ACIDITY (free fatty acid) | fat matrix

DEFINITION AND SCOPE

The acid content of edible fats is given by the quantity of free fatty acids deriving from the hydrolytic rancidity of triglycerides. As this alteration occurs in unsuitable conditions for the processing and preservation of fats, acidity represents a basic indicator of the genuineness of the product. The test is particularly important during the refining of oils and fats, for the assessment of the processing cycle and for the definition of product categories. This test has the same accuracy and yet it is easier than AOCS Official Method Ca 5a-40.

PRINCIPLE

Free fatty acids of the sample, at pH<7,0, react with a chromogenous compound and decrease its color. The decreasing of color, read at 630 nm, is proportional to the acid concentration of the sample, expressed as % of oleic acid.

COMPOSITION OF THE KIT AND REAGENTS

Reagent test kit *300128, suitable for 10 tests, contains:
- 1 bag includes 10 pre-filled cuvettes with reagent (mixture of alcohols and chromogenous compounds).
Reagent test kit *300125, suitable for 100 tests, contains:
- a box includes 10 x reagent test kit *300128.
For samples, with high acidity, where a dilution is requested, use the proper test kit:
Reagent test kit *300129, suitable for 100 tests, contains:
- bulk with 100 mL of a proper diluents
- 100 empty cuvettes
- 100 caps
Stability / Storage conditions: Reagent is stable through expiration date if stored at 2-8°C.

SAMPLE AND MEASURING RANGE

Liquid fats: collect the sample without any treatment.
For samples like solid fats, butter, margarine, cream, nuts, flours and other extracted fat matrix, refer to “preparation of test sample for fat matrix analysis”.

<table>
<thead>
<tr>
<th>Curve</th>
<th>Measuring range (% oleic acid)</th>
<th>Sample volume</th>
<th>Resolution (% oleic acid)</th>
<th>Accuracy</th>
<th>Repeatability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid. 10µL</td>
<td>0,01 - 0,30</td>
<td>10 µL</td>
<td>0,01</td>
<td>+/- 5%</td>
<td>CV &lt;3%</td>
</tr>
<tr>
<td>Acid. 5µL</td>
<td>0,01 - 0,59</td>
<td>5 µL</td>
<td>0,01</td>
<td>+/- 5%</td>
<td>CV &lt;3%</td>
</tr>
<tr>
<td>Acid. 2,5µL</td>
<td>0,03 - 1,10</td>
<td>2,5 µL</td>
<td>0,01</td>
<td>+/- 5%</td>
<td>CV &lt;3%</td>
</tr>
<tr>
<td>Acid. 1µL</td>
<td>0,90 - 3,50</td>
<td>1 µL</td>
<td>0,01</td>
<td>+/- 5%</td>
<td>CV &lt;3%</td>
</tr>
<tr>
<td>Acid. dil - 100µL</td>
<td>1,00 – 13,01</td>
<td>2,5 µL diluted *</td>
<td>0,01</td>
<td>+/- 5%</td>
<td>CV &lt;3%</td>
</tr>
<tr>
<td>Acid. dil - 50µL</td>
<td>5,00 – 26,03</td>
<td>2,5 µL diluted **</td>
<td>0,01</td>
<td>+/- 5%</td>
<td>CV &lt;3%</td>
</tr>
</tbody>
</table>

*Application method for curve Acid. dil - 100: Take 100 µL of oil, using the specific pipette (see Note 1) and add it to the diluent. Mix the oil with diluent, after adding, by inverting test cuvette. Use 2,5 uL of diluted sample for testing.

**Application method for curve Acid. dil - 50: Take 50 µL of oil, using the specific pipette (see Note 1) and add it to the diluent. Mix the oil with diluent, after adding, by inverting test cuvette. Use 2,5 uL of diluted sample for testing.

Note 1: In order to do a correct dilution of the sample, it is recommended to use the specific precision positive-displacement pipette, set up to 50 or 100 µL, supplied by CDR.
CALIBRATION CURVE / CORRELATION DATA

FoodLab method shows a very good correlation with AOCS Official Method Ca 5a-40.

![FFA correlation curve](image)

**TEST PROCEDURE**

**Reagent preparation**

1. Pre-filled test cuvettes are ready to use.
   Incubate pre-filled test cuvette in the incubation cells for at least **5 minutes**.
   
   **Note:** The stability of the reagent R1 declines if pre-warmed exceeding 2 hours.

2. Where a dilution of the sample is requested, before starting an analysis session, it’s necessary to prepare a number of test cuvettes. Each cuvette is suitable for one single test.
   Follow the instruction below:
   - Dispense exactly 1 mL of diluent in each cuvette and close with its supplied cap.
   
   **Note:** In order to verify the correct filling level, make sure that diluent level matches the arrow tip on the reading side.

3. Select the appropriate **Acidity** curve, depending on the expected value of the sample, confirm your selection by pressing **ENTER** (on display shows INSERT BLANK).

4. Invert the incubated cuvette to mix before inserting in the reading cell 1. Green LED is on, press **ENTER** to read blank value. **Repeat for subsequent blanks.** A session of analysis allows reading up to 14 blanks/samples continuously.

   **Note:** It is recommended to homogenize reagent in the cuvette before reading blank. If the display shows **BLANK KO** after reading, it indicates that the cuvette in the reading cell is invalid (e.g. wrong or expired reagent, used cuvette, damaged reagent).

5. At the end, press **ARROW KEY UP** to stop the blanks reading session (on display shows INSERT SAMPLE).

**Sample adding and reading**

6. Using the specific pipette, add in proper amount of sample, depending on the range of acidity. Mix the reagent with the sample, immediately after adding, by inverting the cuvette. Insert the cuvette in the reading cell 1 and start the reading by pressing **ENTER. Repeat for subsequent samples.**

   **Note:** Homogenize samples in the bottle before taking it.
   It is recommended to use a positive displacement pipette for oil samples for higher accuracy.
   Remove excess oil by wiping the outer surface of pipette tip gently using a blotting paper.
   Immerse the pipette tip in the reagent while dispensing sample. Press and release the piston of pipette several times to ensure all sample has been transferred.
   Mix the sample with reagent, after adding, by inverting the cuvette several times.

7. At the end of the analysis session, results, expressed as % of oleic acid, will be displayed and printed.

**SYSTEM STANDARDIZATION**

The system is supplied pre-calibrated and ready for use.
Results are expressed in accordance with the reference method.
It is also possible to standardize the system using samples with a known value.
For information on the operating procedure, see the manual provided with the system.