/mole})(\frac{1}{0.84\ \text{g/ml}})}{(\text{ml Sample})} \times 100$$

$$= \frac{(\text{ml Titrant})(0.00084\ \text{g})(\frac{1}{0.84\ \text{g/ml}})}{(\text{ml Sample})} \times 100$$

$$= \frac{(\text{ml Titrant})(0.0010\ \text{ml})}{(\text{ml Sample})} \times 100$$

$$= \frac{(\text{ml Titrant})}{(\text{ml Sample})} \times 0.1$$

where

(Net ml $KBrO_3$-KBr) = (ml $KBrO_3$-KBr for Sample – ml $KBrO_3$-KBr for Blank)

For 25 ml juice sample titrated with 0.0247 N $KBr_3$-KBr

$$\% \text{ Oil (v/v)} = \frac{(\text{Net ml } KBrO_3 \text{ - KBr})(0.0010 \text{ ml})}{(25 \text{ ml})} \times 100$$

$$= (\text{Net ml } KBrO_3 \text{ - KBr}) \times 0.004$$

VI. Reference

Official Methods of Analysis. 1999. 16th Edition, 5th Reversion, AOAC International, Gaithersburg, MD, method 968.20, 939.12, and 947.13.

Scott, W.C. and M.K. Veldhuis. 1966. Rapid estimation of recoverable oil in citrus juices by bromate titration. J. AOAC. 49:628 – 633.

FMC Technologies, Inc., FMC FoodTech, Citrus Systems.

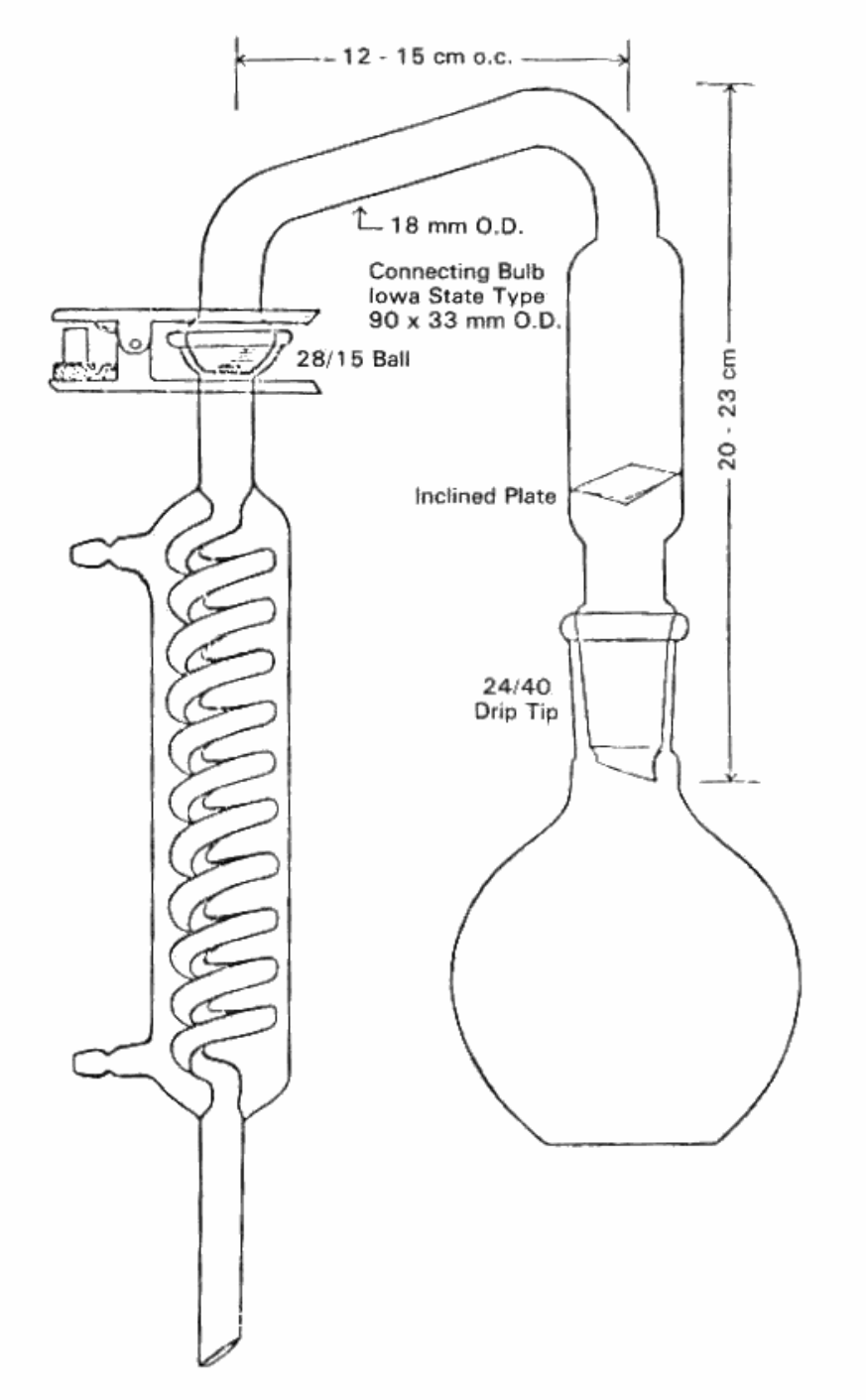



Figure IV – 10. Distillation apparatus used for Scott oil test.

# 11. Recoverable Oil (Distillation Method)

I. Apparatus

Electric heating mantle
All-glass still with 2000-ml boiling flask of standard taper 24/40 joint, modified oil separatory trap connected to 500-ml round-bottom flask through standard taper 24/40 joint, and tight fitting condenser having projection at bottom to facilitate return of oil to trap (see Figure IV – 11)
Hot glove or pad
Magnetic stirrer and Teflon® coated stirring bar
Glass bead

II. Chemicals

Antifoam agent

III. Reagents

None

IV. Procedure

To a 2-liter boiling flask, add:

- For juice: 1000 ml.
- For concentrate: 400 g concentrate plus 1000 ml distilled water.

Add a few glass beads or a little antifoam (use sparingly).
Close the stopcock on the oil trap and fill oil trap with water to overflowing, connect to boiling flask and condenser.
Run cold water through the condenser from bottom to top.
Turn on the heater and boil sample for 1 h. Control heating so that water condensation appears on no more than 75% of the condenser wall and the condensate flow approaches, but does not exceed, 50 drops per minute.
Turn off heater and let stand for several minutes.
Release enough water from trap with stopcock to low oil layer within graduation portion.
Let stand 5 min to complete drainage.
Adjust the bottom of the lower meniscus of the column of oil to exactly the zero calibration mark.
Read amount of oil at the highest point of the upper meniscus, estimating the third decimal place.

V. Calculations

$$\% \text{ Oil} = \frac{(\text{ml Oil})}{(\text{Sample Quantity})} \times 100$$

For 1000 ml juice:

$$\% \text{ Oil (v/w)} = \frac{(\text{ml Oil})}{10}$$

For 400 g concentrate:

$$\% \text{ Oil (v/w)} = \frac{(\text{ml Oil})}{4}$$

VI. Reference

Official Methods of Analysis. 1999. 16th Edition, 5th Reversion, AOAC International, Gaithersburg, MD, method 944.06.

Citrus Handbook. 1998. Agricultural Marketing Service, USDA. Washington, D.C.

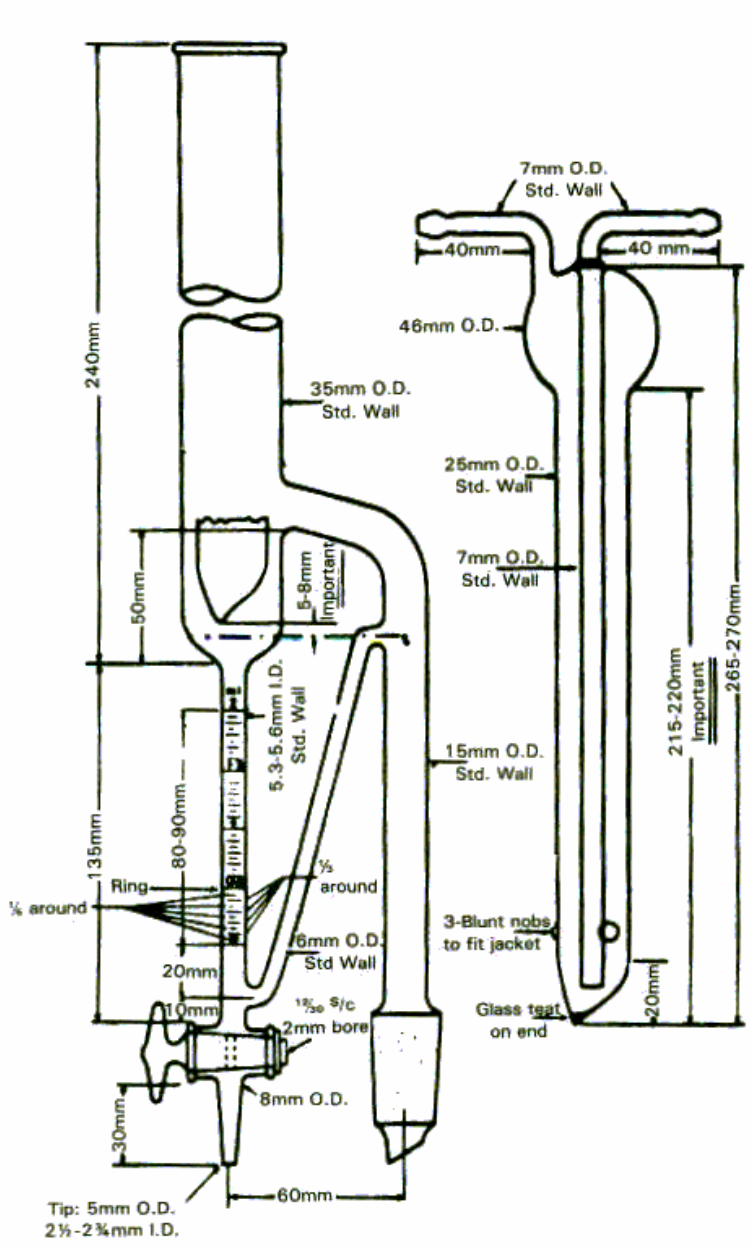



Figure IV – 11. Oil separatory trap used for Clevenger method (source: USDA Citrus Handbook).

# 12. Screened Pulp

I. Apparatus

FMC FoodTech Quick Fiber Device (see Figure V – 1)

20 mesh screen: dish shaped, approximately 5″ diameter and 2 ¾″ deep made with woven stainless steel wire 0.015″ in diameter and containing 20 openings, 0.033″ square, per linear inch of screen

60 mesh screen: same as above but wire of 0.009″ in diameter and containing 60 openings, 0.0077″ square, per linear inch of screen

Top loading analytical balance

500 ml graduate cylinder

II. Chemicals

None

III. Reagents

None

IV. Procedure

Prepare 500 ml of single-strength or reconstituted juice.
Wet the 20 mesh screen with water or juice to simulate the juice residue on the screen.
Shake the screen by hand and then blot the bottom with paper tissue.
Place the wet screen on a balance and tare.
Place the screen in the device.
Pour juice through the screen, permitting free juice to drain.
Turn on device to shake for 2 min.
If automatic shaker is unavailable, shake by hands until pulp retained on the screen 'balls up' and is free of excess juice.
Remove screen from the shaker.
Blot off juice adhering to the bottom of screen with paper tissue.
Weigh the pulp-containing screen.
Rinse pulp off the screen and repeat step 3 to 9 for the next sample.

If desired, collect the screened juice and repeat the step 2 to 9 with a 60 mesh screen for 20 ~ 60 mesh screened pulp (commonly referred as 60 mesh pulp).

V. Calculations

$$\%\,\text{Screened Pulp (w/v)} = \frac{(\text{Weight of Pulp and Basket}) - (\text{Weight of Basket})}{(\text{Volume of Juice})} \times 100$$

$$= \frac{\text{g Pulp}}{(500\ \text{ml})} \times 100$$

$$= (\text{g Pulp}) \times 0.2$$

or

$$\%\,\text{Screened Pulp (g/l)} = \frac{(\text{Weight of Pulp and Basket}) - (\text{Weight of Basket})}{(\text{Volume of Juice})} \times (1000\ \text{ml})$$

$$= \frac{\text{g Pulp}}{(500\ \text{ml})} \times (1000\ \text{ml})$$

$$= (\text{g Pulp}) \times 2$$

or

If concentrate of 42°$Brix_C$ in the 6-oz can is used and diluted with water to make 24 oz juice (710 ml), grams of screened pulp per 24-oz single-strength orange juice (SSOJ) can be calculated as:

$$\text{Screened Pulp (g/24-oz SSOJ)} = \%\ \text{Screened Pulp} \times 7.1$$

VI. Reference

FMC Technologies, Inc., FMC FoodTech, Citrus Systems.

Citrus Handbook. 1998. Agricultural Marketing Service, USDA. Washington, D.C.

# 13. Suspended Pulp

I. Apparatus

20 mesh Screen (see Chapter IV, 12)
Laboratory/clinical centrifuge
50 ml Graduated centrifuge tube with conical bottom

II. Chemicals

None

III. Reagents

None

IV. Procedure

Bring juice sample to 27±1°C (80 ± 2°F). A 5°C (10°F) difference will make about 1.0% difference in pulp reading

Pour ~ 100 ml of juice through a 20 mesh screen or use the 20 mesh screened juice (see Chapter IV, 12).

Fill a centrifuge tube with 50 ml of the screened juice.

Place the tubes in the centrifuge with the graduated scale facing the direction of rotation for easier reading of pulp volume after centrifugation. Make sure load is balanced.

Centrifuge for 10 min after reaching a centrifugation force of 365 $\times g$ or the speed specified in Table IV – 13 based on rotor operation diameter. Once the time required for acceleration is known, the combined time can be used at the time of starting the centrifuge.

Read pulp volume after centrifugation. For uneven pulp surface, use the average of readings of pulp top layer at its highest and lowest points.

V. Calculations

$$\%\,\text{Suspended Pulp (v/v)} = \frac{\text{Volume of Pulp}}{\text{Volume of Juice}} \times 100$$

$$= \frac{\text{ml Pulp}}{50\,\text{ml}} \times 100$$

$$= (\text{ml Pulp}) \times 2$$

VI. Reference

Citrus Handbook. 1998. Agricultural Marketing Service, USDA. Washington, D.C.

Table IV – 13. Centrifuge speed selection for determining suspended pulp using various rotor sizes.

| Operation Diameter* | | Approximate Speed (rpm) | Operation Diameter* | | Approximate Speed (rpm) |
|---|---|---|---|---|---|
| Inches | Centimeters | | Inches | Centimeters | |
| 10.0 | 25.4 | 1609 | 15.5 | 39.4 | 1292 |
| 10.5 | 26.7 | 1570 | 16.0 | 40.6 | 1271 |
| 11.0 | 27.9 | 1534 | 16.5 | 41.9 | 1252 |
| 11.5 | 29.2 | 1500 | 17.0 | 43.2 | 1234 |
| 12.0 | 30.5 | 1468 | 17.5 | 44.4 | 1216 |
| 12.5 | 31.8 | 1438 | 18.0 | 45.7 | 1199 |
| 13.0 | 33.0 | 1410 | 18.5 | 47.0 | 1182 |
| 13.5 | 34.3 | 1384 | 19.0 | 48.3 | 1167 |
| 14.0 | 35.6 | 1359 | 19.5 | 49.5 | 1152 |
| 14.5 | 36.8 | 1336 | 20.0 | 50.8 | 1137 |
| 15.0 | 38.1 | 1313 | | | |

* Operation Diameter is the distance between the bottoms of opposing centrifuging tubes in horizontal operation position.

** Relative centrifugal force ($\times g$) is calculated as: RCF = (1.118)(radius in mm)(rpm/1000)$^2$.

# 14. Clarification (Percent Light Transmission Method)

I. Apparatus

Spectrophotometer with cuvet or test tube cuvet
Laboratory/clinical centrifuge
Stopwatch or timer
50 ml graduated centrifuge tube with conical bottom

II. Chemicals

None

III. Reagents

None

IV. Procedure

If the supernatant from the Suspended Pulp test is used, go to step 4.
If the juice sample has not been centrifuged, fill a 50-ml centrifuge tube to the mark.
Centrifuge for 10 min after the centrifuge reaches a centrifugation force of 365 ×*g* or a speed based on rotor operation diameter as specified in Table IV –13.
Carefully decant about 20 ml of the supernatant, through gauze or coarse cheese cloth, into a small beaker. Be sure that the pulp layer at the bottom is not disturbed and all coarse floating pulp particles are removed by the straining.
Adjust the colorimeter to 100% light transmission at 650 nm against distilled water in a cuvet or test tube cuvet.
Decant cuvet or test tube cuvet, rinse with some supernatant, and then fill with the supernatant.
Read percent light transmission of the supernatant.

V. Calculations

Report percentage light transmission as read.

VI. Reference

Citrus Handbook. 1998. Agricultural Marketing Service, USDA. Washington, D.C.

Huggart, R.L., Moore, E.L., and Wenzel, L.W. 1951. The measurement of clarification in concentrated citrus juice. Proc. Fla. State Hortic. Soc. 64:185 – 188.

Table IV – 14. Citrus juice clarification in relation to percentage of light transmission.

| Juice Clarification | Light Transmission (%) | |
|---|---|---|
| | Orange | Grapefruit |
| None | 0 – 24 | 0 – 35 |
| Slight | 25 – 35 | 36 – 50 |
| Definite | 36 – 60 | 51 – 72 |
| Extreme | 61 – 100 | 73 – 100 |

# 15. Defects

I. Apparatus

1000 ml glass beaker with a diameter of 100 mm (4″)
Microscope

II. Chemicals

None

III. Reagents

None

IV. Procedure

Pour 710 ml (24 fluid ounces) of single-strength or reconstituted juice sample into a clean 1000 ml glass beaker.
Allow juice to stand for 5 min.
Hold beaker over a strong light.
Examine the bottom of the beaker and count the number of seed bits and any dark specks (see Table IV – 15).
To determine the origins of the dark specks (burnt product or equipment fall-off), examine the piecement on a piece of white paper under microscope of ~30 magnifications.
The examination of hesperidin defects can be facilitated by mixing 3 – 4 drops of blue or black vegetable dye into the juice.

V. Calculations

Grade defect according to USDA Grade Standards listed in the Table IV – 15.

VI. Reference

Citrus Handbook. 1998. Agricultural Marketing Service, USDA. Washington, D.C.

Table IV – 15. Citrus juice defect description and scores.

| Defect Type | Definition | Defect Count | Description | Defect Score |
|---|---|---|---|---|
| Seeds and Portions Thereof | Very small particles of membrane, core or seeds (can pass through round perforation of less than 1/8' or 3.2 mm) | < 3 | Practically free of defects | 18 – 20 |
| | Very small particles of membrane, core or seeds Palatability not substantially detracted | 4 – 7 | Slightly defects | 16 – 17 |
| Dark Specks | Specks from charred or burnt product, fruit scale, black rot, equipment fall-off, etc. | See Figure IV – 15A | | |
| Hesperidin | Hesperidin | See Figure IV – 15B | | |

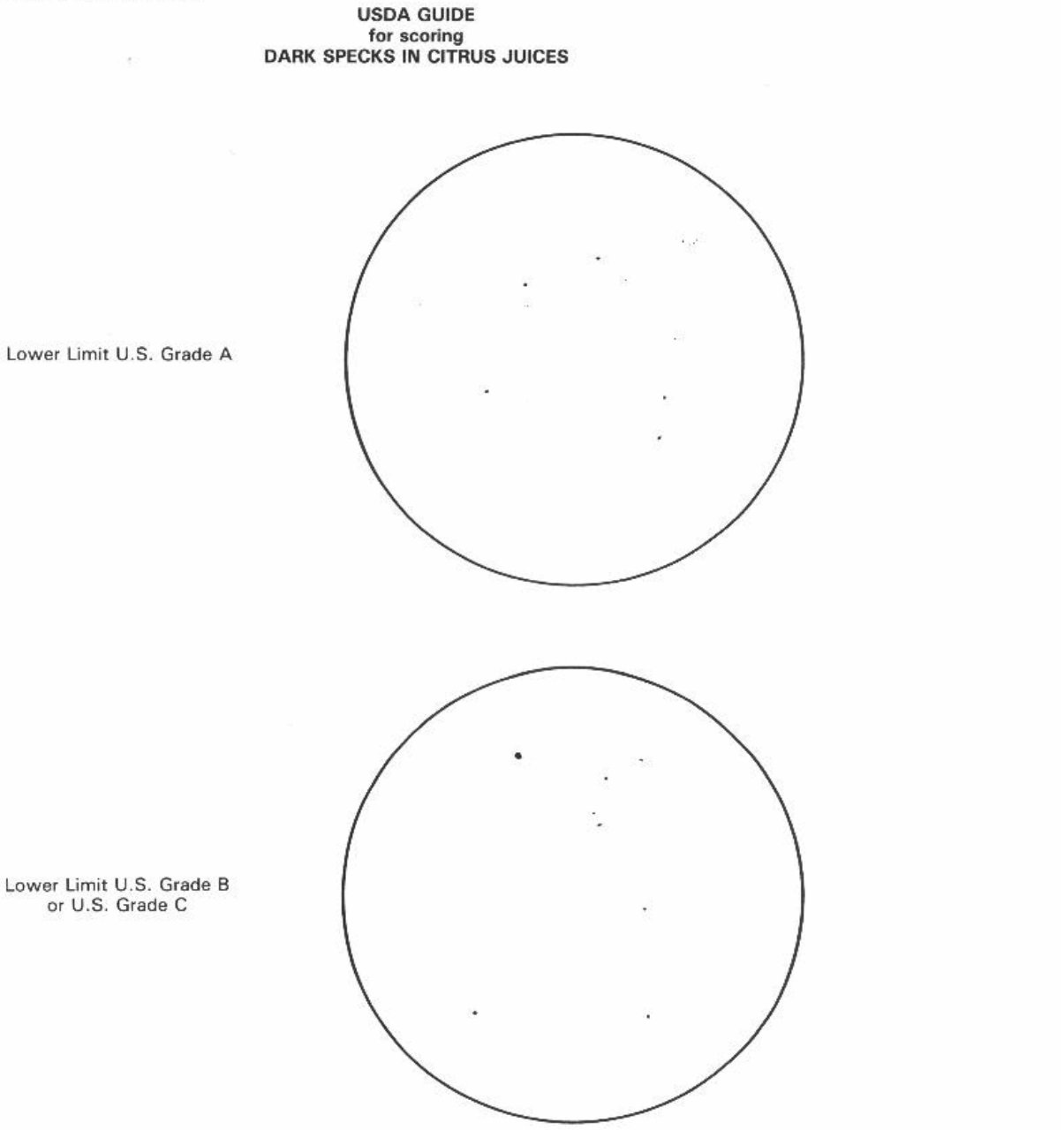



Figure IV – 15A. Juice defect – Scoring guide for dark specks in citrus juice (source: USDA Citrus Handbook).

Special Note: This is for illustration purpose only, not for using as USDA inspection device.

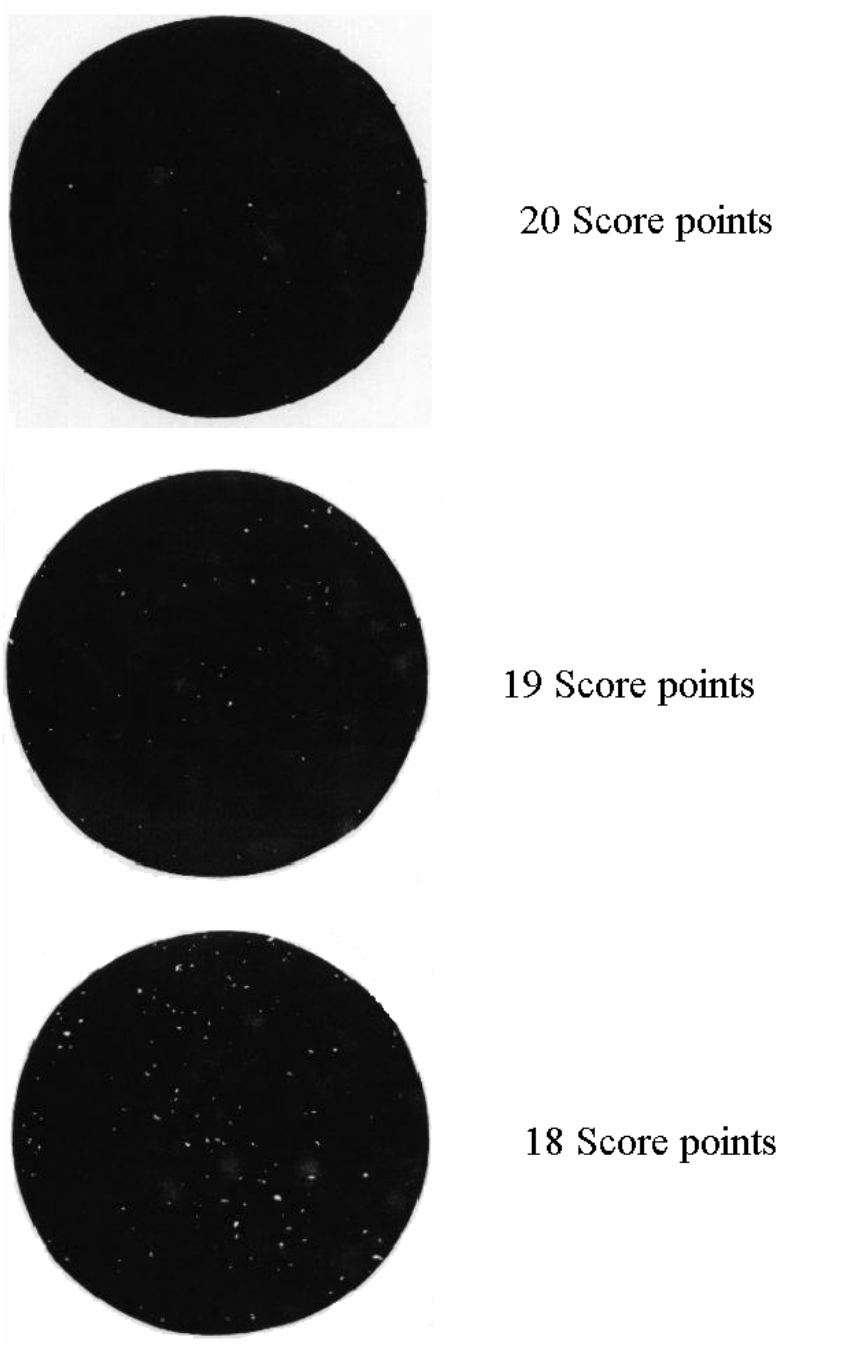



Figure IV – 15B. Juice defect – Scoring guide for hesperidin for frozen concentrated orange juice and concentrated orange for manufacturer (source: USDA Citrus Handbook).

Special Note: This is for illustration purpose only, not for using as USDA inspection device.

# 16. Gelation of Juice Concentrates

I. Apparatus

Water bath with temperature control
Petri dish or beaker of 100 mm (4″) in diameter, 50 mm (2″) maximum in height
Can opener

II. Chemicals

None

III. Reagents

None

IV. Procedure

Collect frozen product sample (42°Brix concentrate in 6-oz cans) or prepare sample by filling 6-oz cans with concentrate from production line (may require constitution to 42 °Brix) and freezing the cans for a desired storage period.
Thaw samples (two 6-oz cans) in running water (21–27°C/70–80°F) for 30 min.
Place one can in a water bath of 27 ± 1°C (80 ± 2 F°) for 24 h.
Carefully open another can with a can opener. Pay attention to see if pressure builds up inside due to fermentation.
Cover the can with a Petri dish and invert the can while holding the two together.
Slowly pull the can straight upward.
Grade the degree of gelation (*Initial Gel Test*).
Repeat steps 4 to 8 with the can in water bath at the end of incubation (*24-Hours Gel Test*).
If either fermentation or No. 3 gelation occurs, retest by thawing a 6-oz can to 4°C (40°F) and holding at that temperature for 6 days and then examine samples as above.

V. Calculations

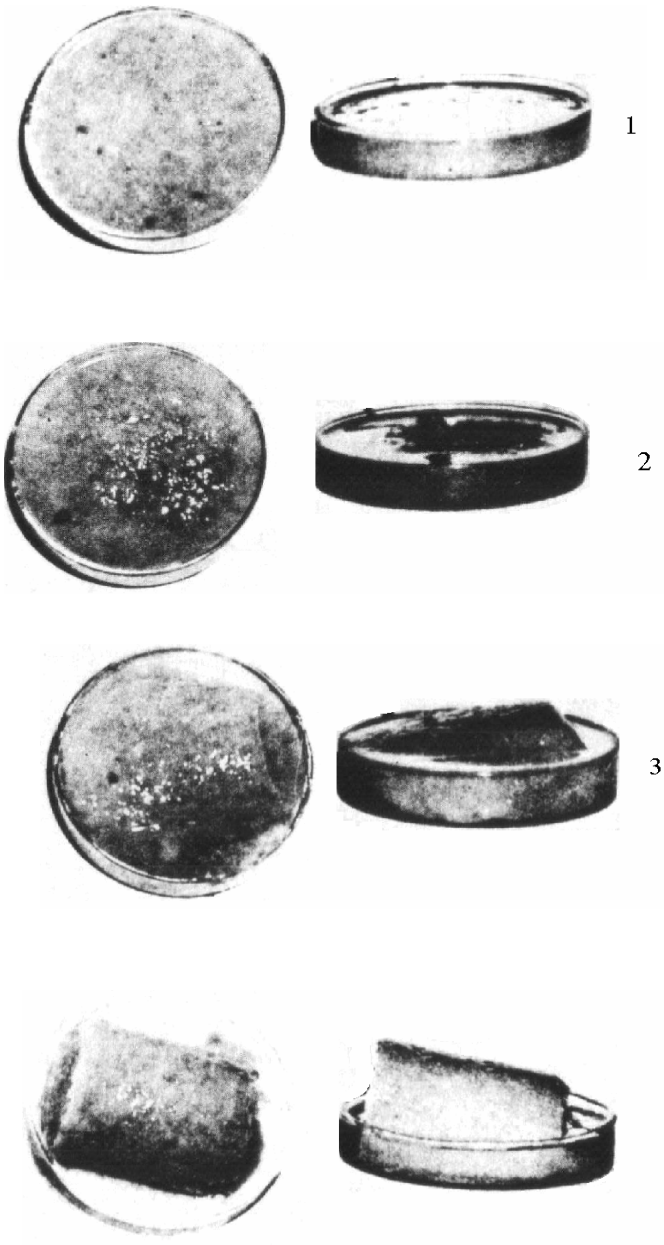
Gelation of concentrate is rated according to the following table. If No. 3 gel occurs during retesting, the product is substandard according to Florida Statues.

VI. Reference

Citrus Handbook. 1998. Agricultural Marketing Service, USDA. Washington, D.C.

Table IV – 16. Gel scale for frozen concentrate orange juice and frozen orange juice for manufacture.

| Degree of Gelation | Description |
|---|---|
| Zero gel | Concentrate is uniform in appearance and contains non gelled lumps |
| No. 1 gel | Concentrate contains a few small gelled lumps, however, is completely fluid and has no tendency to mound |
| No. 2 gel | Concentrate contains many gelled lumps and shows resistance of flow, however, no portion of the concentrate retains the shape of any part of the can. When poured, concentrate has a tendency to mound |
| No. 3 gel | Definite degree of gel formation is evident in the concentrate as indicated by any portion of the product showing and retaining the shape of any part of the can |




Questionable whether gel present or not.
Definite gel lumps.
Definite gel which holds to shape of can but breaks up partially upon pouring into another container.
Definite gel which retains shape of can upon placing in another container.

Figure IV – 16. Stages of citrus concentrate gel formation (source: Florida Citrus Experiment Station).

Special Note: This is for illustration purpose only, not for using as inspection device.

# 17. Separation Test (FMC FoodTech Method)

I. Apparatus

100 ml Graduated glass cylinder

II. Chemicals

None

III. Reagents

None

IV. Procedure

Bring concentrated juice to ambient temperature.
Reconstitute the concentrate juice to the appropriate °$Brix_C$ (see Chapter III).
Thoroughly mix the juice and place 100 ml of juice into the glass cylinder.
Allow juice to stand for 30 min.
Read the volume of the top clear juice serum.

V. Calculations

The separation test results are reported as percent separation:

$$\%\,\text{Separation} = \frac{\text{Volume of Serum}}{\text{Volume of Juice}} \times 100$$

$$= (\text{ml Serum})$$

VI. Reference

FMC Technologies, Inc., FMC FoodTech, Citrus Systems.

Table IV – 17. Citrus juice separation scale.

| Separation Scale | Volume of Juice Serum (ml) |
|---|---|
| None | 0 |
| Slight | 0 to 10 |
| Moderate | 10 to 20 |
| Severe | 20 to 40 |
| Extreme | > 40 |

# 18. Separation Test (USDA Method)

I. Apparatus

250 ml Graduated glass cylinder of 31 mm (1.25″) in diameter

II. Chemicals

None

III. Reagents

None

IV. Procedure

Reconstitute the concentrate juice to the appropriate °$Brix_C$ (see Chapter III).
Place the juice in a 250 ml glass cylinder.
Allow juice to stand for 4 h at ambient temperature of not less than 20°C (68°F).
Examine the degree of separation.

V. Calculations

USDA's guidelines for juice separation scoring are listed in Table IV – 18.

VI. Reference

Citrus Handbook. 1998. Agricultural Marketing Service, USDA. Washington, D.C.

Table IV – 18. USDA separation scores for concentrated citrus juices.

| Separation of Reconstituted Juice | Score | Acceptance/Rejection |
|---|---|---|
| None | 0 | |
| Slight | 1 | |
| Definite | 2 | substandard |
| Extreme | 3 | substandard |

# 19. Cloud Stability

I. Apparatus

Constant temperature incubator
pH meter
Centrifuge
Screw cap clear glass bottle, 200 ml

II. Chemicals

Citric acid (anhydrous) ($C_6H_8O_7$)
Benzoic acid ($C_7H_6O_2$)
Sodium benzoate ($C_6H_5CO_2Na$)
Barium chloride ($BaC1_2 \cdot H_2O$)
Rapid-set (2.5 min) pectin
Barium hydroxide ($Ba(OH)_2 \cdot 8H_2O$)

III. Reagents

A. Citric acid solution (50%): Dissolve 500 g of $C_6H_8O_7$ and 1 g of $C_7H_6O_2$ (0.10%) in distilled water and make up to 1000 ml.
B. Sodium benzoate solution (23%): Dissolve 230 g of $C_6H_5CO_2Na$ in distilled water and make up to 1000 ml.
C. Barium chloride solution (19%): Dissolve 190 g of $BaC1_2$ in distilled water and make up to 1000 ml.
D. Pectin solution (2.75%): Dissolve 27.5 g of rapid-set pectin and 1.5 g of $C_7H_6O_2$ (0.15%) in distilled water and make up to 1000 ml. Allow solution to stand for 2 h before use. There should be no gel formation.
E. Barium hydroxide solution (2%): Dissolve 20 g of $Ba(OH)_2 \cdot 8H_2O$ in distilled water and make up to 1000 ml.

IV. Procedure

Bring reconstituted juice to ambient temperature.
Mix juice and reagents as shown in the following table into bottles.
Add 14 ml of mixture to the bottles.
Keep bottles in an incubator at 49°C (120°F) for 24 h.
Gently invert the bottles 3 times at the end of incubation.
Add 9 ml of distilled water to the bottles and mix thoroughly.
Centrifuge at 900 ×g for 2 min.
Zero spectrophotometer with distilled water.
Read light transmission at 660 nm of the supernatant.

| Adding Order | Solution | Quantity (ml) | |
|---|---|---|---|
| | | Orange, Grapefruit, and Tangerine | Lemon |
| 1 | Juice | 93 | 49 |
| 2 | Citric acid | sufficient to give pH 3.15 – 3.20 | – |
| 3 | Sodium Benzoate | 1 | 1 |
| 4 | Pectin | 4 | 4 |
| 5 | Barium chloride | 4 | – |
| 6 | Barium hydroxide | – | 50 |

V. Calculations

The implications of the accelerated cloud stability test are shown in Table IV – 19. The supernatant liquid following centrifugation should retain a good cloud. A clear serum following centrifugation would be indicative of enzyme action.

VI. Reference

Holland, R.R, S.K. Reeder, and D.E. Pritchett. 1976. Cloud stability test for pasteurized citrus juice. J. Food Sci. 41:812 – 815.

# 20. Pectinesterase Activity

I. Apparatus

Burette with 0.1 ml division
pH meter
Magnetic stirrer and Teflon® covered stir bar
Water bath with temperature control
Stop watch readable to seconds
Disposable plastic pipet or dropper
Blender (full speed, 20,000 rpm; low speed 15,000 rpm)
FMC FoodTech Quick Fiber Device with 40 mesh screen (see Chapter V, 1)
150 ml Beaker
1000 ml graduate cylinder
1000 ml Plastic bottle

II. Chemicals

Sodium chloride (NaCl)
Sodium hydroxide, carbonate free (NaOH)
Powdered high-ester pectin from citrus

III. Reagents

A. Sodium chloride solution (0.15 M): Dissolve 8.766 g of NaCl in distilled water and make up to 1000 ml.
B. High ester pectin solution (1%): Warm NaCl solution to 50 – 55°C (122 – 131°F) and pour a portion into a blender; while run at slow speed, slowly add 10 g of powdered pectin, blend till powder is well dissolved, make up to 1000 ml with NaCl solution, and mix thoroughly. Store solution in a refrigerator.
C. Sodium hydroxide solution for pH-adjustment (1 N): Dissolve 40 g of NaOH in distilled water and make up to 1000 ml.
D. Sodium hydroxide solution for pH-adjustment (0.2 N): Mix 200 ml of 1 N NaOH with 800 ml of distilled water. Store in plastic bottle.
E. Sodium hydroxide solution for pH-adjustment (0.02 N): Mix 100 ml of 0.2 N NaOH with 900 ml of distilled water. Store in plastic bottle.
F. Sodium hydroxide solution for titration (0.02 N): Mix 100 ml of 0.2 N NaOH with 900 ml of distilled water. Store in plastic bottle. This solution should be carbonate-free and standardized to ± 0.0001 N. For standardization, see Chapter IV, 2.

## IV. Procedure

Warm pectin solution to 30°C (86°F).
Comminute 200 ml of single-strength or reconstituted juice at full speed for 3 min in a blender. If only pectinesterase activity in pulp-free juice is of interest, remove pulp from juice sample by shaking in a 40 mesh screen for 3 min using a Quick Fiber device.
Fill burette with 0.02 N NaOH titration solution.
Accurately weigh 10 g of well-mixed juice into a 150 ml beaker.
Add 100 ml of 1% pectin solution.
On a stirrer, adjust stirring speed to produce a slight vortex. Always use the same speed setting.
Insert a pH meter electrode into the beaker.
Add 1.0 N NaOH drop-wise to bring solution pH to 6.5.
Then add 0.2 N NaOH drop-wise to bring solution pH to 7.5.
Then add 0.02 N NaOH drop-wise to bring solution pH to 7.8 and maintain at this pH for approximately 1 min to establish reaction equilibrium.
With the pH at exactly 7.8 start stopwatch and start adding 0.02 N NaOH from the burette to maintain solution at this pH. Do not exceed limits of 7.7 to 7.9. Any variation above 7.8 should be compensated by an equal variation below 7.8 and vice versa.
Stop the titration after adding 5 ml titrant if the enzyme activity is low and 10 ml if the activity is high. The pH must be 7.8 at titration determination. A convenient way to stop the titration is to anticipate the last addition of alkali so that this addition will raise the pH to 7.9. Stop the stopwatch when the pH drops to exactly 7.8.
Record ml of NaOH and the titration time.

## V. Calculations

Pectinesterase (PE) activity is calculated and reported as PE units (PEU). One unit will release 1.0 molar equivalent of acid from pectin per minute at pH 7.8 and 30°C (86°F).

$$\text{PE Activity} = \mu\text{PEU per gram soluble solids}$$

$$= \text{PEU} \times 10^3 \text{ per gram soluble solids}$$

$$= \frac{(\text{ml NaOH})(\text{N NaOH})}{(\text{min})(\text{g Sample})(\frac{^\circ\text{Brix}_C}{100})} \times 1000$$

$$= \frac{(\text{ml NaOH})(0.02)}{(\text{min})(\text{g Sample})(\frac{°\text{Brix}_C}{100})} \times 1000$$

$$= \frac{(\text{ml NaOH})}{(\text{min})(\text{g Sample})(°\text{Brix}_C)} \times 2000$$

For 10 g of juice

$$\text{PEU} (\times 10^3 / \text{g SS}) = \frac{(\text{ml NaOH})}{(\text{min})(°\text{Brix}_C)} \times 200$$

## VI. Reference

Rouse, A.H. and Atkin, C.D. 1955. Pectinesterase and pectin in commercial citrus juices as determined by methods used at the Citrus Experiment Station. Tech. Bull. 570. University of Florida. Agric. Exp. Sta., Gainesville, Florida.

# Water Soluble Pectin (m-Hydroxydiphenyl Method)

VII. Apparatus

Spectrophotometer with cuvet or tube
Vortex mixer
Thermostatically controlled water bath
In-hood stove or heater for boiling water
Stopwatch or timer
Glass marble
Dispenser
16 × 150 mm Test tube
100 ml graduate cylinder

VIII. Chemicals

Sodium tetraborate decahydrate (borax, $Na_2B_4O_7 \cdot 10H_2O$)
Sodium hydroxide (NaOH)
Sulfuric acid ($H_2SO_4$)
*m*-hydroxydiphenyl ($C_{12}H_{10}O$)
Galacturonic acid ($C_6H_{10}O_7$)
Sulfamic acid ($NH_3O_3S$)
Potassium hydroxide (KOH)

IX. Reagents

A. Borax-sulfuric acid solution (0.0125 M): Dissolve 47.671 g of $Na_2B_4O_7 \cdot 10H_2O$ in 1000 ml of concentrated $H_2SO_4$ with stirring overnight. Keep in an iced water bath.
B. Sodium hydroxide solution (0.5%): Dissolve 5 g of NaOH in 1000 ml of distilled water.
C. *m*-Hydroxydiphenyl (HDP) solution (0.15%): Dissolve 150 mg of HDP in 100 ml of 0.5% NaOH solution. Store in dark in a refrigerator.
D. Galacturonic acid (GA) standard solution: Dissolve 1 g of GA in distilled water and make up to 1000 ml. Dilute to make 0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml standard solutions. Store in a refrigerator.
E. Sulfamic acid solution: Dissolve 38.8 g of $NH_3O_3S$ in distilled water and make up to 100 ml. Titrate to pH 1.6 with saturated potassium hydroxide solution.

X. Procedure

1. Heat 95% and 65% ethanol to 75°C (167°F).
2. Measure 15 ml of single-strength juice into 50 ml centrifuge tube.
3. Add hot 95% ethanol into tube to make up to 40 ml.

4. Heat for 10 min at 85°C (185°F) in a water bath, occasionally stir with a glass rod.
5. Rinse pectin off the glass rod into a centrifuge tube with 95% ethanol and make up to 50 ml.
6. Centrifuge for at least 15 min at 2000 ×*g*.
7. Decant the supernatant.
8. Repeat steps 4 to 7 with hot 63% ethanol.
9. Add 5 ml of distilled water to the centrifuge tube with the precipitate.
10. Stir up the precipitate with a rubber policeman.
11. Rinse the policeman with 30 ml of distilled water.
12. Vigorously agitate the mixture with a stir bar until precipitate is well dissolved.
13. Rinse the stir bar and add water to bring to 40 ml.
14. Centrifuge for 15 min at 2000 ×*g*.
15. Collect the supernatant in a 100 ml graduated flask.
16. Repeat the water extraction (steps 2 to 7)
17. Collect the second supernatant into the same graduate flask.
18. Add distilled water to make up to 100 ml and mix.
19. Filter the solution and use the filtrate for total pectin analysis.
20. Label a set of test tubes, in triplicate, for the followings:

    - Blank
    - Galacturonic acid standards
    - Samples

21. Place tubes in a rack in an ice water bath about 3 cm (1 inch) deep.
22. Pipet 0.5 ml of proper solutions to the designated test tube, add water for blanks.
23. (add 0.050 ml of sulfamic acid solution to each tube to reduce background if needed)
24. Add 2.5 ml of cold borax-sulfuric acid solution into each tube and mix quickly by vortex mixer or shaking.
25. Return tube to the rack in ice water bath.
26. Cover each test tube with a glass marble.
27. Place tubes, together with the rack, in a boiling water bath for 10 min.
28. Immediately place tubes, together with the rack, back in an ice water bath to cool.
29. If sample reaction solutions have yellow or pink color, zero spectrophotometer with the blank and read the sugar interference absorbance at 520 nm. If not, skip this step.
30. Add 0.050 ml of m-hydroxydiphenyl solution to:

    - Galacturonic acid standards
    - Samples

    Add 0.050 ml of 0.5% NaOH to:

    - Blank

31. Mix the solutions with a vortex mixer and allow to stand at ambient temperature for 20 min.
32. Zero spectrophotometer with the blank.

33. Read the absorbance at 520 nm

XI. Calculations

Pectin content is calculated from the sample absorbance based on the linear regression equation of the absorbances ($A_{520\ nm\ GA}$) and concentrations of galacturonic acid standards. For samples showed color before adding *m*-hydroxydiphenyl solution, the final absorbances are subtracted of the sugar interference absorbance before used for calculation.

Linear regression of galacturonic acid standards (see Appendixes, 3)

$$A_{520\ nm\ Standard} = a + b \times Concentration_{Standard}\ (\mu g/ml)$$

Pectin level in undiluted sample

$$\text{Pectin Level}\,(\text{mg GA/l})$$

$$= (\text{Net } A_{520\ nm\ Sample})\left(\frac{A_{520\ nm\ Standard} - a}{b}\ \mu g/ml\right)\left(\frac{1\,mg}{1000\ \mu g}\right)(1000\,ml)$$

$$= (\text{Net } A_{520\ nm\ Sample})\left(\frac{A_{520\ nm\ Standard} - a}{b}\right)$$

where

(Net $A_{520\ nm\ Sample}$) = ($A_{520\ nm}$ of Filtrate) – ($A_{520\ nm}$ of Sugar Inference)

Pectin level in 100 ml filtrate contains 15 ml juice sample

$$\text{Pectin Level (mg GA/l)} = \frac{(\text{Net } A_{520\text{ nm Sample}})\left(\frac{A_{520\text{ nm Standard}} - a}{b}\right)}{(\text{ml Sample per ml Filtrate})}$$

$$= \frac{(\text{Net } A_{520\text{ nm Sample}})\left(\frac{A_{520\text{ nm Standard}} - a}{b}\right)}{\frac{(15\text{ ml Sample})}{(100\text{ ml Filtrate})}}$$

$$= (\text{Net } A_{520\text{ nm Sample}})\left(\frac{A_{520\text{ nm Standard}} - a}{b}\right) \times 6.67$$

where

(Net $A_{520\text{ nm Sample}}$) = ($A_{520\text{ nm}}$ of Filtrate) – ($A_{520\text{ nm}}$ of Sugar Inference)

## XII. Reference

Blumenkrantz, N and G. Asbpe-Hansen. 1973. New method for quantitative determination of uronic acids. Anal. Biochem. 54:484 – 489.

Paul K. Kinter, III, and J. P. Van Buren. 1982. Carbohydrate interference and its correction in pectin analysis using the *m*-hydroxydiphenyl method. J. of Food Science, 47:756 – 764.

Rouse, A.H. and C.D. Alkins. 1955. Pectinesterase and pectin in commercial citrus juices as determined by methods used at the Citrus Experiment Station. Technical Bulletin 570. University of Florida, Agric. Exp. Sta., Gainesville, Florida.

# 21. Water Soluble Pectin (Carbazole Method)

I. Apparatus

Spectrophotometer
Vortex mixer
Thermostatically controlled water bath
Filter paper
Stopwatch or timer
16 × 150 mm Test tube
Dispenser

II. Chemicals

Ethanol ($C_2H_6O$)
Carbazole ($C_{12}H_9N$)
Sodium hydroxide (NaOH)
Sulfuric acid ($H_2SO_4$)
Galacturonic acid ($C_6H_{10}O_7$)

III. Reagents

A. Ethanol (63%): Dilute 630 ml of 95% ethanol with distilled water to 950 ml.
B. Alcoholic carbazole solution (0.1%): Dissolve 0.1 g of carbazole in ethanol and make up to 100 ml. A mixture of 1 ml of water, 0.5 ml of alcoholic carbazole solution, and 6 ml of concentrated sulfuric acid must be water clear or almost so.
C. Sodium hydroxide solution (1 N): Dissolve 40 g of NaOH in 1000 ml of distilled water.
D. Galacturonic acid (GA) standard solution: Dissolve 0.1205 g of GA, previously dried for 5 h in vacuum at 30°C or at 20°C over $P_2O_5$, in distilled water, add 0.5 ml of 1 N NaOH, and make up to 1000 ml with distilled water to make a 100 μg/ml stock solution of galacturonic acid. Let mixture stand overnight. Dilute with distilled water to make 10, 20, 40, 60, and 80 μg/ml standard solutions. Store in a refrigerator.

IV. Procedure

1. Heat 95% and 65% ethanol to 75°C (167°F).
2. Measure 15 ml of single-strength juice into 50 ml centrifuge tube.
3. Add hot 95% ethanol into tube to make up to 40 ml.
4. Heat for 10 min at 85°C (185°F) in a water bath, occasionally stir with a glass rod.
5. Rinse pectin off the glass rod into a centrifuge tube with 95% ethanol and make up to 50 ml.
6. Centrifuge for at least 15 min at 2000 ×*g*.

7. Decant the supernatant.
8. Repeat steps 4 to 7 with hot 63% ethanol.
9. Add 5 ml of distilled water to the centrifuge tube with the precipitate.
10. Stir up the precipitate with a rubber policeman.
11. Rinse the policeman with 30 ml of distilled water.
12. Vigorously agitate the mixture with a stir bar until precipitate is well dissolved.
13. Rinse the stir bar and add water to bring to 40 ml.
14. Centrifuge for 15 min at 2000 $\times g$.
15. Collect the supernatant in a 100 ml graduated flask.
16. Repeat the water extraction (steps 2 to 7)
17. Collect the second supernatant into the same graduate flask.
18. Add 5 ml of 1 N NaOH to the combined supernatants.
19. Add distilled water to make up to 100 ml and mix.
20. Let stand for at least 15 min with occasional shaking.
21. Filter the solution. The filtrate is used for analysis.
22. Add 5 ml of 1 N NaOH.
23. Add distilled water to make up to 100 ml.
24. Let stand for at least 15 min with occasional shaking.
25. Filter the solution. The filtrate is used for water soluble pectin analysis.
26. Label a set of test tubes, in triplicate, for the followings:

    - Ethanol reagent blank
    - Carbazole reagent blank
    - Samples in ethanol
    - Samples in carbazole

27. Add 1 ml of distilled water to each tube of :

    - Ethanol reagent blank
    - Carbazole reagent blank

    Add 1 ml of pectin filtrate to each tube of:

    - Samples in ethanol
    - Samples in carbazole

28. Add 0.5 ml of ethanol to each tube of :

    - Ethanol reagent blank
    - Samples in ethanol

    Add 0.5 ml of carbazole solution to each tube of:

    - Carbazole reagent blank
    - Samples in carbazole

29. Dispense 6 ml of $H_2SO_4$, over a period of 7 seconds, to each of all the test tubes with continual shaking.
30. Immediately place test tubes in an 85°C water bath for 5 min.
31. Remove from water bath to cool at ambient temperature for 15 min.
32. Zero spectrophotometer with combined ethanol reagent blank.
33. Immediately read absorbance at 525 nm of sample in ethanol.
34. Zero spectrophotometer with combined carbazole reagent blank.
35. Immediately read samples in carbazole.

V. Calculations

Pectin content is calculated from the sample absorbance and the linear regression equation of the absorbances ($A_{525\text{ nm Standard}}$) and concentrations of galacturonic acid standards.

Linear regression of galacturonic acid standards (see Appendixes, 3)

$$A_{525\text{ nm Standard}} = a + b \times \text{Concentration}_{\text{Standard}}\ (\mu g/ml)$$

Pectin level in undiluted sample

$$\text{Pectin Level (mg GA/l)}$$

$$= (\text{Net } A_{525\text{ nm Sample}})\left(\frac{A_{525\text{ nm Standard}} - a}{b}\ \mu g/ml\right)\left(\frac{1\text{ mg}}{1000\ \mu g}\right)(1000\text{ ml})$$

$$= (\text{Net } A_{525\text{ nm Sample}})\left(\frac{A_{525\text{ nm Standard}} - a}{b}\right)$$

where

(Net $A_{525\text{ nm Sample}}$) = ($A_{525\text{ nm}}$ of Sample in Carbazole) – ($A_{525\text{ nm}}$ of Sample in Ethanol)

Pectin level in 100 ml filtrate contains 15 ml juice sample

$$\text{Pectin Level (mg GA/l)} = \frac{(\text{Net } A_{525\text{ nm Sample}})\left(\frac{A_{525\text{ nm Standard}} - a}{b}\right)}{(\text{ml Sample per ml Filtrate})}$$

$$= \frac{(\text{Net } A_{525\text{ nm Sample}})\left(\frac{A_{525\text{ nm Standard}} - a}{b}\right)}{\frac{(15\text{ ml Sample})}{(100\text{ ml Filtrate})}}$$

$$= (\text{Net } A_{525\text{ nm Sample}})\left(\frac{A_{525\text{ nm Standard}} - a}{b}\right) \times 6.67$$

where

(Net $A_{525\text{ nm Sample}}$) = ($A_{525\text{ nm}}$ of Sample in Carbazole) – ($A_{525\text{ nm}}$ of Sample in Ethanol)

## VI. Reference

Rouse, A.H. and C.D. Alkins. 1955. Pectinesterase and pectin in commercial citrus juices as determined by methods used at the Citrus Experiment Station. Technical Bulletin 570. University of Florida, Agric. Exp. Sta., Gainesville, Florida.

# 22. Total Pectin (Carbazole Method)

I. Apparatus

See Water Soluble Pectin in Chapter IV, 22

II. Chemicals

See Water Soluble Pectin in Chapter IV, 22

III. Reagents

A. See Water Soluble Pectin in Chapter IV, 22

IV. Procedure

1. Follow steps 1 to 9 as in Water Soluble Pectin in Chapter IV, 22
2. Add 5 ml of distilled water to the centrifuge tube with the precipitate.
3. Stir up the precipitate with a rubber policeman.
4. Transfer precipitate to a 100 ml graduated flask.
5. Rinse the centrifuge tube and policeman with distilled water and add water to ~ 50 ml.
6. Vigorously agitate the mixture with a stir bar until precipitate is well dissolved.
7. Rinse the stir bar.
8. Add 5 ml of 1 N NaOH.
9. Add distilled water to make up to 100 ml and mix.
10. Let stand for at least 15 min with occasional shaking.
11. Filter the solution. The filtrate is used for total pectin analysis.
12. Measure the pectin level in filtrate following steps 26 to 32 as in Water Soluble Pectin in Chapter IV, 22

V. Calculations

See Water Soluble Pectin in Chapter IV, 22

VI. Reference

See Water Soluble Pectin in Chapter IV, 22

# 23. Diacetyl

I. Apparatus

Spectrophotometer with cuvet or tube
Distillation apparatus (see Figure IV – 10)
25 × 150 ml test tube
100 ml beaker

II. Chemicals

Diacetyl ($C_4H_6O_2$)
Potassium hydroxide (KOH)
α-Naphthol ($C_{10}H_8O$)
Isopropanol (99%) ($C_3H_8O$)
Creatine ($C_4H_9N_3O_2$)

III. Reagents

A. α-Naphthol solution (5%): Dissolve 5 g of α-Naphthol in 100 ml 99% isopropanol.
B. Creatine-potassium hydroxide solution (0.3%): Dissolve 40 g of KOH in about 60 ml distilled water, cool, and add 0.3 g of creatine or 0.5 g of creatine hydrate. Make up to 100 ml with distilled water. This solution is stable for at least 3 days at 4°C (40°F).
C. Diacetyl standard solutions: Make a stock solution of 1 mg/ml (1000 ppm) in distilled water and dilute with distilled water to make 0.5, 1, 2, 3, 5, 7, and 10 ppm solutions.

IV. Procedure

1. Transfer 300 ml single-strength or reconstituted juice to a boiling flask.
2. Distill and collect distillate, at a rate of ~5 ml/min, by letting it flow down the side of a graduated cylinder.
3. Collect three 25-ml portions of distillate.
4. Discard the second 25-ml portion of the distillate in 100 ml beakers.
5. Label a set of test tubes, in triplicate, for the followings:

   - Blank
   - Diacetyl standards
   - First 25-ml distillate
   - Third 25-ml distillate

6. Pipette 10 ml of the proper solutions into the designated test tubes, avoiding the floating peel oil in the distillates, add distilled water for blank.

7. Add 5 ml α-Naphthol solution to each tube.
8. Add 2 ml of creatine-KOH solution to each tube.
9. Stopper tubes and mix thoroughly by inverting (about 15 seconds).
10. Wait 5 min and mix again.
11. Zero the instrument with the blank.
12. Read absorbance at 530 nm.

V. Calculations

Diacetyl content is calculated from the sample absorbance corrected for acetylemethylcarbinol based on the linear regression equation of the absorbance peak area ($PA_{Standard}$) and concentrations of diacetyl standards. Acetylmethylcarbinol distills over at a uniform rate in the described test procedure, and therefore, presents an equal quality in the first and third 25-ml portions of the distillate. Acetylemethylcarbinol is a fermentation product and reacts with the creatine to give the same color produced with diacetyl. Correction for acetylemethylcarbinol is done by subtracting the absorbance of the third 25-ml distillate from that of the first 25-ml distillate.

Linear regression of diacetyl standards (see Appendixes, 3)

$$A_{530\ nm\ Standard} = a + b \times Concentration_{Standard}\ (\mu g/ml)$$

Diacetyl level in juice sample with correction for acetylemethylcarbinol

$$\text{Diacetyl Level (ppm)}$$

$$= (\text{Net } A_{530\ nm\ Sample})\left(\frac{A_{Standard} - a}{b}\ ppm\right)\left(\frac{\text{Volume of Distillate}}{\text{Volume of Sample}}\right)$$

$$= (\text{Net } A_{530\ nm\ Sample})\left(\frac{A_{Standard} - a}{b}\ ppm\right)\left(\frac{25\ ml}{300\ ml}\right)$$

$$= (\text{Net } A_{530\ nm\ Sample})\left(\frac{A_{Standard} - a}{b}\ ppm\right) \div 12$$

where

(Net $A_{530\ nm\ Sample}$) = ($A_{530\ nm}$ of 3rd Distillate) – ($A_{530\ nm}$ of 1st Distillate)

## VI. Reference

Citrus Handbook. 1998. Agricultural Marketing Service, USDA. Washington, D.C.

Byer, E.M. 1954. Visual detection of either diacetyl or acetyl-methyl-carbinol in frozen concentrated orange juice. Food Tech. 8:174 – 174.

Hill, E.C., Wenzel, F.W., and Barreto, A. 1954. Colorimetric method for detection of microbiological spoilage in citrus juices. Food Tech. 3:168 – 171.

# 24. Ascorbic Acid by Indophenol Titration

I. Apparatus

50 ml Buret
10 ml Pipette
Magnetic stirrer and Teflon® coated stirring bar
16 × 150 ml test tube
50 ml flask
250 ml amber glass bottle
fluted filter paper, particle retention > 20 μm

II. Chemicals

Sodium 2,6-dichloroindophenol
Sodium bicarbonate ($NaHCO_3$)
Metaphosphoric acid ($HPO_3$)
Acetic acid (glacial) ($C_2H_4O_2$)
Ascorbic acid ($C_6H_8O_6$)

III. Reagents

A. Dye solution (0.5%): Dissolve 0.042 g of $NaHCO_3$ in distilled water and then add 0.050 g of sodium 2,6-dichloroindolphenol, shake vigorously. When dye dissolves, make up to 200 ml. Filter through fluted paper into an amber glass bottle and stored capped in a refrigerator. The solution is good until it fails to give a distinct endpoint.
B. Acid stabilization solution (3%): Dissolve, with shaking, 15 g of $HPO_3$ in a mixture of 40 ml of glacial acetic acid and 200 ml of distilled water and make up to 500 ml with distilled water. Filter solution rapidly through filter paper into a glass bottle. The solution remains stable for 7 to 10 days when stored in a refrigerator.
C. Ascorbic acid standard solution (1 mg/ml): Accurately weigh 0.100 g of ascorbic acid into a 100-ml volumetric flask. Immediately before use, dissolve in 100 ml of acid stabilization solution.

IV. Procedure

1. Label a set of 50-ml glass flasks, in triplicate, for:

- Blank
- Ascorbic acid standards
- Juice samples

2. Add 5 ml of acid stabilization solution to each flask.

3. Add 2 ml of the proper solutions to the designated test tube, for the blank add distilled water.
4. Titrate rapidly with the dye solution until a light but distinct rose pink color persists for at least 5 seconds.

V. Calculations

$$\text{Ascobic Acid (mg/100 ml)}$$

$$= \frac{(\text{Net ml Titrant for Sample})\,(\text{Ascorbic Acid Equivalent})}{(\text{Sample Volume})}$$

$$= \frac{(\text{Net ml Titrant for Sample})\,\dfrac{(\text{mg Ascorbic Acid in Stardand})}{(\text{Net ml Titrant for Standard})}}{(\text{ml Sample})} \times 100$$

$$= \frac{(\text{Net ml Titrant for Sample})\,\dfrac{(\text{ml Standard})(\text{mg/ml Standard})}{(\text{Net ml Titrant for Standard})}}{(\text{ml Sample})} \times 100$$

$$= \frac{(\text{Net ml Titrant for Sample})(\text{ml Standard})(\text{mg/ml Standard})}{(\text{Net ml Titrant for Standard})(\text{ml Sample})} \times 100$$

where

(Net ml Titrant for Sample) = (ml Titrant for Sample) – (ml Titrant for Blank)

(Net ml Titrant for Standard) = (ml Titrant for Standard) – (ml Titrant for Blank)

For analysis of both juice and standard using the same analyte volume (i.e., 2 ml in this test) and using ascorbic standard solution of 1 mg/ml, the ascorbic acid level is:

$$\text{Ascorbic Acid (mg/100 ml)} = \frac{(\text{Net ml Titrant for Sample})}{(\text{Net ml Titrant for Standard})} \times 100$$

## VI. Reference

Official Methods of Analysis. 1999. 16th Edition, 5th Reversion, AOAC International, Gaithersburg, MD, method 967.21.

# 25. Ascorbic Acid by HPLC

I. Apparatus

HPLC system with a reverse phase column (Zorbax ODS, 250 mm × 4.6 mm, 5 μm particle size), Zorbax ODS guard column (4 mm × 3.4 mm, 5 μm particle size), UV-visible detector, and integrator.

Centrifuge
C18 Sep-Pak cartridge
1.2 μm Glass fiber filter
25 μl Syringe
10 ml Syringe
16 × 150 mm test tube

II. Chemicals

L - Ascorbic acid ($C_6H_8O_6$)
Metaphosphoric acid ($HPO_3$)
Potassium phosphate, monobasic ($KH_2PO_4$)
Acetonitrile (HPLC grade)($C_2H_3N$)
Methanol ($CH_3OH$)
Water (HPLC grade)
Quinic acid ($C_7H_{12}O_6$)

III. Reagents

A. Mobile phase solution (2%): Dissolve 20 g of $KH_2PO_4$ in HPLC grade water and make to 1000 ml (pH 2.4). Filter through 0.45 μm nylon filter and degas with vacuum.
B. Metaphosphoric acid solution (2.5%): Dissolve 25 g of $HPO_3$ in distilled water and make up to1000 ml.
C. Ascorbic acid standard stock solution (100 ppm): Dissolve 100 mg of ascorbic acid in 100 ml of 2.5% $HPO_3$ to make a 1000 ppm stock solution and dilute with 2.5% $HPO_3$ (1:9, v/v) make a 100 ppm stock solution. Keep solution frozen and in the dark.
D. Ascorbic acid standard solution (10 ppm or 1 mg/100 ml): Dilute 1 ml of the 100 ppm stock solution with 9 ml of 2.5% $HPO_3$. Prepare this standard solution just before use.

IV. Procedure

1. Mix 5 ml of juice with 5 ml of 2.5% $HPO_3$ solution in test tube.
2. Centrifuge mixture at 5000 ×g for 10 min at 5°C (41°F).

3. Dilute 0.5 ml of the supernatant with 2.5% $HPO_3$ solution to 10 ml (if internal standard is desired, include 1 ml of 15% quinic acid in 2.5% $HPO_3$ within the 10 ml final volume).
4. Filter the mixture through a 0.45 μm nylon filter using a 10-ml syringe and into a LC vial.
5. Set the HPLC system at:

   Flow rate = 0.5 ml/min
   Detection wavelength = 245 nm
   Run time = 15 min

6. Gradually bring the system solvent from the 70% acetonitrile to 5% acetonitrile.
7. Change to and equilibrate system with the mobile phase.
8. Make duplicate 10 μl-injections for each standard and juice sample.
9. After using, bring system solvent back to 5% acetonitrile and then 70% acetonitrile.

V. Calculations

1. Ascorbic acid in samples is identified by comparison of retention time with a standard and its concentration (mg/100 ml) is calculated from the following formula:

$$\text{Ascorbic Acid (mg/100 ml)}$$

$$= \frac{(\text{Peak Area for Sample})\,(\text{Ascorbic Acid Equivalent})}{\dfrac{(\text{Sample Volume})}{(\text{Sample Dilution Factor})}}$$

$$= \frac{(\text{Peak Area for Sample})\,\dfrac{(\text{Standard Volume})(\text{Standard Concentration})}{(\text{Peak Area for Standard})}}{\dfrac{(\text{Sample Volume})}{(\text{Sample Dilution Factor})}}$$

2. For analysis of both juice and standard using the same injection volume and using ascorbic standard solution of 1 mg/100 ml or 10 ppm, the ascorbic acid level is calculated using the following formula. Ascorbic acid in samples is identified by comparison of retention time with a standard.

$$\text{Ascorbic Acid (mg/100 ml)} = \frac{(\text{Sample Peak Area})}{(\text{Standard Peak Area})} \times 400$$

## VI. Reference

Lee, H.S. and G.A. Coates. 1999. Vitamine C in frozen, freshly squeezed, unpasteurized polyethylene-bottled orange juice: a storage study. Food Chemistry 65:165 – 168.

Lee, H.S. and G.A. Coates. 1987. Liquid chromatographic determination of vitamin C in commercial Florida orange juice J. Micronutrient Analysis 3:199 – 209.

# 26. Ascorbic Acid by Iodine Titration

I. Apparatus

25 ml Buret with a 0.1 ml graduation, prefer a Digital buret
25 ml Pipette
Magnetic stirrer and Teflon® coated stirring bar
150 ml Glass beaker

II. Chemicals

Iodine ($I_2$)
Potassium iodide (KI)
Sulfuric acid (concentrate, $H_2SO_4$)
Starch
Salicylic acid ($C_7H_6O_3$)

III. Reagents

A. Iodine solution (0.1 N): Dissolve 12.69 g of $I_2$ and 19.52 g of KI in 50 ml of distilled water and then dilute to 1000 ml. Store solution in dark brown glass bottle away from light.

To standardize the solution, accurately measure 50 ml of the standard 0.1 N $As_2O_3$ solution (see Chapter IV, 10) into a 150-ml beaker, add 2 g solid $NaHCO_3$, add 0.5 ml of 1% starch solution as indicator, and titrate with the iodine solution.

$$\text{Normality of Iodine Solution} = \frac{(\text{ml } As_2O_3)(\text{N } As_2O_3)}{(\text{ml } I_2)}$$

B. Starch solution (1%): Mix 10 g of soluble starch with 100 ml of distilled water. Add to 900 ml of boiling water under continuous stirring. Cool and salicylic acid can be added as a preservative. Store in a refrigerator.

C. Starch-acid solution: Pre-mix 977 ml of distilled water, 17 ml of 1% starch solution, and 19 ml (35 g at density of 1.84 g/ml) of concentrated $H_2SO_4$ to make 1000 ml of solution.

IV. Procedure

1. To a 150-ml glass beaker, add 35 ml of starch-acid solution.
2. Add 25 ml of juice sample (replace juice with distilled water for blank).
3. Titrate with 0.1 N iodine solution from buret (covered from light) while under stirring until the first stable blue color appears.

V. Calculations

Since one mole of ascorbic acid reacts with one mole of iodine, each ml of 0.1 N iodine is equivalent to 8.806 g of ascorbic acid (MW 176.12):

$$\text{Ascorbic Acid (mg/100 ml)}$$

$$= \frac{(\frac{\text{Net ml Titrant}}{1000\ \text{ml/l}})(\text{N Titrant})(\frac{1}{2})(\text{MW of Ascorbic Acid})(1000\ \text{mg/g})}{(\text{ml Sample})} \times 100$$

$$= \frac{(\frac{\text{Net ml Titrant}}{1000\ \text{ml/l}})(0.1\ \text{N})(\frac{1}{2})(176.12)(1000\ \text{mg/g})}{(\text{ml Sample})} \times 100$$

$$= \frac{(\text{Net ml Titrant})(8.806\ \text{mg})}{(\text{ml Sample})} \times 100$$

$$= \frac{(\text{Net ml Titrant})}{(\text{ml Sample})} \times 880.6$$

where $(\text{Net ml Titrant}) = (\text{ml Titrant for Sample}) - (\text{ml Titrant for Blank})$

For 25 ml juice

$$\text{Ascorbic Acid (mg/100 ml)} = (\text{Net ml Iodine}) \times 35.2$$

For 25 ml of juice the equivalent % US RDI of 60 mg ascorbic acid per service of 8 fluid ounces (240 ml) is:

$$\text{Ascorbic Acid (\%US RDI per 8 Fluid Ounces)}$$

$$= \frac{(\text{Net ml Iodine})(8.806\ \text{mg/ml})}{(25\ \text{ml})} \times \frac{(240\ \text{ml/8 oz})}{(60\ \text{mg})} \times 100$$

$$= (\text{Net ml Iodine}) \times 141$$

## VI. Reference

Official Methods of Analysis. 1999. 16th Edition, 5th Reversion, AOAC International, Gaithersburg, MD, method 939.13 and 966.18.

Food Chemicals Codex. 1996. 14th ED., Food and Nutrition Board Institute of Medicine National Academy of Sciences, National Academy Press, Washington, D.C., p. 33.

# 27. Naringin (Davis Test)

I. Apparatus

Spectrophotometer
1 ml pipette graduated in 0.01 ml
25 × 150 mm Test tube with screw cap
Centrifuge

II. Chemicals

Naringin ($C_{27}H_{32}O_{12}$)
Diethylene glycol ($C_4H_{10}O_3$)
Sodium hydroxide (NaOH)

III. Reagents

A. Diethylene glycol solution (90%): To 900 ml of diethylene glycol, add distilled water to 1000 ml and mix thoroughly.
B. Sodium hydroxide solution (4 N): Dissolve 16 g of NaOH in distilled water and make up to 100 ml.
C. Naringin standard solutions: Recrystallize commercial naringin in isopropanol, dried at 85°C (185°F), and store in a desiccator. Prepare a 1000 ppm stock solution by dissolving 100 mg of naringin in 100 ml of warm distilled water. Dilute with distilled water to make 100, 200, 300, 400, 500, and 600 ppm standard solutions.

IV. Procedure

1. Centrifuge single-strength or reconstituted juice at centrifugation force of 365 ×*g* for 10 min (see also Table IV – 4). Use the supernatant for analysis.
2. Label a set of test tubes, in triplicate, for:

   - Reagent blank
   - Naringin standards
   - Samples

3. Add 25 ml of diethylene glycol to each tube.
4. Add 0.5 ml of the proper solution to each tube, for reagent blank add distilled water.
5. Cap tubes and mix thoroughly by inversion.
6. Zero instrument at 420 nm with the reagent blank.
7. Read at 420 nm the backgroup absorbance of samples (Sample Blank).
8. Add 0.5 ml 4 N NaOH to the followings:

- Reagent blank
- Naringin standards
- Samples

9. Cap tubes and mix thoroughly by inversion.
10. Allow tubes to stand for 10 min at ambient temperature until yellow color fully develops.
11. Zero instrument at 420 nm again with the reagent blank.
12. Read absorbance at 420 nm of naringin standards and samples.

## V. Calculations

Naringin concentration (ppm) in sample is calculated from sample absorbance based on a linear regression equation of the standard curve of absorbance at 420 nm against concentration of naringin standards.

Linear regression of naringin standards (see Appendixes, 3)

$$A_{450\ nm\ Standard} = a + b \times Concentration_{Standard}\ (ppm)$$

Concentration of naringin in juice sample

$$Naringin\ Level\ (ppm) = (Net\ A_{450\ nm\ Sample})\left(\frac{A_{450\ nm\ Standard} - a}{b}\ ppm\right)$$

$$= (Net\ A_{450\ nm\ Sample})\left(\frac{A_{450\ nm\ Standard} - a}{b}\right)$$

where

(Net $A_{450\ nm}$) = ($A_{450\ nm}$ of Sample) – ($A_{450\ nm}$ of Sample Blank)

## VI. Reference

Citrus Handbook. 1998. Agricultural Marketing Service, USDA. Washington, D.C.

Davis, W.B. 1947. Determination of flavanones in citrus fruits. Anal. Chem. 19:476 – 478.

# 28. Naringin by HPLC

I. Apparatus

HPLC system with a reverse phase column (Microsorb-MV, 150 mm × 4.6 mm, 5 μm particle size) and UV-visible detector.
Centrifuge
1.2 μm Glass fiber filter
25 μl Syringe
10 ml Syringe
16 × 150 mm test tube

II. Chemicals

Naringin ($C_{27}H_{32}O_{12}$)
Acetonitrile (HPLC grade)($C_2H_3N$)
Glacial acetic acid ($C_2H_4O_2$)
Water (HPLC grade)
Desired standard compounds

III. Reagents

A. Mobile phase solution: Mix, by volume, 79.5 parts of acetonitrile, 20 parts of water, and 0.5 parts of glacial acetic acid. Prepare the mobile phase 3-4 days in advance to allow for equilibrium or degass with vacuum.
B. Naringin standard solutions: Dissolving 50 mg of naringin in 100 ml of mobile phase in a volumetric flask to make a 500 ppm stock solution. Prepare weekly standard solutions by diluting the stock solution to 10, 50, 100, 150, and 250 ppm with the mobile phase.

IV. Procedure

1. Centrifuge approximately 10 ml of juice sample at 2500 ×*g* for 10 min.
2. Dilute 1 ml of supernatant with 9 ml of HPLC grade water and mix thoroughly.
3. Filtrate mixture through a glass fiber filter using a 10-ml syringe directly into a LC vial.
4. Set the HPLC system at:

   Flow rate = 1.0 ml/min
   Detection wavelength = 280 nm
   Run time = 10 min

5. Equilibrate system with mobile phase for at least 30 min.

6. Make duplicate 10 µl-injections for each standard and juice sample.
7. After using, bring system solvent back to acetonitrile.

V. Calculations

Naringin is identified by comparison of retention time with a standard. Naringin concentration (ppm) in sample is calculated from sample absorbance based on a linear regression equation of the standard curve of absorbance peak area (PA) at 280 nm against concentration of naringin standards.

Linear regression of naringin standards (see Appendixes, 3)

$$PA_{Standard} = a + b \times Concentration_{Standard}\ (ppm)$$

Concentration of naringin in juice sample

$$\text{Naringin Level}\,(ppm) = (PA_{Sample})\left(\frac{PA_{Standard} - a}{b}\ ppm\right)(\text{Sample Dilution Factor})$$

$$= (PA_{Sample})\left(\frac{PA_{Standard} - a}{b}\right) \times 10$$

VI. Reference

Rouseff, R.L. 1988. Liquid chromatographic determination of naringin as a detector of grapefruit juice in orange juice J. Assoc. Off. Anal. Chem. 71:798 – 802.

# 29. Limonin by HPLC

I. Apparatus

HPLC system with a reverse phase column (Microsorb $C_{18}$, 150 mm × 4.6 mm, 5 μm particle size), $C_{18}$ guard column (30 mm × 2 mm, 5 μm), a UV-visible detector, and an integrator.

Hot plate or stove
Centrifuge
$C_{18}$ Cartridge
0.45 μm nylon filter
25 μl Syringe
10 ml Syringe
50 ml centrifuge tube

II. Chemicals

Limonin
Acetonitrile (HPLC grade)
Methanol (HPLC grade)
Tetrahydrafuran (HPLC grade)
Water (HPLC grade)

III. Reagents

A. Mobile phase solution: Mix, by volume, 67.5 parts of water, 17.5 parts of acetonitrile, and 15 parts of tetrahydrofuran. Make 3-4 days in advance to allow for equilibrium.
B. Limonin standard solutions: Prepare a stock solution of 50 ppm by dissolving 5.0 mg of limonin in 2.0 ml of acetonitrile in a volumetric flask and make to 100 ml with methanol. Prepare standard solutions weekly by diluting the stock solution to 1, 5, 10, 15, and 25 ppm with the mobile phase.

IV. Procedure

1. Heat juice sample of about 60 ml in boiling water bath for 3 – 5 min to develop limonin. Heating is not needed for concentrate and pasteurized juice samples.
2. Centrifuge 25 ml of the juice at 2500 ×g for 10 min
3. Precondition $C_{18}$ cartridges by passing through 2.5 ml of acetonitrile followed by 5 ml of HPLC grade water under vacuum until all water just enters the $C_{18}$ bed.
4. Load 2.5 ml of juice supernatant on the preconditioned $C_{18}$ cartridge. For samples with low limonin content, increase load volume accordingly.
5. Slowly filtrate the juice supernatant under vacuum or pressure.
6. Rinse cartridges with 5 ml of HPLC grade water and free the $C_{18}$ bed of water.

7. Slowly elute limonin from the cartridge with 2.5 ml of acetonitrile.
8. Filtrate acetonitrile effluent through a 0.45 μm nylon filter and into a LC vial.
9. Set the HPLC system at:

   Flow rate = 1.5 ml/min
   Detection wavelength = 210 nm
   Run time = 10 min

10. Make duplicate 10 μl-injections for each standard and filtrated sample.
11. After using, bring the system back to acetonitrile.

V. Calculations

Limonin is identified by comparison of retention time with a standard. Limonin concentration (ppm) is calculated from sample absorbance based on a linear regression equation of the standard curve of absorbance peak area (PA) against concentration of limonin standards.

Linear regression for limonin standards (see Appendixes, 3)

$$PA_{Standard} = a + b \times Concentration_{Standard}\ (ppm)$$

Concentration of limonin from 25 ml of juice in 2.5 ml of acetonitrile

$$\text{Limonin Level}\,(ppm) = (PA_{Sample})\left(\frac{PA_{Standard} - a}{b}\ ppm\right)(\text{Sample Dilution Factor})$$

$$= (PA_{Sample})\left(\frac{PA_{Standard} - a}{b}\right) \times 10$$

VI. Reference

Shaw, P.E. and Wilson, C.W. 1984. A rapid method for determination of limonin in citrus juices by high performance liquid chromatography. J. Food Sci. 49:1216 – 1218.

# 30. Headspace Volatiles by GC

I. Apparatus

GC system with a polyethylene glycol column (DB-Wax, 60 m × 0.53 mm, 1 μm film thickness), sample heating block, a flame ionization detector, and an integrator
25 μl Syringe
20 ml GC vial with crimp seal cap
Cap crimper and decrimper

II. Chemicals

Helium
Desired standard compounds

III. Reagents

A. Standard solutions: Prepare standard compounds in distilled water or enrich juice sample with standard solutions.

IV. Procedure

1. Set the GC system at:

Injector temperature = 160°C
Detector temperature = 220°C
Oven temperatures = start at 40°C for 6 min, increases to 180°C at a rate of 4°C/min, stays at 180°C for 5 min
Flow rate = 0.985 ml/min
Run time = 46 min

2. Add 5 ml of standard mixture in a 20-ml GC vial and seal vial.
3. Add 5 ml of thoroughly mixed juice in a 20-ml GC vial and seal vial.
4. Heat standard and juice sample individually at 85°C (185°F) for 15 min right before injection.
5. Make duplicate 20 μl-injections for each standard and samples.

V. Calculations

Juice headspace volatile compounds are identified by comparison of retention time with standards and by enrichment of the individual compound. Juice headspace volatile concentrations (ppm) are calculated from a linear regression equation of the standard curve of absorbance peak area (PA) against concentration of the respect standards.

Linear regression of standards for a specific compound (see Appendixes, 3)

$$PA_{Standard} = a + b \times Concentration_{Standard}\ (ppm)$$

Concentration of the specific compound under the test conditions

$$\text{Compound Level}\,(ppm) = (PA_{Sample})\left(\frac{PA_{Standard} - a}{b}\ ppm\right)$$

$$= (PA_{Sample})\left(\frac{PA_{Standard} - a}{b}\right) \times 10$$

VI. Reference

Nisperos-Carriedo, M.O. and P.E. Shaw. 1990. Volatile flavor components of fresh and processed orange juices. Food Tech. 134 – 138.

FMC Technologies, Inc., FMC FoodTech, Citrus Systems.

# Chapter V. Pulp Analysis

## 1. Quick Fiber (PulpView™ Method)

I. Apparatus

- PulpView™
- 12″ Pulp sampling tube with mark lines at 8″ from the insert end
- 14″ Plunger rod

II. Chemicals

None

III. Reagents

None

IV. Procedure

1. Make sure the sample port in the magnet assembly and the sampling tube and rod are clean and dry.
2. Turn on the PulpView™.
3. Scroll to select the proper mode by pressing the MODE button:

   QF ----- for orange pulp
   SI ----- for grapefruit pulp
   PD ----- for pulp density

4. Mix the bulk pulp sample.
5. Insert the tube into the pulp while pulling back the plunger rod simultaneously until the pulp fills to the 8" mark line. There should be no large air cavity in the sample.
6. Wipe off any pulp on the outside of the tube.
7. Insert filled tube into the sample port of the magnet assembly until the 8" mark line is at the edge of sample port.
8. Zero the instrument by press the CAL button on the electronic assembly, more than once if required. (display should read 0 Hz)
9. Press the RUN button.
10. The displayed value is the pulp dryness measurement for the selected mode.

V. Calculations

Value in the QF mode is the QF value (ml)
Value in the SI mode is the saturation index. The SI plus 100 equals the QF value.
Value (g/L) in the PD mode is the pulp density.

VI. Reference

FMC Technologies, Inc., FMC FoodTech, Citrus Systems. Instruction manual, model 2150 PulpView™ magetic resonance analyzer, Discover 2000.

Table V – 1. Industrial guideline of pulp dryness in relationship to quick fiber values.

| Pulp Condition | Pulp Quick Fiber Value (ml) |
|---|---|
| Very tight finish | < 130 |
| Tight finish | 130 – 150 |
| Loose finish | 150 – 180 |
| Very loose finish | 180 – 210 |


Figure V – 1. Magnetic assembly and instrument panels of the FMC FoodTech PulpView™.

# 2. Quick Fiber (FMC FoodTech Shaker Method)

I. Apparatus

- FMC FoodTech Quick Fiber Device with a 40 mesh screen of 5″ diameter and 2 ¾″ deep, made with woven stainless steel wire 0.010″ in diameter and containing 40 openings, 0.015 square inches, per linear inch of screen
- Timer or stopwatch
- Spatula (25 mm /1″ width) with flat edge
- 1000 ml beaker
- 250 ml graduated cylinder

II. Chemicals

None

III. Reagents

None

IV. Procedure

1. Collect representative pulp samples fresh from finishers (within 30 min of production).
2. Mix sample thoroughly.
3. Weight 200 g of pulp into an 1-liter beaker.
4. Add 200 ml of water.
5. Mix by hand-stirring using a spatula for 1 min.
6. Allow the mixture to stand for 3 min.
7. Stir again for 1 min.
8. Immediately transfer the mixture to the 40 mesh screen placed in the Quick Fiber Device.
9. Shake for 3 min.
10. Pure the free liquid in the collection tray to a 250-ml graduated cylinder.
11. Read free liquid volume in millimeters.

V. Calculations

Quick Fiber = Free Liquid Volume (ml)

## VI. Reference

The Minute Maid Company, The Coca Cola Company.

FMC Technologies, Inc., FMC FoodTech, Citrus Systems.

Ting, S.V. and R.L. Rouseff. 1986. Citrus fruits and their products. p. 65 – 66. Marcel Dekker, Inc. New York.

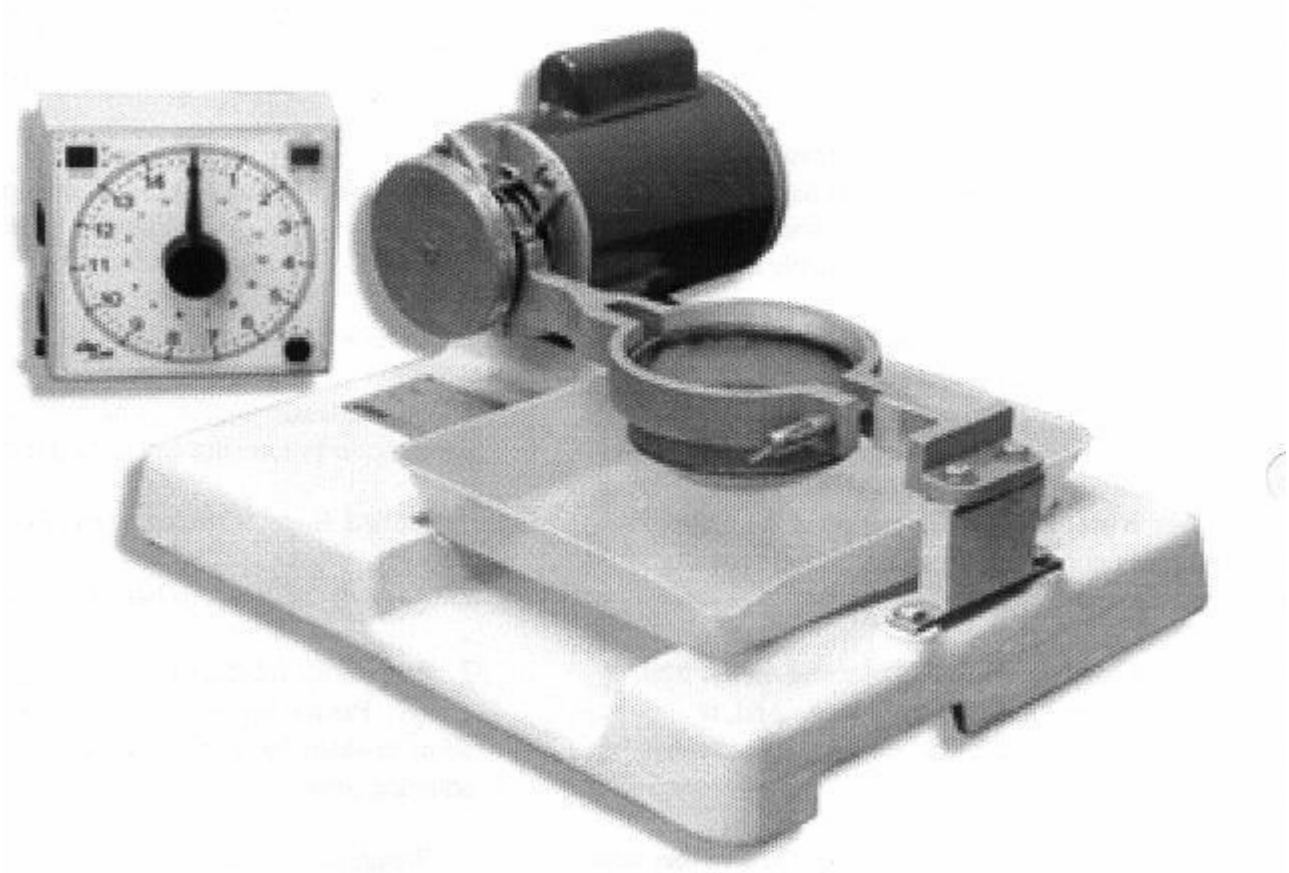


Figure V – 2. FMC FoodTech Quick Fiber Device.

# 3. Defects (FMC FoodTech Method)

I. Apparatus

- 1000 ml Standard glass beaker with a 100 mm (4″) diameter
- 60 mesh Screen
- Spatula (25 mm/1″ width) with flat edge

II. Chemicals

None

III. Reagents

None

IV. Procedure

1. If necessary, thaw pulp sample at room temperature.
2. Drain pulp on a 60 mesh screen to eliminate excess juice.
3. Weigh 50 g of pulp into a 1000 ml beaker.
4. Add distilled water to bring volume to 1000 ml.
5. Mix thoroughly with a spatula.
6. Let the beaker stand for at least 5 min.
7. Hold beaker above head and underneath a good ceiling light or illuminate the bottom of the beaker with a strong flashlight.
8. Count the defects at the bottom of the beaker.

V. Calculations

Defects are materials that affect the appearance of the pulp, including particles of membrane, core materials, peel, and seeds.

VI. Reference

FMC Technologies, Inc., FMC FoodTech, Citrus Systems.

# 4. Concentration

I. Apparatus

- FMC FoodTech Quick Fiber Device with a 20 mesh screen (see Chapter V, 1 and IV, 12)
- Analytical balance
- 500 ml graduated cylinder

II. Chemicals

None

III. Reagents

None

IV. Procedure

1. If necessary, thaw the juicy pulp sample at room temperature.
2. Wet a 20 mesh screen with some juice or water and shake off excess liquid by hand.
3. Tare the scale with the wet screen on.
4. Replace the screen on a quick fiber device.
5. Weigh 500 ml or 500 g of pulp into the screen.
6. Turn on the quick fiber device to shake for 2 min.
7. Remove the screen and blot its bottom with a paper towel.
8. Weigh the screen with pulp.

V. Calculations

- For 500 ml of juicy pulp

$$\text{Pulp Concentration (g/l)} = \frac{(\text{Weight of Pulp, g})}{(\text{Volume of Juicy Pulp, l})}$$

$$= \frac{(\text{g Pulp})}{0.5} \times 2$$

$$= (\text{g Pulp}) \times 2$$

where

(g Pulp) = (g Pulp Plus Basket) – (g Wet Basket)

- For 500 g of sample

$$\%\ \text{Pulp (w/w)} = \frac{\text{Weight of Pulp}}{\text{Weight of Juicy Pulp}} \times 100$$

$$= \frac{(\text{g Pulp})}{(500\ \text{g})} \times 100$$

$$= (\text{g Pulp}) \times 0.2$$

where

(g Pulp) = (g Pulp Plus Basket) – (g Wet Basket)

VI. Reference

FMC Technologies, Inc., FMC FoodTech, Citrus Systems.

# 5. Liquid °Brix

I. Apparatus

- Refractometer with degrees Brix scale and ATC
- Cheesecloth
- 100 ml beaker

II. Chemicals

None

III. Reagents

None

IV. Procedure

1. Place about 100 g of pulp sample in a piece of cheesecloth.
2. Wrap up the pulp in the cheesecloth and squeeze out some liquid into a beaker.
3. Apply an aliquot of sample (~3 drops) to the refractometer prism, avoiding air bubbles and large pulp particles.
4. Cover the sample with the fogged glass and position the light beam to shine through the fogged glass.
5. Adjust the shadow to the cross hairs.
6. Read the °Brix.

V. Calculations

The information is used for monitoring °Brix of pulp (i.e., $Pulp_{in}$ and $Pulp_{out}$) in pulp washing system for the secondary solids recovery (see Chapter VIII, 3).

VI. Reference

FMC Technologies, Inc., FMC FoodTech, Citrus Systems.

# 6. Visual Analysis in Beaker

I. Apparatus

- Scale
- 1000 ml Glass beaker
- Glass rod

II. Chemicals

None

III. Reagents

None

IV. Procedure

1. Fill glass beaker with tap water.
2. Weigh 10 g of pulp into the beaker.
3. Gently stir water with a glass rod to obtain even pulp distribution in water.
4. Examine defects and pulp size and integrity.

   * If warm water is used or the pulp suspension solution is poured several times between two beakers, more pulp cells will float near the surface.

V. Calculations

None

VI. Reference

FMC Technologies, Inc., FMC FoodTech, Citrus Systems.

# 7. Visual Analysis in Petri Dish

I. Apparatus

- 60 mesh Screen (see Chapter IV, 12)
- Analytical balance
- 140 mm (5 1/2″) Diameter glass or disposable Petri dish

II. Chemicals

None

III. Reagents

None

IV. Procedure

1. Bring the pulp sample to ambient temperature.
2. Drain pulp on a 60 mesh screen to eliminate excess juice.
3. Weigh 2 g of pulp into a Petri dish.
4. Add 25 ml of distilled water to the Petri dish.
5. Swirl the Petri dish to obtain even pulp distribution.
6. Place the Petri dish against a black background.
7. Examine for defects, pulp size, and pulp integrity.

* this procedure can also be used for pulp in juice or stained pulp

V. Calculations

Defects are materials that affect the appearance of the pulp, including particles of membranous and core materials, peel, and seeds.

VI. Reference

FMC Technologies, Inc., FMC FoodTech, Citrus Systems.

# 8. Staining (FMC FoodTech Method)

I. Apparatus

- 100 ml Glass beaker
- 20 and 60 mesh Screen
- Analytical balance
- Spatula (25 mm/1" width) with flat edge

II. Chemicals

- Crystal violet

III. Reagents

A. Crystal violet solution (0.012%): Dissolve 0.12 g of crystal violet in 1000 ml of distilled water.

IV. Procedure

1. Bring pulp sample to ambient temperature.
2. If the pulp contains excess amount of juice, drain sample on a 60 mesh screen.
3. Place 15 g of pulp in a beaker.
4. Add 50 ml of crystal violet solution.
5. Mix gently with a flat edge spatula.
6. Let the mixture stand for minimum of 10 min.
7. Shake in a 20 mesh screen for 1 min.
8. The stained pulp can be used for preparation of agar or paper specimens or visual analysis.

V. Calculations

None

VI. Reference

FMC Technologies, Inc., FMC FoodTech, Citrus Systems.

# 9. Specimen on Agar or Paper

I. Apparatus

- 150 × 15 mm Petri dish
- Analytical balance
- 90 or 150 mm Diameter filter paper, Whatman No 41 or one with coarse porosity, fast flow rate and 20 – 25 μm particle retention
- 90 or 150 mm Diameter Buchner funnel

II. Chemicals

- Agar (high-gel strength)

III. Reagents

A. Agar Gel solution (0.4%): Dissolve 2 g of agar in 500 ml of hot distilled water.

IV. Procedure

1. Agar Gel Specimen

a). Prepare stained pulp as in Chapter V, 7.
b). Weigh ~ 0.5 g of stained pulp into a Petri dish. Vary the amount of pulp so to give a good pulp separation.
c). Pipet 10 ml of hot agar over the pulp in the Petri dish.
d). Swirl the Petri dish to obtain even pulp distribution in agar.
e). Allow mixture to solidify at room temperature.

2. Paper Specimen

a). Prepare stained pulp as in Chapter V, 7.
b). Weigh stained pulp into a 300 ml beaker, 0.7 g for 15 cm diameter filter paper and 0.3 g for 9 cm diameter filter paper.
c). Add 200 ml of water.
d). Swirl beaker to obtain even pulp distribution.
e). Label and place two filter paper disks inside a Buchner funnel.
f). Wet filter with some water.
g). Apply a low vacuum to the funnel.
h). Pour pulp suspension into the funnel.
i). Tap the funnel on the side to help obtain uniform distribution of pulp on the filter.
j). Apply vacuum for ~ 30 s after filtration is completed.
k). Carefully remove top filter paper and place it on plane surface to air dry.

## V. Calculations

None

## VI. Reference

FMC Technologies, Inc., FMC FoodTech, Citrus Systems.

# 10. Recoverable Oil

I. Apparatus

- See Recoverable Oil in Chapter IV, 10 and Recoverable Oil in Oil Recovery System in Chapter VI, 2

II. Chemicals

- See Recoverable Oil in Chapter IV, 10

III. Reagents

A. See Recoverable Oil in Chapter IV, 10

IV. Procedure

1. Blend 500 g of pulp sample with 1500 ml of cold distilled water for 3 min at ~ 1800 rpm in a 4-liter blender.
2. While the blender is running, carefully transfer about 15 g of the slurry into the distillation flask with a plastic disposable pipette.
3. Weight sample to the nearest 0.01 g.
4. Determine the emulsion oil content using 0.0247 N potassium bromide-bromate solution as titrant as in Recoverable Oil (Chapter IV, 10).

V. Calculations

$$\%\,\text{Oil (w/w)} = \frac{\dfrac{\text{Oil Weight in Sample}}{(\text{g Sample})}}{(\text{Sample Dilution Factor})} \times 100$$

$$= \frac{\dfrac{(\text{Titrant Volume})(\text{Titrant Oil Equivalent})(\text{Oil Specific Gravity})}{(\text{g Sample})}}{(\text{Sample Dilution Factor})} \times 100$$

$$= \frac{(\text{Net ml Titrant})(\text{Calculation Factor})}{(\text{g Sample})}$$

where

(Net ml Titrant) = (ml Titrant for Sample) – (ml Titrant for Blank)

and the Calculation Factors are shown below.

| Fruit | Dilution Factor | Specific Gravity Used (g/ml) | Calculation Factor | |
|---|---|---|---|---|
| | | | 0.0247 N $KBrO_3$-KBr | 0.100 N $KBrO_3$-KBr |
| Orange | 4 | 0.840 | 0.336 | 1.36 |
| Grapefruit<br>Lemon<br>Tangerine | 4 | 0.850 | 0.340 | 1.376 |
| Lime | 4 | 0.880 | 0.352 | 1.424 |

## VI. Reference

Official Methods of Analysis. 1999. 16th Edition, 5th Reversion, AOAC International, Gaithersburg, MD, method 968.20, 939.12, and 947.13.

FMC Technologies, Inc., FMC FoodTech, Citrus Systems.

# 11. Pectinesterase Activity

I. Apparatus

- See Chapter IV, 20

II. Chemicals

- See Chapter IV, 20

III. Reagents

A. See Chapter IV, 20

IV. Procedure

1. Blend 500 g of pulp sample with 500 ml of cold distilled water at ~ 1800 rpm for 3 min in a 4-liter blender.
2. Following the steps in Chapter IV, 20 and replace the juice sample with 1 g of homogenate of fresh pulp or 10 g of homogenate of pasteurized pulp sample.

V. Calculations

1. Pectinesterase (PE) activity is calculated and reported as PE units (PEU). One unit will release 1.0 molar equivalent of acid from pectin per minute at pH 7.8 and 30°C (86°F).

$$\text{PE Activity} = \mu\text{PEU per gram pulp}$$

$$= \text{PEU} \times 10^3 \text{ per gram pulp}$$

$$= \frac{(\text{ml NaOH})(\text{N NaOH})}{(\text{min})(\text{g Sample})} \times (\text{Dilution Factor}) \times 1000$$

$$= \frac{(\text{ml NaOH})(0.02\ \text{N})}{(\text{min})(\text{g Sample})} \times 2 \times 1000$$

$$= \frac{(\text{ml NaOH})}{(\text{min})(\text{g Sample})} \times 40$$

2. PE activity for accurately weighed sample is calculated as:

- For 1 g of fresh pulp homogenate

$$\text{PE Activity}\,(\text{PEU}\times 10^3/\text{g}) = \frac{(\text{ml NaOH})}{(\text{min})}\times 40$$

- For 10 g of pasteurized pulp homogenate

$$\text{PE Activity}\,(\text{PEU}\times 10^3/\text{g}) = \frac{(\text{ml NaOH})}{(\text{min})}\times 4$$

## VI. Reference

Rouse, A.H. and Atkin, C.D. 1955. Pectinesterase and pectin in commercial citrus juices as determined by methods used at the Citrus Experiment Station. Fla. Agr. Exp. Sta. Tech. Bull. 570, Gainesville, Florida.

# Chapter VI. Oil Analysis of Fruit and Byproducts

## 1. Whole Fruit Available Oil

I Apparatus

A) Four litter blender ((low speed, 15 000 rpm; high speed , 2,0000 rpm)

or

B) Forty eight litter (~13 Gal) vertical cutter mixer (1800 rpm) equipped with wave cut knives and a mixing baffle.

II Chemical

See Recoverable Oil in Chapter IV, 10

III Reagents

See Recoverable Oil in Chapter IV, 10

IV Procedure

A) Fruit collections

1) Collect a 45 Lb (20.4 Kg) sample of the fruit lot to be analyzed making sure that the sample is representative of the size distribution. Count the number of fruit in a given weight and then calculate the number of fruit in 90 pounds of fruit.

B) Slurry Preparation and Oil Determination

Using a four liter blender.

1. Select 16 fruit making sure they represent the sample size distribution.
2. Using a sharp knife, cut each fruit into quarter from the stem to the blossom end.
3. Keep one quarter from each fruit and discard the rest.
4. Weigh the 16 quarters to the nearest 0.1 gram, place them in the blender, add an equal weight of chilled water (2-7 °C/35-45 ° F) and attached the lid.
5. Blend for 3 minutes at low speed and 1 minute at high speed.

6. Place 500 ml distillation flask on a balance and tare.
7. Stop the blender and transfer 5 g (~ 6 ml) of the fruit water slurry into the distillation flask using a plastic disposable pipette.
8. Record the aliquot weight (to the nearest 0.01 g) and determine the oil content, using 0.0247 N potassium bromide-bromate solution as titrant, as described in the Recoverable Oil Section (Chapter IV, 10)

   Using a 48 liter cutter mixer.

9. Weigh 20 to 25 lbs of fruit, place it in the mixer and add an equal weight of cold tap water.
10. If the mixer has a low and a high speed, blend 2 minutes at high speed and 8 at low speed otherwise blend for 10 minutes.
11. After the mixer has completely stopped transfer a ~ 500 ml aliquot of the slurry into a plastic container by tilting the bowl.
12. Place 500 ml distillation flask on a balance and tare it.
13. Mixed or shake the slurry aliquot and place ~5 g of the fruit slurry into the distillation flask, using a plastic pipette, and record its weight to the nearest 0.01 g.
14. Determine the oil content, using 0.0247 N potassium bromide-bromate solution as titrant, as described in the Recoverable Oil Section (Chapter IV, 10)

C) Lemon Fruit.

It is recommended to prepare the fruit slurry by blending 20 lbs of fruit (~ 9 Kg) with an equal amount of cold tap water using a vertical cutter/mixer, as described in the previous section.

## V Calculations

1. The fruit available oil is calculated based on 1 ml of 0.0247 N $KBrO_3$-KBr solution equaling 0.0010 ml of *d*-limonene (Titrant Oil Equivalent) and oil specific weights of 0.840 g/ml for orange, 0.850 g/ml for grapefruit, lemon, and tangerine and 0.880 g/ml for lime (see also Chapter IV, 10).

$$\text{Available Oil (g/g fruit)}$$

$$= \frac{\text{Oil Weight in Fruit Homogenate}}{\text{Fruit Weight in Fruit Homogenate}}$$

$$= \frac{(\text{Titrant Volume})(\text{Titrant Oil Equivalent})(\text{Oil Specific Gravity})}{(\text{Fruit Homogenate Weight})(\text{Fruit Content in Fruit Homogenate})}$$

$$= \frac{(\text{Net ml Titrant})(\text{Titrant Oil Equivalent})(\text{Oil Specific Gravity})}{(\text{g Fruit Homogenate})\{\frac{(\text{g Fruit})}{(\text{g Fruit}) + (\text{g Water})}\}}$$

$$= \frac{(\text{Net ml Titrant})(\text{Oil Specific Gravity})}{(\text{g Fruit Homogenate})} \times 0.002$$

or

$$\text{Available Oil (kg /MT Fruit)} = \frac{(\text{Net ml } KBrO_3 - KBr)(\text{Oil Specific Gravity})}{(\text{g Fruit Homogenate})} \times 2$$

$$\text{Available Oil (lb/ST Fruit)} = \frac{(\text{Net ml } KBrO_3 - KBr)(\text{Oil Specific Gravity})}{(\text{g Fruit Homogenate})} \times 4$$

Where:

(Net Titrant Volume) = (ml Titrant for Sample) – (ml Titrant for Blank)

- For orange fruit

$$\text{Available Oil (kg /MT Fruit)} = \frac{(\text{Net ml } KBrO_3 \text{ - KBr})(0.840\ \text{g/ml})}{(\text{g Fruit Homogenate})} \times 2$$

$$= \frac{(\text{Net ml } KBrO_3 \text{ - KBr})}{(\text{g Fruit Homogenate})} \times 1.68$$

$$\text{Available Oil (lb/ST Fruit)} = \frac{(\text{Net ml } KBrO_3 \text{ - KBr})}{(\text{g Fruit Homogenate})} \times 3.36$$

- For grapefruit, lemon, and tangerine fruit

$$\text{Available Oil (kg/MT Fruit)} = \frac{(\text{Net ml } KBrO_3 \text{ - KBr})(0.850\ \text{g/ml})}{(\text{g Fruit Homogenate})} \times 2$$

$$= \frac{(\text{Net ml } KBrO_3 \text{ - KBr})}{(\text{g Fruit Homogenate})} \times 1.7$$

$$\text{Available Oil (lb/ST Fruit)} = \frac{(\text{Net ml } KBrO_3 \text{ - KBr})}{(\text{g Fruit Homogenate})} \times 3.40$$

- For lime fruit

$$\text{Available Oil (kg/MT Fruit)} = \frac{(\text{Net ml } KBrO_3 - KBr)(0.880\ \text{g/ml})}{(\text{g Fruit Homogenate})} \times 2$$

$$= \frac{(\text{Net ml } KBrO_3 - KBr)}{(\text{g Fruit Homogenate})} \times 1.76$$

$$\text{Available Oil (lb/ST Fruit)} = \frac{(\text{Net ml } KBrO_3 - KBr)}{(\text{g Fruit Homogenate})} \times 3.52$$

2. Available oil for accurately weighed sample titrated with 0.0247 N $KBrO_3$-KBr is calculated as:

- For 5 g of orange fruit homogenate

$$\text{Available Oil (kg /MT Fruit)} = (\text{Net ml } KBrO_3 - KBr) \times 0.336$$

$$\text{Available Oil (lb/ST Fruit)} = (\text{Net ml } KBrO_3 - KBr) \times 0.660$$

- For 5 g of grapefruit, lemon, or tangerine fruit homogenate

$$\text{Available Oil (kg/MT Fruit)} = (\text{Net ml } KBrO_3 - KBr) \times 0.340$$

$$\text{Available Oil (lb/ST Fruit)} = (\text{Net ml } KBrO_3 - KBr) \times 0.680$$

- For 5 g of lime fruit homogenate

$$\text{Available Oil (kg/MT Fruit)} = (\text{Net ml } KBrO_3 - KBr) \times 0.352$$

$$\text{Available Oil (lb/ST Fruit)} = (\text{Net ml } KBrO_3 - KBr) \times 0.704$$

## VI Reference

Official Methods of Analysis. 1999. 16th Edition, 5th Reversion, AOAC International, Gaithersburg, MD, method 968.20, 939.12, and 947.13.

Table VI – 1. Normal peel oil level in some citrus fruits grown in Florida.

| Cultivars | Available Oil Content | |
|---|---|---|
| | (lb/ST) | (kg/MT) |
| Hamlin orange | 6 – 10 | 3 – 5 |
| Parson Brown orange | 7 – 12 | 3.5 – 6 |
| Pineapple orange | 8 – 12 | 4 – 6 |
| Valencia orange | 10 – 15 | 5 – 7.5 |
| Temples orange | 6 – 10 | 3 – 5 |
| Duncan grapefruit | 4 – 7 | 2 – 3.5 |
| Marsh grapefruit | 5 – 8 | 2 – 4 |
| Ruby Red grapefruit | 5 – 8 | 2.5 – 4 |
| Dancy tangerines | 10 – 20 | 5 – 10 |
| Orlando tangelos | 9 – 13 | 4.5 – 6.5 |
| Persian lime | 7 – 10 | 3.5 – 5 |
| Lemon | 10 – 23 | 5 – 11.5 |

Source: Kesterson, J.W. and R.J. Braddock. 1975. Total peel oil content of the major Florida citrus cultivars. J. Food Sci. 40: 931 – 933.

# 2. Recoverable Oil in Oil Recovery System and Juice

VII Apparatus

- 4-Liter blender (low speed, 15,000 rpm; medium speed, 18,300 rpm; high speed, 20,000 rpm)
- See Recoverable Oil in Chapter IV, 10

VIII Chemicals

See Available Oil in Chapter IV, 10

IX Reagents

See Available Oil in Chapter IV, 10

X Procedure

1. Collect representative bulk samples at the desired processing points in the approximate quantities shown in Table VI – 2A.
2. Mix samples thoroughly and prepare analysis samples as shown in Table VI – 2B.
3. Weigh each sample accurately into a boiling flask while under stirring. The normal sample sizes at different oil recovery stages are shown below:

| Original, Blended, or Diluted Samples | Analysis Sample Size | |
|---|---|---|
| | 0.0247 N Titrant | 0.1000 N Titrant |
| Juice | 25 g | |
| Peel, Core, Frit, or Pulp | | 15 g |
| Oil emulsions | | 2 g |
| Desludger or Breaker heavy phase | | 25 g |
| Desludger or Breaker sludge | | 10 g |
| Desludger light phase | | 0.1 g |
| Breaker light phase | | 0.05 g or 5 g of dilution solution |
| Polisher heavy phase | | 2 g |
| Polisher sludge | | 0.1 g |
| Polisher light phase | | 0.05 g or 5 g of dilution solution |

The proper sample size can be determined using the following equation based the approximate/expected oil level in the sample and the using of 0.100 N $KBrO_3$-KBr as titrant. In most cases, the titrant volume used should be in the range of 5 to 10 ml.

One way to obtain the approximate % oil in sample is to briefly spin an aliquot of the sample using a centrifuge or a hand hold spinning device (oil spin test).

$$\text{Approximate Sample Size (g)} = \frac{3}{\text{Approximate \% Oil in Sample}}$$

For example, if a desludger light phase may have an oil level of about 50% recoverable oil, the sample size is about 0.06 g (= 3 ÷ 50).

| Approximate % Oil in Sample | Sample Size (g) |
|---|---|
| 0.5 | 6.000 |
| 1 | 3.000 |
| 2 | 1.500 |
| 5 | 0.600 |
| 10 | 0.300 |
| 20 | 0.150 |
| 30 | 0.100 |
| 40 | 0.075 |
| 50 | 0.060 |
| 60 | 0.050 |
| 70 | 0.043 |
| 80 | 0.038 |
| 90 | 0.033 |
| 100 | 0.030 |

4. Determine the sample oil content as in Recoverable Oil of Chapter IV, 10.

## XI Calculations

The recoverable oil in oil emulsion and oil bearing materials is calculated as following based on 0.0247 N or 0.100 N potassium bromide-bromate titrant and the fruit oil specific gravity.

$$\%\,\text{Oil (w/w)} = \frac{\dfrac{\text{Oil Weight in Sample}}{(\text{g Sample})}}{(\text{Sample Dilution Factor})} \times 100$$

$$= \frac{\dfrac{(\text{Titrant Volume})(\text{Titrant Oil Equivalent})(\text{Oil Specific Gravity})}{(\text{g Sample})}}{(\text{Sample Dilution Factor})} \times 100$$

$$= \frac{(\text{Net ml Titrant})(\text{Calculation Factor})}{(\text{g Sample})}$$

where

(Net Titrant Volume) = (ml Titrant for Sample) – (ml Titrant for Blank)

and

Calculation Factor is listed in Table VI – 2C, based on 1 ml of 0.0247 N $KBrO_3$-KBr titrant equaling 0.0010 ml or 0.00084 g of *d*-limonene.

## XII Reference

Official Methods of Analysis. 1999. 16th Edition, 5th Reversion, AOAC International, Gaithersburg, MD, method 968.20, 939.12, and 947.13.

Citrus Systems Division, FoodTech, FMC Corporation.

Table VI – 2A. Quantity of bulk sample to be collected at different processing points for recoverable oil analysis in oil recovery systems.

| Processing and Oil Recovery System Stage (common terminology) | Preparation Material Quantity | Note |
|---|---|---|
| Extractor Discharges | | (FMC citrus extractor) |
| Juice (raw juice, unfinished juice) | – | juice stream |
| Oil slurry | – | oil recovery stream |
| Peel | 2 kg (4 lb) | cup discharge |
| Core | 2 kg (4 lb) | orifice tube discharge |
| From Extractor Discharge: Juice | | |
| Juice Finisher Discharges | | (screw or paddle separator) |
| Juice (pulpy juice) | 2 kg (4 lb) | juice free of peel and membrane |
| Sludge (core) | 2 kg (4 lb) | solids discharge |
| From Extractor Discharge: Oil Slurry | | |
| Emulsion Separator Discharges[Z] | | (screen or screw separator) |
| Primary oil emulsion (oil emulsion) | 500 ml | aqueous phase of oil slurry |
| Peel fragment (frit) | 2 kg (4 lb) | peel fragments in oil slurry |
| Secondary Oil Emulsion (frit wash) | 500 ml | aqueous phase of the wash slurry |
| Washed Frit | 2 kg (4 lb) | peel fragments in the wash slurry |
| From Emulsion Separator Discharges: Oil Emulsions | | |
| Desludger Discharges | | (first stage centrifuge) |
| Heavy phase | 500 ml | aqueous phase |
| Light phase (cream) | 100 ml | oil rich emulsion |
| Sludge (bowl shoot) | 2 kg (4 lb) | solid discharge |
| Breaker Discharges[Y] | | (second stage centrifuge) |
| Heavy phase | 500 ml | aqueous phase |
| Light phase (cream) | 100 ml | oil rich emulsion |
| Sludge (bowl shoot) | 2 kg (4 lb) | solid discharge |
| Polisher Discharges | | (third stage centrifuge) |
| Heavy phase | 500 ml | aqueous discharge |
| Light phase | 100 ml | oil |
| Sludge[w] | 500 ml | solid discharge |

[Z] The primary and secondary oil emulsions can be combined before taking sample for recoverable oil analysis if the distribution in the two emulsions is not of interest. The

primary oil emulsion or the combined emulsions also are called desludger feed or weak emulsion.

[Y] For oil recovery system of only two centrifugation stages, there is no second desludger or breaker.

[X] When collecting sludge, take sample only during initial discharge that is free of operating water).

[W] Only if the centrifuge has three outlets.

Table VI – 2B. Preparation of analysis sample for recoverable oil in oil recovery systems.

| Samples | Quantity | Preparation |
|---|---|---|
| Peel, Core, Frit, or Pulp | 500 g | Blend with 3 times weight of cold distilled water (1:3) at low speed for 3 min at ~ 1800 rpm in a 4-liter blender |
| Juice<br>Oil emulsions<br>Desludger heavy phase<br>Desludger sludge<br>Breaker heavy phase<br>Breaker sludge<br>Polisher heavy phase | – | Use directly |
| Desludger light phase<br>Breaker light phase<br>Polisher light phase | 0.05 g | Use directly or |
| | 5 g | Dilute with 300 ml of isopropanol (235 g) and, while under stirring, slowly add distilled water to 500 g |

Table VI – 2C. Calculation factors for recoverable oil in oil recovery systems and available oil in fruit.

| Fruit | Sample | Dilution Factor | Specific Gravity (g/ml) | Calculation Factor | |
|---|---|---|---|---|---|
| | | | | 0.0247 N $KBrO_3$-KBr | 0.100 N $KBrO_3$-KBr |
| Orange | Whole fruit | 2 | 0.840 | 0.168 | 0.68 |
| | Peel, Core, Frit, Pulp | 4 | | 0.336 | 1.36 |
| | Juice<br>Oil emulsions<br>Desludger/Breaker/Polisher heavy phases and sludges | 1 | | 0.084 | 0.340 |
| | Oil rich emulsion Desludger/Breaker/Polisher light phases | 1 | | 0.084 | 0.340 |
| | | 100 | | 8.4 | 34.0 |
| Grapefruit Lemon Tangerine | Whole fruit | 2 | 0.850 | 0.170 | 0.688 |
| | Peel, Core, Frit, Pulp | 4 | | 0.340 | 1.376 |
| | Juice<br>Oil emulsions<br>Desludger/Breaker/Polisher heavy phases and sludges | 1 | | 0.085 | 0.344 |
| | Oil rich emulsion Desludger/Breaker/Polisher light phases | 1 | | 0.08 | 0.344 |
| | | 100 | | 8.5 | 34.4 |
| Lime | Whole fruit | 2 | 0.880 | 0.176 | 0.712 |
| | Peel, Core, Frit, Pulp | 4 | | 0.352 | 1.424 |
| | Juice<br>Oil emulsions<br>Desludger/Breaker/Polisher heavy phases and sludges | 1 | | 0.088 | 0.356 |
| | Oil rich emulsion Desludger/Breaker/Polisher light phases | 1 | | 0.088 | 0.356 |
| | | 100 | | 8.8 | 35.6 |

Table VI – 2D. Target recoverable oil level in citrus oil recovery systems.

| Oil Sample | Available Oil (%, w/w) |
|---|---|
| Oil emulsions | 0.5 – 2.5 |
| Desludger/Breaker heavy phases | 0.03 – 0.1 |
| Desludger/Breaker sludges | 0.1 – 0.5 |
| Desludger/Breaker light phases | 25 – 90 |
| Polisher heavy phase | 1 – 2 |
| Polisher sludge[Z] | 2 – 10 |
| Polisher light phase | > 95 |

[Z] Only if the centrifuge has three outlets.

# 3. Oil-Rich Emulsion Spin Test

XIII Apparatus

- Laboratory/clinical centrifuge
- 50 ml graduated centrifuge tube with conical bottom

XIV Chemicals

None

XV Reagents

None

XVI Procedure

1. Fill a centrifuge tube with 50 ml of oil-rich emulsion sample (i.e., light phases of desludger, breaker, or polisher).
2. Place the tubes in the centrifuge. Make sure load is balanced.
3. Centrifuge for 10 min after reaching a centrifugation force of 365 $\times g$ or the speed specified in Table IV – 13 based on rotor operation diameter. Once the time required for acceleration is known, the combined time can be used at the time of starting the centrifuge.
4. After centrifugation, remove tubes from centrifuge.
5. Read the bottom water phase volume in milliliters.

XVII Calculations

This test is only for rough estimation of oil level in oil-rich emulsion (i.e., cream) and cannot be used as a substitute of the Scott oil test.

$$\text{Approximate \% Oil (v/v)} = \frac{\text{Volume of Oil}}{\text{Volume of Emulsion}} \times 100$$

$$= \frac{(\text{Volume of Emulsion}) - (\text{Volume of Water Phase})}{50\ \text{ml}} \times 100$$

$$= (50 - \text{ml Water Phase}) \times 2$$

## XVIII Reference

Citrus Systems Division, FoodTech, FMC Corporation.

# 4. Total Solids in Oil Emulsion

XIX Apparatus

- 25 or 50 ml Buret with 0.1 ml graduation and Teflon® stopcock
- Magnetic stirrer and Teflon® coated stirring bar
- Drying dishes (glass or aluminum foil)
- Analytic balance with sensitivity of 1 mg
- Tongs
- Desiccator and desiccant/Drierite

XX Chemicals

None

XXI Reagents

None

XXII Procedure

1. Obtain a representative sample of about 500 ml of an oil emulsion.
2. Weight and label drying dishes.
3. While continuously stirring the sample on a magnetic stirrer, transfer about 6 ml into a pre-weighed dish with a disposable pipette.
4. Weigh the dish with sample again.
5. Dry the samples to a constant weight in a drying oven overnight at 93°C (200°F) or 4 to 6 hours in a vacuum drying oven at 70°C (158°F) and 25 mm Hg (3.3 K Pa).
6. Weigh sample after transferring dishes using a pair of tongs from the oven into a desiccator to cool.

XXIII Calculations

$$\%\text{Total Solid (w/w)} = \frac{(\text{Weight of Dried Sample and Dish}) - (\text{Weight of Dish})}{(\text{Weight of Wet Sample and Dish}) - (\text{Weight of Dish})} \times 100$$

XXIV Reference

Citrus Systems Division, FoodTech, FMC Corporation.

# Chapter VII. Cold Pressed Oil Analysis

## 1. Refractive Index

I. Apparatus

- Refractometer, bench model, with refractive index (RI) scale range of 1.32000 – 1.7000 and accuracy to ±0.0001 RI (prefer with automatic temperature compensation).

II. Chemicals

- Isopropanol ($C_3H_8O$)

III. Reagents

None

IV. Procedure

1. Calibrate the refractometer against a standard provided by the manufacturer. For daily use, check with distilled water (RI of 1.3330 at 20°C/68°F and 1.3325 at 25°C/76°F).
2. Condition the oil sample and refractometer to 20°C (68°F).
3. The temperature should be carefully adjusted and maintained since the refractive index varies significantly with temperature.
4. Clean prism by wiping with cotton pad moistened with a solvent (e.g., isopropanol) and let air dry.
5. Apply a couple drops of sample and allow time for temperature equilibrium between instrument and sample.
6. Adjust board line so that it falls on point of intersection of cross hairs.
7. Read RI.

V. Calculations

The reading is presented as the result.

If measurement is performed at prism temperatures other than the recommended 20°C, the observed refractive index is corrected by adding or subtracting 0.00045 for orange oil and 0.00046 for lemon oil for each degree centigrade above or below 20°C.

## VI. Reference

Official Methods of Analysis. 1999. 16th Edition, 5th Reversion, AOAC International, Gaithersburg, MD, method 921.141.

Kesterson, J.W., Hendrickson. R., and Braddock, R.J. 1971. Florida citrus oil. Bulletin 749 (technical). University of Florida, Gainesville, Florida., 24 – 27, 114 – 127.

# 2. Optical Rotation

I. Apparatus

- Polarimeter with a standard 100 mm tube and a sodium vapor lamp

II. Chemicals

None

III. Reagents

None

IV. Procedure

1. Adjust oil sample and instrument temperature to 20°C (68°F).
2. Fill the polarimeter tube with oil and wipe off excess oil on the exterior.
3. Place the tube in the polarimeter.
4. Slowly turn the analyzer until both halves of the field, viewed through the telescope, show equal intensities of illumination. Better perform in dark.
5. Read rotation degree together with the direction of rotation from the zero position {counter-clockwise, (-) and clockwise, (+)}.

V. Calculations

The reading is presented as result.

If measurement is performed at prism temperatures other than the recommended 20°C, the observed optical rotation is corrected by adding or subtracting 0.22° for orange and grapefruit oils and 0.14° for lemon oil for each degree centigrade above or below 20°C.

VI. Reference

Official Methods of Analysis. 1999. 16th Edition, 5th Reversion, AOAC International, Gaithersburg, MD, method 921.142.

Kesterson, J.W., Hendrickson. R., and Braddock, R.J. 1971. Florida citrus oil. Bulletin 749 (technical). University of Florida, Gainesville, Florida., 24 – 27, 114 – 127.

# 3. Specific Gravity

I. Apparatus

- Pycnometer, glass, conical body, narrow mouth preferred
- Analytic balance with resolution of ± 1 mg
- Water bath with temperature control

II. Chemicals

None

III. Reagents

A. Water: Recently boiled or degassed distilled water.

IV. Procedure

1. Thoroughly clean a pycnometer.
2. Adjust the temperature of degassed water to about 15°C (59°F).
3. Fill the pycnometer with water.
4. Adjust the temperature of the filled pycnometer to 20°C (68°F).
5. Place the cap on.
6. Carefully wipe off the excess water from exterior.
7. Immediately weigh the pycnometer containing the water.
8. Repeat steps 2 to 7 with oil.

V. Calculations

$$\text{Oil Specific Gravity (g/ml)}$$

$$= \frac{\text{Oil Density}}{\text{Water Density}}$$

$$= \frac{\dfrac{(\text{Weight of Oil and Pycnometerl}) - (\text{Weight of Pycnometer})}{\text{Volume of Pyconometer}}}{\dfrac{(\text{Weight of Water and Pycnometer}) - (\text{Weight of Pycnometer})}{\text{Volume of Pycnometer}}}$$

$$= \frac{(\text{Weight of Oil and Pycnometer}) - (\text{Weight of Pycnometer})}{(\text{Weight of Water and Pycnometer}) - (\text{Weight of Pycnometer})}$$

$$= \frac{(\text{g Oil and Pycnometerl}) - (\text{g Pycnometer})}{(\text{g Water and Pycnometerl}) - (\text{g Pycnometer})}$$

The water density need not be determined each time. The standard density of water at 20°C is 0.998203 g/ml. If measurement is performed at temperatures other than the recommended 20°C, the observed specific gravity is corrected by adding or subtracting 0.00078 for orange oil and 0.00077 for lemon oil for each degree centigrade above or below 20°C.

VI. Reference

Official Methods of Analysis. 1999. 16th Edition, 5th Reversion, AOAC International, Gaithersburg, MD, method 920.140.

Kesterson, J.W., Hendrickson. R., and Braddock, R.J. 1971. Florida citrus oil. Bulletin 749 (technical). University of Florida, Gainesville, Florida., 24 – 27, 114 – 127.

# 4. Ultraviolet Absorbance

I. Apparatus

- Spectrophotometer with UV detector and 1 cm quartz cell/cuvet
- Analytic balance with resolution of ± 1 mg
- 100 ml volumetric flask

II. Chemicals

- Isopropanol ($C_3H_8O$)

III. Reagents

None

IV. Procedure

1. Place 250 mg of the oil, accurately weighed, in a 100 ml volumetric flask.
2. Add alcohol to volume mark and mix thoroughly.
3. Zero the baseline of the instrument with alcohol in a quartz cuvet.
4. Replace alcohol in cuvet with oil-alcohol sample solution.
5. Determine the ultraviolet absorption spectrum in the wavelength range from 260 nm to 400 nm at 1 nm interval with automatic scanning.

   If a manual instrument is used, read the absorbances at 5 nm intervals from 260 nm to a point about 12 nm from the expected maximum absorbance, then at 3 nm intervals for 3 readings, and at 1 nm intervals to a point about 5 nm beyond the maximum, and then at 10 nm intervals to 400 nm.

V. Calculations

1. Plot the absorbances as ordinates against wavelength on the abscissa, and draw the absorption spectrum or spectrogram (see Figure VII – 4).
2. Draw a base-line tangent to the abscissa linking points of minimum absorbance (A and B).
3. Locate the point of maximum absorbance (C) and draw a vertical line from it the abscissa, that intersects line AB at D.
4. Absorbances corresponding to point D and C. The difference in absorbance between point C and D is the ultraviolet absorbance of the oil on the basis of a 250 mg sample.
5. If oil sample weight is not exactly 250 mg, standardize the observed CD value using the following formulation:

$$CD_{250\,mg} = CD_{observed} \times \frac{250\,mg}{Weight\ of\ Sample, mg}$$

VI. Reference

Official Methods of Analysis. 1999. 16th Edition, 5th Reversion, AOAC International, Gaithersburg, MD, method 953.09.

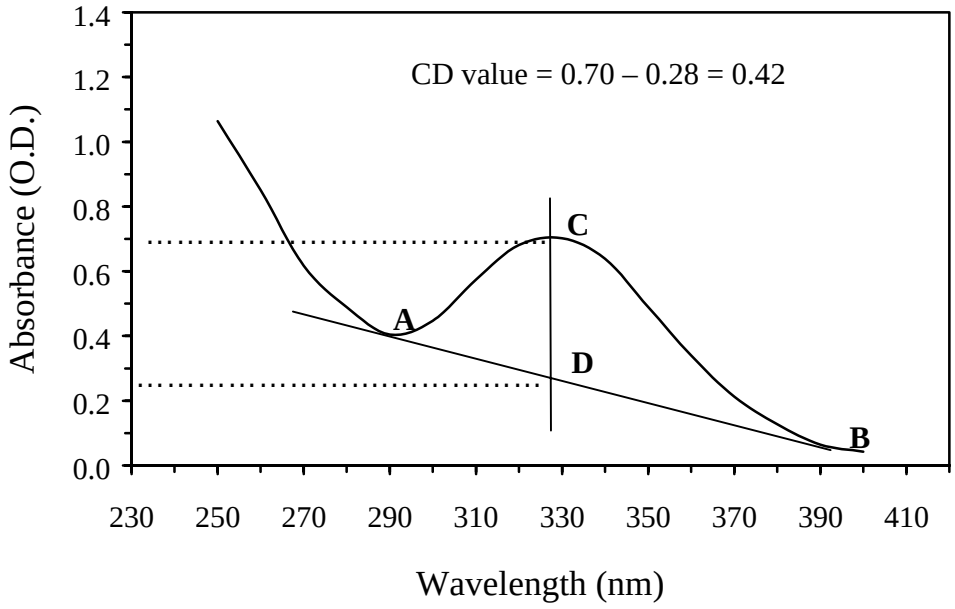



Figure VII – 4.Method of obtaining ultraviolet absorption (CD Value) of citrus oil.

# 5. Evaporation Residue

I. Apparatus

- Steam bath
- Glass evaporation dish of 80 mm diameter and 45 mm deep
- Analytic balance
- Tongs

II. Chemicals

None

III. Reagents

None

IV. Procedure

1. Label and place a glass dish in a desiccator for 30 min.
2. Accurately weigh the glass dish.
3. Accurately weigh the specified quantity of sample (see the following table) into the dish.
4. Dry oil sample by placing the dish on a steam bath for the length of time specified.
5. Continue to dry oil sample in an oven if required.
6. Remove the dish with tongs and place in a desiccator to cool to ambient temperature.
7. Weigh the dish containing oil evaporation residues.

| Oil Source | Sample Size (g) | Period of Steam Heating (h) |
|---|---|---|
| Orange* | 5 | 4.5 |
| Lemon | 5 | 4.5 |
| Tangerine | 5 | 5 |
| Mandarin | 5 | 5 |
| Grapefruit | 5 | 6 |
| Lime | 5 | 6 |

* USP method includes additional oven hating at 105°C (221°F) for 2 h.

V. Calculations

$$\% \text{ Evaporaion Residue (w/w)}$$

$$= \frac{(\text{Weight of Residure and Dish}) - (\text{Weight of Dish})}{(\text{Weight of Oil and Dish}) - (\text{Weight of Dish})} \times 100$$

$$= \frac{(\text{g Residure and Dish}) - (\text{g Dish})}{(\text{g Oil and Dish}) - (\text{g Dish})} \times 100$$

VI. Reference

Langenau, E.E. 1950. The examination and analysis of essential oils, synthetics, and isolates. In: The essential oils. ed. E. Guenther. Vol. 1, D. Van Nostrand Company, Inc., New York.

United States Pharmacopeia. 1975. 20th Revision, The National Formulary, 15th Edition, United States Pharmacopeial Convention, Inc. Rockwille, MD.

# 6. Total Aldehyde (AOAC Method)

I. Apparatus

- Analytic balance with resolution of ± 1 mg
- pH Meter with resolution of 0.01
- 10 ml Buret with 0.05 ml graduations
- Magnetic stirrer and Teflon® coated stirring bar
- 50 ml Glass-stoppered graduate
- 1000 ml Graduated cylinder

II. Chemicals

- Potassium hydroxide (KOH)
- Isopropanol (aldehyde free) ($C_3H_8O$)
- Bromophenol blue
- Hydroxylamine hydrochloride ($H_3NO \cdot HCl$)
- Hydrochloric acid (HCl)

III. Reagents

A. Isopropanol (60%): Dilute 632 ml of 95% isopropanol with distilled water to 1000 ml.
B. Alcoholic potassium hydroxide (0.5 M): Dissolve 28.06 g of KOH in 60% ethanol and make up to 1000 ml with the same solvent.
C. Bromophenol blue solution (0.01%): Triturate and dissolve 0.1 gm of bromophenol blue in 5 ml of 0.05 N NaOH and make up to 100 ml with 60% isopropanol.
D. Hydroxylamine hydrochloride solution (0.5 N): Dissolve 34.745 g of $H_3NO \cdot HCl$ in 875 ml of 60% isopropanol, add 1.5 ml of bromophenol blue solution and enough 0.5 M alcoholic KOH solution to give permanent blue solution, and make to 1000 ml with 60% isopropanol.

IV. Procedure

1. Accurately weigh, to the nearest 10 mg, about 10 g of oil sample into glass-stoppered 50 ml graduate.
2. Add 7 ml of hydroxylamine solution.
3. Add 0.1 ml of bromophenol blue solution.
4. Mix thoroughly.
5. Titrate with 0.5 M alcoholic KOH to a permanent full alkaline color of the bromophenol blue solution (in the lower layer separated after shaking vigorously for 2 min). Reaction time is complete in about 15 min and KOH solution used should be less than 5 ml.

V. Calculations

1. The major aldehyde component present in orange oil and lemon oil are decanal and citral, respectively. Accordingly, each ml of 0.5 M alcoholic KOH is equivalent to 0.07813 g of decanal or 0.07612 g of citral.

$$\text{Percent Aldehyde (w/w)} = \frac{(\frac{\text{ml Titran}}{1000\ \text{ml/l}})(\text{M Titrant})(\text{MW of Aldehyde})}{(\text{Sample Weight})} \times 100$$

or

$$\%\ \text{Aldehyde (citral, w/w)} = \frac{(\frac{\text{ml KOH}}{1000\ \text{ml/l}})(0.5\ \text{M KOH})(152.23\ \text{g/mole})}{(\text{g Sample})} \times 100$$

$$= \frac{(\text{ml KOH})}{(\text{g Sample})} \times 7.612$$

and

$$\%\ \text{Aldehyde (decanal, w/w)} = \frac{(\frac{\text{ml KOH}}{1000\ \text{ml/l}})(0.5\ \text{M KOH})(156.27\ \text{g/mole})}{(\text{g Sample})} \times 100$$

$$= \frac{(\text{ml KOH})}{(\text{g Sample})} \times 7.813$$

2. The % Aldehyde for accurately weighed oil samples is calculated as:

   - 10 g of cold-pressed lemon oil

$$\% \text{ Aldehyde (citral, w/w)} = (\text{ml KOH}) \times 0.7612$$

   - 10 g of cold-pressed orange oil

$$\% \text{ Aldehyde (decanal, w/w)} = (\text{ml KOH}) \times 0.7813$$

VI. Reference

Official Methods of Analysis. 1999. 16th Edition, 5th Reversion, AOAC International, Gaithersburg, MD, method 955.32 and 942.13.

# 7. Total Aldehyde (USP Method)

I. Apparatus

- Analytic balance with resolution of ± 1 mg
- pH Meter with resolution of 0.01
- 10 ml Buret with 0.05 ml graduations
- Magnetic stirrer and Teflon® coated stirring bar
- 150 ml Flasks with ground glass stoppers

II. Chemicals

- Hydroxylamine hydrochloride ($H_3NO \cdot HCl$)
- Potassium hydroxide (KOH)
- Tertiary butyl alcohol
- Isopropanol ($C_3H_8O$)

III. Reagents

A. Alcoholic potassium hydroxide solution (0.5 M): Dissolve 28.055 g of KOH in 1000 ml of 95% isopropanol.

B. Hydroxylamine hydrochloride solution: Dissolve 45 g of $H_3NO \cdot HCl$ in 130 ml of distilled water, add 850 ml of tertiary butyl alcohol. Mix and adjust pH to 3.4 with 0.5 N alcoholic KOH solution.

IV. Procedure

1. Weigh accurately 5 g of oil into a 250-ml flask with stopper.
2. Add 50 ml of hydroxylamine hydrochloride solution into the flask.
3. Stopper the flask right away and swirl to mix the solution.
4. Allow solution to stand for 30 minutes at ambient temperature with occasional swirling.
5. Titrate samples, drop-wise and slowly, with 0.5 M alcoholic KOH to pH 3.4.
6. Read volume of titrant used.

V. Calculations

1. The aldehyde content in citrus oils is expressed in equivalent of the major aldehyde component present in the type of oil. For orange and lemon oils, they are decanal and citral, respectively. One mole of KOH reacts with one mole of aldehyde and therefore, each ml of 0.5 M alcoholic KOH is equivalent to 0.07813 g of decanal or 0.07612 g of citral.

$$\text{Percent Aldehyde (w/w)} = \frac{(\frac{\text{ml Titrant}}{1000\ \text{ml/l}})(\text{M Titrant})(\text{MW of Aldehyde})}{(\text{Sample Weight})} \times 100$$

or

$$\%\ \text{Aldehyde (citral, w/w)} = \frac{(\frac{\text{ml KOH}}{1000\ \text{ml/l}})(0.5\ \text{M KOH})(152.23\ \text{g/mole})}{(\text{g Sample})} \times 100$$

$$= \frac{(\text{ml KOH})}{(\text{g Sample})} \times 7.612$$

and

$$\%\ \text{Aldehyde (Decanal, w/w)} = \frac{(\frac{\text{ml KOH}}{1000\ \text{ml/l}})(0.5\ \text{M KOH})(156.27\ \text{g/mole})}{(\text{g Sample})} \times 100$$

$$= \frac{(\text{ml KOH})}{(\text{g Sample})} \times 7.813$$

2. The % Aldehyde for accurately weighed oil samples is calculated as:

   - 5 g of cold-pressed lemon oil

$$\%\ \text{Aldehyde (citral, w/w)} = (\text{ml KOH}) \times 1.522$$

- 5 g of cold-pressed orange oil

$$\% \text{ Aldehyde (Decanal, w/w)} = (\text{ml KOH}) \times 1.563$$

VI. Reference

United States Pharmacopeia. 1985. 21th Revision and The National Formulary. 1985. 15th Edition, p. 1572 – 1573 and 1583. United States Pharmacopeial Convention, Inc. Rockwille, MD.

# 8. Volatile Composition by GC

I. Apparatus

- GC system with a 5% phenyl methylpolysiloxane column (DB-5, 60 m × 0.25 mm, 0.25 μm film thickness), flame ionization detector, and integrator.
- 5 μl Syringe

II. Chemicals

- Helium gas
- Desired standard compounds

III. Reagents

A. Standard solution: Prepare mixed standard of desired compounds in pure *d*-limonene.

IV. Procedure

1. Make sure oil sample is clear, filter if needed.
2. Allow oil sample to be in equilibrium with ambient temperature.
3. Set GC system as:

   Injector temperature = 270°C
   Detector temperature = 270°C
   Oven temperatures = start at 60°C for 8 min, increase to 200°C at a rate of 3°C/min, then hold at 200°C for 5 min
   Flow rate = 310 ml/min
   Split ratio = 250:1
   Run time = 60 min

4. Inject standard mixture and oil sample into GC, 0.5 μl each.

V. Calculations

1. Compounds are identified by comparing retention time with standard compounds and confirmed by sample enrichment of the standards. The levels of compounds in oil samples are normally expressed as percent of total peak area ($PA_{Total}$).

$$\text{Compound Level (\% Peak Area)} = \left(\frac{PA_{Compound}}{PA_{Total}}\right) \times 100$$

2. Actual compound concentrations can be calculated from linear regression of standard curves of absorbance peak area against concentration of the respect standards.

- Linear regression of standards for a specific compound (see Appendixes, 3)

$$PA_{Standard} = a + b \times Concentration_{Standard}\ (ppm)$$

- Concentration of the specific compound under the test conditions

$$\text{Compound Level}\,(ppm) = (PA_{Sample})\left(\frac{PA_{Standard} - a}{b}\ ppm\right)$$

$$= (PA_{Sample})\left(\frac{PA_{Standard} - a}{b}\right) \times 10$$

VI. Reference

Citrus System Division, FoodTech, FMC Corporation.

# Chapter VIII. Processing Evaluation

## 1. Juice and Pulp Yield Standardization

1. To convert the actual juice yield and pulp yield to standard juice and pulp yields of a standard pulp quick fiber of 160 ml,

   a). With the quick fiber value of the pulp, determined as in Chapter V – 1, look up the modified correct factor (MCF) in Table V – 1A for converting the actual pulp weight to an expected pulp weight if the pulp has a quick fiber value of 160 ml.
   b). Calculate the standard juice yield using the following formula:

$$\text{Standard Finished Juice Weight} = \text{Finished Juice Weight} + (\text{Pulp Weight} \times \text{MCF})$$

   c). Calculate the standard pulp yield using the following formula:

$$\text{Standard Pulp Weight} = \text{Pulp Weight} - (\text{Pulp Weight} \times \text{MCF})$$

2. MFC can be calculated as:

$$\text{MCF} = (-0.66570) + (0.0041628 \times \text{Quick Fiber})$$

Table VIII – 1A. Modified correction factors (MCF) for estimating juice and pulp yield to a standard quick fiber value of 160 ml based on actual quick fiber values (QF, ml).

| QF | MCF | QF | MCF | QF | MCF | QF | MCF | QF | MCF |
|---|---|---|---|---|---|---|---|---|---|
| 100 | -0.249 | 129 | -0.129 | 158 | -0.008 | 187 | 0.113 | 216 | 0.234 |
| 101 | -0.245 | 130 | -0.124 | 159 | -0.004 | 188 | 0.117 | 217 | 0.238 |
| 102 | -0.241 | 131 | -0.121 | 160 | 0.000 | 189 | 0.121 | 218 | 0.242 |
| 103 | -0.238 | 132 | -0.117 | 161 | 0.005 | 190 | 0.125 | 219 | 0.246 |
| 104 | -0.232 | 133 | -0.112 | 162 | 0.008 | 191 | 0.130 | 220 | 0.250 |
| 105 | -0.229 | 134 | -0.108 | 163 | 0.013 | 192 | 0.134 | 221 | 0.254 |
| 106 | -0.225 | 135 | -0.103 | 164 | 0.016 | 193 | 0.138 | 222 | 0.259 |
| 107 | -0.220 | 136 | -0.099 | 165 | 0.021 | 194 | 0.142 | 223 | 0.263 |
| 108 | -0.216 | 137 | -0.096 | 166 | 0.026 | 195 | 0.146 | 224 | 0.267 |
| 109 | -0.213 | 138 | -0.092 | 167 | 0.029 | 196 | 0.150 | 225 | 0.271 |
| 110 | -0.207 | 139 | -0.087 | 168 | 0.034 | 197 | 0.154 | 226 | 0.275 |
| 111 | -0.204 | 140 | -0.083 | 169 | 0.038 | 198 | 0.159 | 227 | 0.279 |
| 112 | -0.200 | 141 | -0.079 | 170 | 0.042 | 199 | 0.163 | 228 | 0.284 |
| 113 | -0.195 | 142 | -0.075 | 171 | 0.046 | 200 | 0.167 | 229 | 0.288 |
| 114 | -0.192 | 143 | -0.071 | 172 | 0.050 | 201 | 0.171 | 230 | 0.292 |
| 115 | -0.187 | 144 | -0.067 | 173 | 0.054 | 202 | 0.175 | 231 | 0.296 |
| 116 | -0.183 | 145 | -0.063 | 174 | 0.059 | 203 | 0.179 | 232 | 0.300 |
| 117 | -0.178 | 146 | -0.058 | 175 | 0.063 | 204 | 0.183 | 233 | 0.305 |
| 118 | -0.175 | 147 | -0.053 | 176 | 0.067 | 205 | 0.188 | 234 | 0.308 |
| 119 | -0.170 | 148 | -0.049 | 177 | 0.071 | 206 | 0.192 | 235 | 0.313 |
| 120 | -0.164 | 149 | -0.045 | 178 | 0.075 | 207 | 0.196 | 236 | 0.317 |
| 121 | -0.162 | 150 | -0.041 | 179 | 0.080 | 208 | 0.201 | 237 | 0.321 |
| 122 | -0.159 | 151 | -0.037 | 180 | 0.084 | 209 | 0.204 | 238 | 0.325 |
| 123 | -0.154 | 152 | -0.033 | 181 | 0.088 | 210 | 0.209 | 239 | 0.329 |
| 124 | -0.149 | 153 | -0.028 | 182 | 0.092 | 211 | 0.213 | 240 | 0.334 |
| 125 | -0.146 | 154 | -0.025 | 183 | 0.096 | 212 | 0.217 | 241 | 0.338 |
| 126 | -0.141 | 155 | -0.021 | 184 | 0.100 | 213 | 0.221 | 242 | 0.342 |
| 127 | -0.136 | 156 | -0.016 | 185 | 0.104 | 214 | 0.225 | 243 | 0.346 |
| 128 | -0.133 | 157 | -0.012 | 186 | 0.109 | 215 | 0.229 | 244 | 0.350 |

* The MCF is derived from the original quick fiber correction factor table (Table VIII – 1B) using the formula: MCF = (1 – Correction Factor).

Table VIII – 1B. Correction factors (CF) for estimating juice and pulp yield to a standard quick fiber value of 160 ml based on actual quick fiber values (QF, ml).

| QF | CF | QF | CF | QF | CF | QF | CF | QF | CF |
|---|---|---|---|---|---|---|---|---|---|
| 100 | 1.249 | 132 | 1.116 | 164 | 0.983 | 196 | 0.850 | 228 | 0.717 |
| 101 | 1.245 | 133 | 1.112 | 165 | 0.979 | 197 | 0.846 | 229 | 0.712 |
| 102 | 1.241 | 134 | 1.108 | 166 | 0.975 | 198 | 0.841 | 230 | 0.708 |
| 103 | 1.237 | 135 | 1.104 | 167 | 0.971 | 199 | 0.837 | 231 | 0.704 |
| 104 | 1.233 | 136 | 1.100 | 168 | 0.966 | 200 | 0.833 | 232 | 0.700 |
| 105 | 1.229 | 137 | 1.095 | 169 | 0.962 | 201 | 0.829 | 233 | 0.696 |
| 106 | 1.224 | 138 | 1.091 | 170 | 0.958 | 202 | 0.825 | 234 | 0.692 |
| 107 | 1.220 | 139 | 1.087 | 171 | 0.954 | 203 | 0.821 | 235 | 0.687 |
| 108 | 1.216 | 140 | 1.083 | 172 | 0.950 | 204 | 0.816 | 236 | 0.683 |
| 109 | 1.212 | 141 | 1.079 | 173 | 0.946 | 205 | 0.812 | 237 | 0.679 |
| 110 | 1.208 | 142 | 1.075 | 174 | 0.941 | 206 | 0.808 | 238 | 0.675 |
| 111 | 1.204 | 143 | 1.070 | 175 | 0.937 | 207 | 0.804 | 239 | 0.671 |
| 112 | 1.199 | 144 | 1.066 | 176 | 0.933 | 208 | 0.800 | 240 | 0.667 |
| 113 | 1.195 | 145 | 1.062 | 177 | 0.929 | 209 | 0.796 | 241 | 0.662 |
| 114 | 1.191 | 146 | 1.058 | 178 | 0.925 | 210 | 0.792 | 242 | 0.658 |
| 115 | 1.187 | 147 | 1.054 | 179 | 0.921 | 211 | 0.787 | 243 | 0.654 |
| 116 | 1.183 | 148 | 1.050 | 180 | 0.916 | 212 | 0.783 | 244 | 0.650 |
| 117 | 1.179 | 149 | 1.045 | 181 | 0.912 | 213 | 0.779 | 245 | 0.646 |
| 118 | 1.175 | 150 | 1.041 | 182 | 0.908 | 214 | 0.775 | 246 | 0.642 |
| 119 | 1.170 | 151 | 1.037 | 183 | 0.904 | 215 | 0.771 | 247 | 0.637 |
| 120 | 1.166 | 152 | 1.033 | 184 | 0.900 | 216 | 0.767 | 248 | 0.633 |
| 121 | 1.162 | 153 | 1.029 | 185 | 0.896 | 217 | 0.762 | 249 | 0.629 |
| 122 | 1.158 | 154 | 1.025 | 186 | 0.891 | 218 | 0.758 | 250 | 0.625 |
| 123 | 1.154 | 155 | 1.020 | 187 | 0.887 | 219 | 0.754 | 251 | 0.621 |
| 124 | 1.150 | 156 | 1.016 | 188 | 0.883 | 220 | 0.750 | 252 | 0.617 |
| 125 | 1.145 | 157 | 1.012 | 189 | 0.879 | 221 | 0.746 | 253 | 0.613 |
| 126 | 1.141 | 158 | 1.008 | 190 | 0.875 | 222 | 0.742 | 254 | 0.608 |
| 127 | 1.137 | 159 | 1.004 | 191 | 0.871 | 223 | 0.737 | 255 | 0.604 |
| 128 | 1.133 | 160 | 1.000 | 192 | 0.866 | 224 | 0.733 | 260 | 0.587 |
| 129 | 1.129 | 161 | 0.995 | 193 | 0.862 | 225 | 0.729 | 265 | 0.567 |
| 130 | 1.125 | 162 | 0.991 | 194 | 0.858 | 226 | 0.725 | 270 | 0.541 |
| 131 | 1.120 | 163 | 0.987 | 195 | 0.854 | 227 | 0.721 | 300 | 0.416 |

# 2. Oil Recovery Efficiency by Centrifuge

1. The approximate efficiency of oil recovery by each centrifuge (i.e., desludger, breaker, or polisher) is calculated using the following formulas without considering the oil presents in the sludges (i.e., solid discharges):

$$\text{Approximate Efficiency (\%)} = \frac{(\%\ \text{Oil of Feed}) - (\%\ \text{Oil of Heavy Phase Discharge})}{(\%\ \text{Oil of Feed})} \times 100$$

2. The overall oil recovery efficiency of the centrifuge system can be estimated as the followings:

   a). Based on the efficiency of all centrifuges within the system

$$\text{Centrifuge System Efficiency (\%)} = \frac{(\text{Efficiency of Desludger})}{100} \times \frac{(\text{Efficiency of Polisher})}{100} \times 100$$

   or

$$\text{Centrifuge System Efficiency (\%)} = \frac{(\text{Efficiency of Desludger})}{100} \times \frac{(\text{Efficiency of Breaker})}{100} \times \frac{(\text{Efficiency of Polisher})}{100} \times 100$$

   For example, the oil recovery efficiency of a centrifuge system consisted of a desludger at 75% and a polisher at 90% efficiencies is 67.5% (= 0.75 × 0.90 × 100).

d). Based on the quantity of oil emulsion, oil collected, and the emulsion oil level.

$$\text{Centrifuge System Efficiency (\%)}$$

$$= \frac{(\text{Weight of Oil})}{(\text{Average \% Oil of Emulsion, w/w})(\text{Weight of Emulsion})} \times 100$$

$$= \frac{(\text{lb Oil})}{(\text{Average \% Oil of Emulsion, w/w})(\text{lb Emulsion})} \times 100$$

# 3. Secondary Solids Recovery Efficiency

I. Approximate Method

1. The efficiency of secondary solids recovery system from pulp wash is calculated using the following formulas:

$$\text{Efficiency}\,(\%) = \frac{(°\text{Brix of Pulp}_{in}) - (°\text{Brix of Pulp}_{out})}{(°\text{Brix of Pulp}_{in})} \times 100$$

2. The efficiency of secondary solids recovery system from pulp wash can be expressed as:

$$\text{Solids Recovery}\,(\text{lb/box}) = (\text{Pulp Yield, lb/box})\frac{(°\text{Brix of Pulp}_{in}) - (°\text{Brix of Pulp}_{out})}{100}$$

or

$$\text{Solids Recovery}\,(\text{lb/box}) = (\text{Pulp Yield, lb/box})(\text{Water/Pulp Ratio})(\frac{°\text{Brix of Strong Extract}}{100})$$

where

$$\text{Water/Pulp Ratio} = \frac{\text{Weight of Water Added}}{\text{Weight of Pulp}_{in}}$$

and the strong extract is the liquid exiting the system and having the highest °Brix value.

II. Theoretical Method

1. The efficiency of secondary solids recovery system from pulp wash is calculated, based on one pound of pulp at a quick fiber value of 200, using the following formulas:

$$\text{Efficiency}\,(\%) = \frac{(\text{°Brix of Pulp}_{in} \times F_{in}) - (\text{°Brix of Pulp}_{out} \times F_{out})}{(\text{°Brix of Pulp}_{in} \times F_{in})} \times 100$$

where

$$F = \text{Factor} = \frac{1}{2 - \frac{QF}{200}}$$

2. The efficiency of secondary solids recovery system from pulp wash can be expressed as:

$$\text{Solids Recovery}\,(\text{lb/box}) = (\text{Pulp Yield, lb/box})\frac{(\text{°Brix of Pulp}_{in}) - (\text{°Brix of Pulp}_{out})}{100}\left(\frac{F_{in}}{F_{out}}\right)$$

or

$$\text{Solids Recovery}\,(\text{lb/box}) = (\text{Pulp Yield, lb/box})(\text{Water/Pulp Ratio})\left(\frac{\text{°Brix of Strong Extract}}{100}\right)\left(\frac{F_{in}}{F_{out}}\right)$$

where

$$\text{Water/Pulp Ratio} = \frac{\text{Weight of Water Added}}{\text{Weight of Pulp}_{in}}$$

and the strong extract is the liquid exiting the system and having the highest °Brix value.

# Appendixes

## 1. Properties of Commonly Used Lab Reagents

Table A – 1A. Concentrations of commonly used lab reagents.

| Reagent | Mol. Wt. | Approx. Wt. | Approx. Norm. | Density (g/l) | Degree, Baume | No. of ml to dilute to 1 L to make 1N Reagent |
|---|---|---|---|---|---|---|
| Acetic acid, glacial | 60.06 | 99.7 | 17.4 | 1.05 | 6.9 | 57.5 |
| Acetic acid | 60.05 | 80.0 | 14.3 | 1.07 | 9.5 | 70.2 |
| Acetic anhydride | 102.09 | 97.0 | - | 1.08 | 10.7 | 48.7 |
| Ammonium hydroxide | 35.05 | 57.6 | 14.8 | 0.90 | 25.6 | 67.6 |
| Formic acid | 46.03 | 98.0 | 26.0 | 1.22 | 26.1 | 38.5 |
| Hydrochloric acid | 36.46 | 37.0 | 12.1 | 1.19 | 23.2 | 82.6 |
| Nitric acid | 63.01 | 70.0 | 15.7 | 1.41 | 42.2 | 63.7 |
| Perchloric acid | 100.46 | 70.0 | 11.6 | 1.67 | 58.2 | 86.2 |
| Perchloric acid | 100.46 | 60.0 | 9.2 | 1.54 | 50.8 | 108.7 |
| Phosphoric acid | 98.00 | 85.0 | 44.0 | 1.69 | 59.2 | 22.7 |
| Sulfuric acid | 98.08 | 95.0 | 35.6 | 1.84 | 66.2 | 28.1 |

Table A – 1B. Physical properties of organic solvents.

| Solvent | Polarity Index (P′) | Viscosity (cP, 25°C) | Refractive Index (25°) | Boiling Point (°C) |
|---|---|---|---|---|
| Acetic acid, glacial | 6.20 | 1.10 | 1.370 | 118 |
| Acetone | 5.40 | 0.30 | 1.356 | 56 |
| Acetonitrile | 6.20 | 0.34 | 1.341 | 82 |
| Isobutyl alcohol | 3.00 | 4.70 | 1.384 | 108 |
| Isopropyl alcohol | 4.30 | 1.90 | 1.384 | 82 |

Table A – 1C. pH of common acids and bases.

| Acids | Molarity (N) | pH |
|---|---|---|
| Acetic | 1 | 2.4 |
| Acetic | 0.01 | 2.9 |
| Acetic | 0.01 | 3.4 |
| Alum | 0.1 | 3.2 |
| Citric | 0.1 | 2.1 |
| Hydrochloric | 1 | 0.1 |
| Hydrochloric | 0.1 | 1.1 |
| Hydrochloric | 0.01 | 2.0 |
| Sulfuric | 1 | 0.3 |
| Sulfuric | 0.1 | 1.2 |
| Sulfuric | 0.01 | 2.1 |

| Bases | Molarity (N) | pH |
|---|---|---|
| Ammonia | 1 | 11.6 |
| Ammonia | 0.1 | 11.1 |
| Ammonia | 0.01 | 10.6 |
| Borax | 0.01 | 9.2 |
| Potassium acetate | 0.1 | 9.7 |
| Potassium bicarbonate | 0.1 | 8.2 |
| Potassium carbonate | 0.1 | 11.5 |
| Potassium hydroxide | 1 | 14.0 |
| Potassium hydroxide | 0.1 | 13.0 |
| Potassium hydroxide | 0.01 | 12.0 |
| Sodium acetate | 0.1 | 8.9 |
| Sodium bicarbonate | 0.1 | 8.4 |
| Sodium carbonate | 0.1 | 11.6 |
| Sodium hydroxide | 1 | 14.0 |
| Sodium hydroxide | 0.1 | 13.0 |
| Sodium hydroxide | 0.01 | 12.0 |
| Trisodium phosphate | 0.1 | 12.0 |

# 2. Metric Prefixes

Table A – 2. Metric prefixes.

| Prefix | Abbreviation | Meaning |
|---|---|---|
| peta- | P | $\times 10^{15}$ |
| tera- | T | $\times 10^{12}$ |
| giga- | G | $\times 10^{9}$ |
| mega- | M | $\times 10^{6}$ |
| kilo- | K | $\times 10^{3}$ |
| deci- | d | $\times 10^{-1}$ |
| centi- | c | $\times 10^{-2}$ |
| milli- | m | $\times 10^{-3}$ |
| micro- | μ | $\times 10^{-6}$ |
| nano- | n | $\times 10^{-9}$ |
| pico- | p | $\times 10^{-12}$ |
| femto- | f | $\times 10^{-15}$ |

# 3. Box Weight of Citrus Fruits

Table A – 3. Citrus box weights: approximate net weight in pounds by fruit type and states.

| State | Oranges | Grapefruit | Tangerines | Lemons | Limes |
|---|---|---|---|---|---|
| Florida | 90[X] | 85 | 95 | 90 | 88 |
| California | 75 | 67[Y] | 75 | 76 | - |
| Texas | 85 | 80 | - | - | - |
| Arizona | 75 | 67 [Y] | 75 | 76 | - |

[X] Includes Temples and tangelos at 90 pounds.
[Y] Arizona was 64 pounds prior to 1993-1994. California was 65 pounds prior to 1993-1994.

# 4. Calculation for Making Reagents

1. Reagent solution concentrations are normally expressed based on volume and on weight.
2. The calculation of solutes for solutions based on volume concentrations commonly used are shown below (MW = molecular weight):

- Molar concentration

$$\text{Solute Weight (g)}$$

$$= (\text{Solute Concentration, M})(\text{Solution Volume, l})(\text{Solute MW, g/mole})$$

- Normal concentration

$$\text{Solute Weight (g)}$$

$$= (\text{Solute Concentration, N})(\text{Solution Volume, l})\frac{(\text{Solute MW, g/mole})}{n}$$

where

n is the number of $H^+$ provided by one molecule of acid or $OH^-$ provided by one molecule of base in acid-base reactions or the number of electron lost by one molecule of oxidizing agent or gained by one molecule of reducing agent in oxidation-reduction reactions.

- Weight-volume percent concentration

$$\text{Solute Weight (g)} = \frac{(\text{Solute Concentration, \%})}{100}(\text{Solution Volume, l})$$

- Part per million concentration

$$\text{Solute Weight (g)} = \frac{(\text{Solute Concentration, ppm})}{1000\ (\text{mg/g})}(\text{Solution Volume, l})$$

# 5. Calculation for Reagent Dilution

1. The amount of stock solutions known of concentration needed for making working solutions of lower concentration can be calculated using the following formula:

$$\text{Stock Solution Volume} = \frac{(\text{Working Solution Concentration})(\text{Working Solution Volume})}{(\text{Stock Solution Concentration})}$$

$$\text{Water Volume} = (\text{Solution Volume}) - (\text{Stock Solution Volume})$$

2. Example:

   a). Calculate the amount of 1.00 N stock solution and distilled water needed to make 5 liters of 0.3125 N working solution.

$$\text{Stock Solution Volume (l)}$$

$$= \frac{(\text{Working Solution Concentration, N})(\text{Working Solution Volume, ml})}{(\text{Stock Solution Concentration, N})}$$

$$= \frac{0.3125 \times 5}{1}$$

$$= 1.562$$

$$\text{Water Volume (l)}$$

$$= (\text{Solution Volume, l}) - (\text{Stock Solution Volume, l})$$

$$= 5 - 1.562$$

$$= 3.438$$

b). Calculate the amount of 100 ppm stock solution and distilled water needed to make 100 ml of 10 ppm working solution.

$$\text{Stock Solution Volume (ml)}$$

$$= \frac{(\text{Working Solution Concentration, ppm})(\text{Working Solution Volume, ml})}{(\text{Stock Solution Concentration, ppm})}$$

$$= \frac{10 \times 100}{100}$$

$$= 10$$

$$\text{Water Volume (ml)}$$

$$= (\text{Solution Volume, ml}) - (\text{Stock Solution Volume, ml})$$

$$= 100 - 10$$

$$= 90$$

# 6. Calculation of Linear Regression Line

1. The best fitting straight line for set of data of standard curve is calculated as the line for which the sum of squares of vertical deviations of observations (i.e., data) from the line is smaller than corresponding sum of squares of deviation from any other lime.
2. The equation of straight line is:

$$Y = a + bX$$

where constant a is intercept at Y axis (X = 0), and constant b is slope of line.

3. Calculation of the constants a and b by least square estimation are:

$$b = \frac{\sum (X_i Y_i) - \frac{\sum X_i \sum Y_i}{n}}{\sum X_i^2 - \frac{(\sum X_i)^2}{n}}$$

$$a = \overline{Y} - b\overline{X}$$

where

$\sum$ = "sum of " the $n$ individual values of indicated operation and $\overline{X}$ and $\overline{Y}$ are the averages of the X and Y points.

4. Example:

Find the best straight regression line relating absorbance (Y) to concentration (X) of the following standard curve data set.

a). Do the calculations as shown below.

| Observation No. ($i$) | Standard Concentration ($X_i$) | Absorbance ($Y_i$) | $X^2_i$ | $X_iY_i$ |
|---|---|---|---|---|
| 1 | 0.20 | 0.150 | 0.04 | 0.03 |
| 2 | 0.40 | 0.300 | 0.16 | 0.12 |
| 3 | 0.60 | 0.450 | 0.36 | 0.27 |
| 4 | 0.80 | 0.600 | 0.64 | 0.48 |
| 5 | 1.00 | 0.750 | 1.00 | 0.75 |
| Totals: | | | | |
| $n = 5$ | $\sum X_i = 3.00$ | $\sum Y_i = 2.25$ | $\sum X^2_i = 2.20$ | $\sum (X_iY_i) = 1.65$ |

and

$$b = \frac{1.65 - \frac{(3.00)(2.25)}{5}}{2.20 - \frac{(3.00)^2}{5}}$$

$$= 0.750$$

$$a = \frac{2.25}{5} - (0.75)(\frac{3.00}{5})$$

$$= 0.000$$

b). Therefore, the best fitting straight regression line for the set of data is:

$$Y = 0.00 + 0.750X$$

c). If for a sample, the absorbance (Y) is 0.500, the corresponding concentration (X) would be:

$$X = \frac{Y - (0.00)}{0.750}$$

$$= \frac{0.500 - (0.00)}{0.750}$$

$$= 0.667$$

# 7. Temperature Conversion

1. The relationships between temperatures in Celsius and Fahrenheit scale are:

$$°C = \frac{5}{9}(°F - 32)$$

$$° F = \frac{9}{5}°C + 32$$

$$K = °C + 273$$

$$K = °F + 460$$

where

K is absolute temperature unit (Kelvin).

2. The numeric equivalents of temperatures in the Celsius and Fahrenheit scales are shown in Table A4 – 1A and Table A4 – 1B.

Table A – 7A. Conversion of temperatures from the Celsius scale to the Fahrenheit scale.

| °C | °F | °C | °F | °C | °F | °C | °F | °C | °F | °C | °F |
|---|---|---|---|---|---|---|---|---|---|---|---|
| -40 | -40.0 | 0 | 32.0 | 40 | 104.0 | 80 | 176.0 | 120 | 248.0 | 160 | 320.0 |
| -39 | -38.2 | 1 | 33.8 | 41 | 105.8 | 81 | 177.8 | 121 | 249.8 | 161 | 321.8 |
| -38 | -36.4 | 2 | 35.6 | 42 | 107.6 | 82 | 179.6 | 122 | 251.6 | 162 | 323.6 |
| -37 | -34.6 | 3 | 37.4 | 43 | 109.4 | 83 | 181.4 | 123 | 253.4 | 163 | 325.4 |
| -36 | -32.8 | 4 | 39.2 | 44 | 111.2 | 84 | 183.2 | 124 | 255.2 | 164 | 327.2 |
| -35 | -31.0 | 5 | 41.0 | 45 | 113.0 | 85 | 185.0 | 125 | 257.0 | 165 | 329.0 |
| -34 | -29.2 | 6 | 42.8 | 46 | 114.8 | 86 | 186.8 | 126 | 258.8 | 166 | 330.8 |
| -33 | -27.4 | 7 | 44.6 | 47 | 116.6 | 87 | 188.6 | 127 | 260.6 | 167 | 332.6 |
| -32 | -25.6 | 8 | 46.4 | 48 | 118.4 | 88 | 190.4 | 128 | 262.4 | 168 | 334.4 |
| -31 | -23.8 | 9 | 48.2 | 49 | 120.2 | 89 | 192.2 | 129 | 264.2 | 169 | 336.2 |
| -30 | -22.0 | 10 | 50.0 | 50 | 122.0 | 90 | 194.0 | 130 | 266.0 | 170 | 338.0 |
| -29 | -20.2 | 11 | 51.8 | 51 | 123.8 | 91 | 195.8 | 131 | 267.8 | 171 | 339.8 |
| -28 | -18.4 | 12 | 53.6 | 52 | 125.6 | 92 | 197.6 | 132 | 269.6 | 172 | 341.6 |
| -27 | -16.6 | 13 | 55.4 | 53 | 127.4 | 93 | 199.4 | 133 | 271.4 | 173 | 343.4 |
| -26 | -14.8 | 14 | 57.2 | 54 | 129.2 | 94 | 201.2 | 134 | 273.2 | 174 | 345.2 |
| -25 | -13.0 | 15 | 59.0 | 55 | 131.0 | 95 | 203.0 | 135 | 275.0 | 175 | 347.0 |
| -24 | -11.2 | 16 | 60.8 | 56 | 132.8 | 96 | 204.8 | 136 | 276.8 | 176 | 348.8 |
| -23 | -9.4 | 17 | 62.6 | 57 | 134.6 | 97 | 206.6 | 137 | 278.6 | 177 | 350.6 |
| -22 | -7.6 | 18 | 64.4 | 58 | 136.4 | 98 | 208.4 | 138 | 280.4 | 178 | 352.4 |
| -21 | -5.8 | 19 | 66.2 | 59 | 138.2 | 99 | 210.2 | 139 | 282.2 | 179 | 354.2 |
| -20 | -4.0 | 20 | 68.0 | 60 | 140.0 | 100 | 212.0 | 140 | 284.0 | 181 | 357.8 |
| -19 | -2.2 | 21 | 69.8 | 61 | 141.8 | 101 | 213.8 | 141 | 285.8 | 182 | 359.6 |
| -18 | -0.4 | 22 | 71.6 | 62 | 143.6 | 102 | 215.6 | 142 | 287.6 | 183 | 361.4 |
| -17 | 1.4 | 23 | 73.4 | 63 | 145.4 | 103 | 217.4 | 143 | 289.4 | 184 | 363.2 |
| -16 | 3.2 | 24 | 75.2 | 64 | 147.2 | 104 | 219.2 | 144 | 291.2 | 185 | 365.0 |
| -15 | 5.0 | 25 | 77.0 | 65 | 149.0 | 105 | 221.0 | 145 | 293.0 | 186 | 366.8 |
| -14 | 6.8 | 26 | 78.8 | 66 | 150.8 | 106 | 222.8 | 146 | 294.8 | 187 | 368.6 |
| -13 | 8.6 | 27 | 80.6 | 67 | 152.6 | 107 | 224.6 | 147 | 296.6 | 188 | 370.4 |
| -12 | 10.4 | 28 | 82.4 | 68 | 154.4 | 108 | 226.4 | 148 | 298.4 | 189 | 372.2 |
| -11 | 12.2 | 29 | 84.2 | 69 | 156.2 | 109 | 228.2 | 149 | 300.2 | 190 | 374.0 |
| -10 | 14.0 | 30 | 86.0 | 70 | 158.0 | 110 | 230.0 | 150 | 302.0 | 191 | 375.8 |
| -9 | 15.8 | 31 | 87.8 | 71 | 159.8 | 111 | 231.8 | 151 | 303.8 | 192 | 377.6 |
| -8 | 17.6 | 32 | 89.6 | 72 | 161.6 | 112 | 233.6 | 152 | 305.6 | 193 | 379.4 |
| -7 | 19.4 | 33 | 91.4 | 73 | 163.4 | 113 | 235.4 | 153 | 307.4 | 194 | 381.2 |
| -6 | 21.2 | 34 | 93.2 | 74 | 165.2 | 114 | 237.2 | 154 | 309.2 | 195 | 383.0 |
| -5 | 23.0 | 35 | 95.0 | 75 | 167.0 | 115 | 239.0 | 155 | 311.0 | 196 | 384.8 |
| -4 | 24.8 | 36 | 96.8 | 76 | 168.8 | 116 | 240.8 | 156 | 312.8 | 197 | 386.6 |
| -3 | 26.6 | 37 | 98.6 | 77 | 170.6 | 117 | 242.6 | 157 | 314.6 | 198 | 388.4 |
| -2 | 28.4 | 38 | 100.4 | 78 | 172.4 | 118 | 244.4 | 158 | 316.4 | 199 | 390.2 |
| -1 | 30.2 | 39 | 102.2 | 79 | 174.2 | 119 | 246.2 | 159 | 318.2 | 200 | 392.0 |

Table A – 7B. Conversion of temperatures from the Fahrenheit scale to the Celsius scale.

| °F | °C | °F | °C | °F | °C | °F | °C | °F | °C | °F | °C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| -40 | -40.0 | 0 | -17.8 | 40 | 4.4 | 80 | 26.7 | 120 | 48.9 | 160 | 71.1 |
| -39 | -39.4 | 1 | -17.2 | 41 | 5.0 | 81 | 27.2 | 121 | 49.4 | 161 | 71.7 |
| -38 | -38.9 | 2 | -16.7 | 42 | 5.6 | 82 | 27.8 | 122 | 50.0 | 162 | 72.2 |
| -37 | -38.3 | 3 | -16.1 | 43 | 6.1 | 83 | 28.3 | 123 | 50.6 | 163 | 72.8 |
| -36 | -37.8 | 4 | -15.6 | 44 | 6.7 | 84 | 28.9 | 124 | 51.1 | 164 | 73.3 |
| -35 | -37.2 | 5 | -15.0 | 45 | 7.2 | 85 | 29.4 | 125 | 51.7 | 165 | 73.9 |
| -34 | -36.7 | 6 | -14.4 | 46 | 7.8 | 86 | 30.0 | 126 | 52.2 | 166 | 74.4 |
| -33 | -36.1 | 7 | -13.9 | 47 | 8.3 | 87 | 30.6 | 127 | 52.8 | 167 | 75.0 |
| -32 | -35.6 | 8 | -13.3 | 48 | 8.9 | 88 | 31.1 | 128 | 53.3 | 168 | 75.6 |
| -31 | -35.0 | 9 | -12.8 | 49 | 9.4 | 89 | 31.7 | 129 | 53.9 | 169 | 76.1 |
| -30 | -34.4 | 10 | -12.2 | 50 | 10.0 | 90 | 32.2 | 130 | 54.4 | 170 | 76.7 |
| -29 | -33.9 | 11 | -11.7 | 51 | 10.6 | 91 | 32.8 | 131 | 55.0 | 171 | 77.2 |
| -28 | -33.3 | 12 | -11.1 | 52 | 11.1 | 92 | 33.3 | 132 | 55.6 | 172 | 77.8 |
| -27 | -32.8 | 13 | -10.6 | 53 | 11.7 | 93 | 33.9 | 133 | 56.1 | 173 | 78.3 |
| -26 | -32.2 | 14 | -10.0 | 54 | 12.2 | 94 | 34.4 | 134 | 56.7 | 174 | 78.9 |
| -25 | -31.7 | 15 | -9.4 | 55 | 12.8 | 95 | 35.0 | 135 | 57.2 | 175 | 79.4 |
| -24 | -31.1 | 16 | -8.9 | 56 | 13.3 | 96 | 35.6 | 136 | 57.8 | 176 | 80.0 |
| -23 | -30.6 | 17 | -8.3 | 57 | 13.9 | 97 | 36.1 | 137 | 58.3 | 177 | 80.6 |
| -22 | -30.0 | 18 | -7.8 | 58 | 14.4 | 98 | 36.7 | 138 | 58.9 | 178 | 81.1 |
| -21 | -29.4 | 19 | -7.2 | 59 | 15.0 | 99 | 37.2 | 139 | 59.4 | 179 | 81.7 |
| -20 | -28.9 | 20 | -6.7 | 60 | 15.6 | 100 | 37.8 | 140 | 60.0 | 181 | 82.8 |
| -19 | -28.3 | 21 | -6.1 | 61 | 16.1 | 101 | 38.3 | 141 | 60.6 | 182 | 83.3 |
| -18 | -27.8 | 22 | -5.6 | 62 | 16.7 | 102 | 38.9 | 142 | 61.1 | 183 | 83.9 |
| -17 | -27.2 | 23 | -5.0 | 63 | 17.2 | 103 | 39.4 | 143 | 61.7 | 184 | 84.4 |
| -16 | -26.7 | 24 | -4.4 | 64 | 17.8 | 104 | 40.0 | 144 | 62.2 | 185 | 85.0 |
| -15 | -26.1 | 25 | -3.9 | 65 | 18.3 | 105 | 40.6 | 145 | 62.8 | 186 | 85.6 |
| -14 | -25.6 | 26 | -3.3 | 66 | 18.9 | 106 | 41.1 | 146 | 63.3 | 187 | 86.1 |
| -13 | -25.0 | 27 | -2.8 | 67 | 19.4 | 107 | 41.7 | 147 | 63.9 | 188 | 86.7 |
| -12 | -24.4 | 28 | -2.2 | 68 | 20.0 | 108 | 42.2 | 148 | 64.4 | 189 | 87.2 |
| -11 | -23.9 | 29 | -1.7 | 69 | 20.6 | 109 | 42.8 | 149 | 65.0 | 190 | 87.8 |
| -10 | -23.3 | 30 | -1.1 | 70 | 21.1 | 110 | 43.3 | 150 | 65.6 | 191 | 88.3 |
| -9 | -22.8 | 31 | -0.6 | 71 | 21.7 | 111 | 43.9 | 151 | 66.1 | 192 | 88.9 |
| -8 | -22.2 | 32 | 0.0 | 72 | 22.2 | 112 | 44.4 | 152 | 66.7 | 193 | 89.4 |
| -7 | -21.7 | 33 | 0.6 | 73 | 22.8 | 113 | 45.0 | 153 | 67.2 | 194 | 90.0 |
| -6 | -21.1 | 34 | 1.1 | 74 | 23.3 | 114 | 45.6 | 154 | 67.8 | 195 | 90.6 |
| -5 | -20.6 | 35 | 1.7 | 75 | 23.9 | 115 | 46.1 | 155 | 68.3 | 196 | 91.1 |
| -4 | -20.0 | 36 | 2.2 | 76 | 24.4 | 116 | 46.7 | 156 | 68.9 | 197 | 91.7 |
| -3 | -19.4 | 37 | 2.8 | 77 | 25.0 | 117 | 47.2 | 157 | 69.4 | 198 | 92.2 |
| -2 | -18.9 | 38 | 3.3 | 78 | 25.6 | 118 | 47.8 | 158 | 70.0 | 199 | 92.8 |
| -1 | -18.3 | 39 | 3.9 | 79 | 26.1 | 119 | 48.3 | 159 | 70.6 | 200 | 93.3 |

# 8. Unit Conversion Factors

Table A – 8. Common unit conversion factors.

| To Convert | Multiply by | To Obtain / To Convert | Multiply by | To Obtain |
|---|---|---|---|---|
| **Length** | | | | |
| centimeter | 0.3937 | inch | 2.54 | centimeter |
| foot | 0.3048 | meter | 3.281 | foot |
| inch | 0.02540 | meter | 39.37 | inch |
| inch | 1000 | mil | 0.0010 | inch |
| meter | 100 | centimeter | 0.01 | meter |
| meter | $1\times10^{9}$ | nanometer | $1\times10^{-9}$ | meter |
| micron | 1 | micrometer | 1 | micron |
| micron | 0.001 | millimeter | 1000 | micron |
| micron | 0.03937 | mil | 25.40 | micron |
| micron | $3.937\times10^{-5}$ | inch | 25400 | micron |
| micrometer | $1\times10^{4}$ | angstrom | $1\times10^{-4}$ | micrometer |
| mil | 0.0254 | millimeter | 39.37 | mil |
| mile | 1.6093 | kilometer | 0.6214 | mile |
| yard | 0.9144 | Meter | 1.0936 | yard |
| **Area** | | | | |
| acre | 4047 | square meter | $2.471\times10^{-4}$ | acre |
| acre | 0.0015625 | square mile | 640 | acre |
| hectare | 2.471 | acre | 0.4047 | hectare |
| hectare | 10000 | square meter | 0.0001 | hectare |
| hectare | 0.003861 | square mile | 259.0 | hectare |
| square centimeter | 0.1550 | square inch | 6.452 | square centimeter |
| square inch | 6.4516 | square centimeter | 0.1550 | square inch |
| square meter | 10.764 | square feet | 0.09290 | square meter |
| square meter | 1.160 | square yard | 0.8361 | square meter |
| square mil | 1.273 | circular mil | 0.78555 | square mil |
| square yard | 0.8361 | square meter | 1.1960 | square yard |
| **Volume** | | | | |
| barrel (U.S. liquid) | 31.5 | gallon | 0.03 | barrel (U.S. liquid) |
| bushel | 2150.4 | cubic inch | $4.650\times10^{-4}$ | bushel |
| bushel | 0.03524 | cubic meter | 28.38 | bushel |
| bushel | 35.24 | liter | 0.02838 | bushel |
| milliliter | 1 | cubic centimeter | 1 | milliliter |
| milliliter | 0.06102 | cubic inch | 16.39 | milliliter |
| milliliter | $2.642\times10^{-4}$ | gallon (U.S. liquid) | 3785 | milliliter |

| To Convert | Multiply by | To Obtain / To Convert | Multiply by | To Obtain |
|---|---|---|---|---|
| milliliter | 0.001057 | quart (U.S. liquid) | 946.1 | milliliter |
| milliliter | 0.002113 | pint (U.S. liquid) | 473.3 | milliliter |
| cubic feet | 0.02832 | cubic meter | 35.31 | cubic feet |
| cubic feet | 7.4805 | gallon (U.S. liquid) | 0.1337 | cubic feet |
| cubic feet | 28.32 | liter | 0.03531 | cubic feet |
| cubic meter | 61023 | cubic inch | $1.639 \times 10^{-5}$ | cubic meter |
| cubic meter | 264.2 | gallon (U.S. liquid) | 0.003785 | cubic meter |
| cubic meter | 1000 | liter | 0.00100 | cubic meter |
| gallon (U.S. liquid) | 231 | cubic inch | 0.004329 | gallon |
| gallon (U.S. liquid) | 128 | fluid ounce | 0.007812 | gallon (U.S. liquid) |
| gallon (U.S. liquid) | 0.8327 | gallon (British) | 1.2009 | gallon (U.S. liquid) |
| liter | 1000 | cubic centimeter | 0.0010 | liter |
| liter | 61.02 | cubic inch | 0.0164 | liter |
| liter | 0.2642 | gallon (U.S. liquid) | 3.7850 | liter |
| liter | 1.057 | quart (U.S. liquid | 0.9461 | liter |
| liter | 2.113 | pint (U.S. liquid) | 0.4733 | liter |
| ounce (U.S. liquid) | 0.02957 | liter | 33.82 | ounce (fluid) |
| pint (U.S. liquid) | 473.2 | cubic centimeter | 0.002113 | pint (U.S. liquid) |
| pint (U.S. liquid) | 28.87 | cubic inch | 0.03464 | pint (U.S. liquid) |
| pint (U.S. liquid) | 0.1250 | gallon (U.S. liquid) | 8 | pint (U.S. liquid) |
| pint (U.S. liquid) | 0.4732 | liter | 2.113 | pint (U.S. liquid) |
| pint (U.S. liquid) | 0.5 | quart | 2 | pint (U.S. liquid) |
| quart (U.S. liquid) | 946.4 | cubic centimeter | 0.001057 | quart (U.S. liquid) |
| quart (U.S. liquid) | 57.75 | cubic inch | 0.01732 | quart (U.S. liquid) |
| quart (U.S. liquid) | 0.25 | gallon (U.S. liquid) | 4 | quart (U.S. liquid) |
| quart (U.S. liquid) | 0.9463 | liter | 1.057 | quart (U.S. liquid) |
| **Weight** | | | | |
| gram | 0.002205 | pound | 453.51 | gram |
| gram | 0.03527 | ounce | 28.35 | gram |
| kilogram | 2.2046 | pound | 0.4536 | kilogram |
| kilogram | 0.0010 | ton | 1000 | kilogram |
| kilogram | $9.842 \times 10^{-4}$ | ton (long) | 1016.05 | kilogram |
| kilogram | 0.001102 | ton (short) | 907.4 | kilogram |
| pound | 453.59 | gram | 0.002205 | pound |
| pound | 0.4536 | kilogram | 2.205 | pound |
| pound | 16 | ounce | 0.06250 | pound |
| pound | 0.0005 | ton (short) | 2000.00 | pound |
| ton (metric) | 1000 | kilogram | 0.0010 | ton (metric) |
| ton (metric) | 2205 | pound | $4.535 \times 10^{-4}$ | ton (metric) |
| ton (metric) | 0.9842 | ton (long) | 1.016 | ton (metric) |
| ton (metric) | 1.102 | ton (short) | 0.9072 | ton (metric) |
| ton (long) | 1016 | kilogram | $9.843 \times 10^{-4}$ | ton (long) |
| ton (long) | 2240 | pound | $4.464 \times 10^{-4}$ | ton (long) |
| ton (long) | 1.12 | ton (short) | 0.8929 | ton (long) |
| ton (short) | 907.18 | kilogram | 0.001102 | ton (short) |

| To Convert | Multiply by | To Obtain / To Convert | Multiply by | To Obtain |
|---|---|---|---|---|
| ton (short) | 2000 | pound | 0.0005 | ton (short) |
| ton (short) | 9.078 | ton (metric) | 0.1102 | ton (short) |
| **Pressure/Force** (weight = weight force) | | | | |
| atmosphere | 76 | centimeter of mercury (0°C) | 0.01316 | atmosphere |
| atmosphere | 29.92 | inch of mercury | 0.03342 | atmosphere |
| atmosphere | 33.90 | feet of water (4°C) | 0.0295 | atmospheres |
| atmosphere | 406.8 | inch of water | 0.002458 | atmospheres |
| atmosphere | 14.70 | pound/sq. inches | 0.06803 | atmospheres |
| atmosphere | 10333 | kilogram/sq. meter | $9.678 \times 10^{-5}$ | atmospheres |
| atmosphere | 1.013 | bars | 0.9869 | atmospheres |
| bar | 10200 | kilogram/sq. meter | $9.804 \times 10^{-5}$ | bars |
| bar | 14.5 | pound/sq. inch | 0.06897 | bars |
| centipoise | 0.1 | poise | 10 | centipoise |
| centipoise | 0.01 | gram/centimeter second | 100 | centipoise |
| centipoise | $6.720 \times 10^{-4}$ | pound/foot second | 1488 | centipoise |
| inch of mercury | 1.133 | feet of water | 0.8826 | inch of mercury |
| inch of mercury | 0.4912 | pound/sq. inch | 2.036 | inch of mercury |
| inch of mercury | 0.03453 | kilogram/sq. centimeter | 28.96 | inch of mercury |
| inch of mercury | 345.3 | kilogram/sq. meter | 0.002896 | inches of mercury |
| inch of water | 0.07356 | inch of mercury | 13.60 | inch of water |
| inch of water | 0.03613 | pound force/sq. inch | 27.68 | inch of water |
| inch of water | 0.00254 | kilogram force/sq. centimeter | 393.7 | inch of water |
| pascal | $9.869 \times 10^{-6}$ | atmosphere | $1.013 \times 10^{-5}$ | pascal |
| pascal | $1 \times 10^{-5}$ | bar | 100000 | pascal |
| pascal | 1 | newton/sq. meter | 1 | pascal |
| pascal | 0.004015 | inch of water | 249.1 | pascal |
| pound/sq. inch | 2.307 | foot water | 0.4335 | pound/sq. inch |
| pound/sq. inch | 70.31 | gram/sq. centimeter | 0.01422 | pound/sq. inch |
| poise | 1 | gram/(centimeter × second) | 1 | poise |
| poise | 0.1 | pascal second | 10 | poise |
| **Flow Rate/Concentration** | | | | |
| gallon/minute | 0.1337 | cubic foot/minute | 7.481 | gallon/minute |
| grams/liter | 0.008345 | pound/gallon (U.S. liquid) | 119.8 | gram/liter |
| grams/ton (metric) | 1.016 | gram/ton (long) | 0.9842 | gram/ton (metric) |
| grams/ton (metric) | 0.9072 | gram/ton (short) | 1.1023 | gram/ton (metric) |
| gram/ton (metric) | | part per million | | gram/ton (metric) |
| liter/second | 15.85 | gallon (U.S.)/minute | 0.06309 | liter/second |
| liter/minute | 0.2642 | gallon (U.S.)/minute | 3.785 | liter/minute |
| milligram/kilogram | 1 | part per million | 1 | milligram/kilogram |
| part per million | 0.03584 | ounce/ton (long) | 27.90 | part per million |
| part per million | 0.03200 | ounce/ton (short) | 31.25 | part per million |
| part per million | 0.0001 | percent | 10000 | part per million |
| pound/ton (short) | 0.5 | kilogram/ton (metric) | 2 | pound/ton (short) |

| To Convert | Multiply by | To Obtain / To Convert | Multiply by | To Obtain |
|---|---|---|---|---|
| **Water** | | | | |
| pound | 27.68 | cubic inch | 0.03613 | pound |
| pound | 0.1198 | gallon (U.S. liquid) | 8.347 | pound |
| **Work** | | | | |
| Btu | 252.0 | gram calorie | 0.03613 | Btu |
| Btu | 0.2931 | watt hour | 3.412 | Btu |
| Btu | | 1/180 of heat required to change temperature of 1 lb. Water from 32°F to 212°F | | Btu |
| **Power** | | | | |
| Btu/hour | 0.2931 | watt | 3.413 | Btu/hour |
| Btu/hour | 0.2520 | kilogram calorie/hour | 3.968 | Btu/hour |
| Btu/hour | $3.902\times10^{-4}$ | horsepower | 2563 | Btu/hour |
| boiler horsepower | 33480 | Btu/hour | $2.98\times10^{-5}$ | boiler horsepower |
| boiler horsepower | 34.5 | lb water evap/hour | 0.02899 | boiler horsepower |
| boiler horsepower | 9.810 | kilowatt | 0.1019 | boiler horsepower |
| horsepower | 7457 | watt | 1.3410 | horsepower |
| horsepower | 550 | foot pound/second | 0.001818 | horsepower |
| horsepower | 2545 | Btu/hour | $3.929\times10^{-4}$ | horsepower |
| ton refrigeration (US) | 12000 | Btu/hour | $8.33\times10^{-5}$ | ton refrigeration (US) |
| ton refrigeration (US) | 3024 | kilogram calorie/hour | $3.306\times10^{-4}$ | ton refrigeration (US) |
| **Yield** | | | | |
| pound/acre | 1.1208 | kilogram/hectare | 0.8922 | pound/acre |
| tone (short)/acre | 2.2417 | metric ton/hectare | 0.4461 | tone/acre |