Peroxide Value Method

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Abstract

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Brief description: This method for peroxide value determination of lipids is based on the original International Dairy Federation standard method. The basic principal of the method is that ferrous(Fe2+) ions are oxidized to ferric(Fe3+) ions by peroxides. The ferric iron reacts with the colorless ammonium thiocyanate to yield ferric thiocyanate (magenta colored). A standard curve can be generated with different levels of ferric iron using ferric chloride. This IDF method was modified by Shantha & Decker for fats and oils. This method has been modified to use a different solvent (methanol:butanol) so that a lot of samples can be processed using disposable plastic cuvettes, it is also a scaled down version. If chloroform is used, you must use quartz or glass cuvettes. Also, chloroform stabilized with amylene cannot be used. This method is more sensitive and uses less sample than the AOCS standard method, but is subject to a little more variation due to the small amount of sample used.

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Guidelines

Working solvent should be made fresh daily.
For some samples, using this method with chloroform-methanol may work better. In this case, make sure not to use plastic cuvettes.
Ferric chloride solution is good for approximately 1 year.
All other reagents (ammonium thiocyanate, ferrous chloride, etc.) are good for approximately 1 month.
A standard curve should be prepared with ferric chloride before samples are run.

Standard curve instructions:
Standard Curve:
1) Using the 10 µg/ml stock of ferric chloride (which was diluted in working solvent from the 1 mg/ml ferric chloride stock), set up duplicate or triplicate tubes with 1 to 15 µg of iron (or more..but using the above solvent system, 15 µg is about the max. that can be used).
2) Add the appropriate amount of solvent to each tube to make 3 mL.
3) Follow Steps 3-6 in the regular procedure (see Steps).
4) Plot µg ferric iron (X) vs A510 (Y) in Excel or another program and determine the slope of the curve using linear regression.
5) Use this slope in the equation for Peroxide Value determination.

Before start

Solutions:

1) Ammonium thiocyanate: Dissolve 7.5 g ammonium thiocyanate in water (~15 ml) and bring to volume to 25 ml.

2) Ferrous chloride:
a. Dissolve 0.2 g barium chloride dihydrate in 25 ml water.
b. Dissolve 0.25 g FeSO4-7H20 in 25 ml water.
c. Add the BaCl2 solution slowly with stirring to the FeSO4 solution. Then add 1 ml 10 N HCl. The excess BaSO4 will precipitate. Let it settle, then either decant or filter and store in a brown bottle.

3) Ferric chloride for standard curve: Dissolve 0.25 g iron powder into 25 ml 10 N HCl (this takes a little time and heat). Add 1 ml 30% hydrogen peroxide solution, remove the excess peroxide by boiling the solution for 5 min. Cool to room temperature, and dilute with water to 250 ml which gives a 1 mg/ml stock that should be stored in a brown bottle. This is diluted 1:100 in the solvent to make the working standard.

4) Working solvent: Methanol mixed with butanol: 2:1 v/v (i.e. 20 ml methanol, 10 ml butanol). Make fresh daily and mix well.

Protocol

Step 1.
1. Weigh 0.01 mg oil into test tube and record exact weight. Solid fat should be melted first. Samples with high PV may need to be serially diluted in working solvent. Make sure to record dilution factor if so. Up to 0.10 g can be used for samples with very low PV.

AMOUNT

0 mg Additional info:

Step 2.
2. Immediately add 3 mL working solvent and vortex to dissolve-make sure it is fully dissolved.

AMOUNT

3 ml Additional info:

Step 3.
3. Prepare a blank tube (solvent in place of sample).

AMOUNT

0 mg Additional info:

Step 4.
4. Add 15 µl ammonium thiocyanate to all tubes, and vortex 2-4 sec.

AMOUNT

15 µl Additional info:

Step 5.
5. Add 15 µl ferrous chloride to all tubes, and vortex 2-4 sec.

AMOUNT

15 µl Additional info:

Step 6.

6. Let sit at room temperature 20 min. Keep out of bright light.

Step 7.

7. Read absorbance on spectrophotometer set to 510 nm.

Step 8.

8. Determine PV using the following calculations:

a. PV (meq peroxide per kg sample) =

(As – Ab) x *m* / (55.84 x *m0* x 2)

As= absorbance of sample

Ab= absorbance of blank

m = slope of standard curve

m0=mass in grams of the sample

55.84 =atomic weight of iron