

## LIPID OXIDATION

The purpose of this laboratory exercise is to observe the effects of trace minerals and antioxidants on the course of lipid oxidation and to become familiar with different procedures utilized to evaluate the extent of lipid oxidation that has occurred in vegetable oil samples. This laboratory exercise will extend until the next scheduled laboratory session and will require an analysis to be conducted during the week to be scheduled with the instructor and laboratory assistants followed by a final analysis of the samples at the next week's scheduled laboratory session.

### Sample Preparation for Oxidation

The instructor will assign each group one of the following samples:

- Corn oil
- Olive oil
- Safflower oil

### PROCEDURE:

Place 100 ml of the oil in each of 4 beakers. To each beaker add the following:

1. Nothing – control
2. One copper penny
3. 100 mg Butylated Hydroxyanisole (BHA)
4.  $\alpha$ -tocopherol

Place the samples in the 60° C oven.

Do a peroxide value analysis on your control oil and on an abused oil sample. During the week and in the next week's laboratory session you will obtain peroxide values on the samples listed above.

### Abused oil sample

Your fresh oil sample should not give measurable quantities for peroxides. A sample of old, abused oil will be made available for you to use. You should perform peroxide and iodine analysis on those samples. This will allow you to gain experience in performing these assays on samples that have oxidized.

## Methods

### Peroxide Values

Weigh 5.00  $\pm$  0.05 g sample into a 250 ml Erlenmeyer flask and then add 30 ml acetic acid - chloroform (3:2) solution (under the hood). Swirl the flask until the sample is dissolved and add 0.5 ml saturated potassium iodide (KI) solution. Allow the solution to stand with occasional swirling for one minute and then add 30 ml distilled water. Slowly titrate with 0.01 N sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3$ ) adding it with constant and vigorous shaking. Continue titrating until the color changes to light yellow. Add 0.5 ml of 1%

soluble starch indicator which will give you a blue color. Continue titrating, shaking the flask vigorously near the endpoint which is a faint blue color to liberate all of the iodine from the chloroform ( $\text{CHCl}_3$ ) layer. Add the sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3$ ) drop-wise until the blue color just disappears.

Calculate the peroxide value as meq of peroxide /kg of oil,  $S \times M \times 1000 / \text{weight of sample in grams}$ , where  $S = \text{ml of } \text{Na}_2\text{S}_2\text{O}_3$ , and  $M = 0.01$ , the concentration of the  $\text{Na}_2\text{S}_2\text{O}_3$ , solution.

## **IODINE VALUE**

### **Apparatus and glassware**

Analytical balance, sensitivity 0.1 mg.

Erlenmeyer flask with ground glass stopper, capacity 300–500 ml.

Burettes, graduated to 0.1 ml, inspected and approved.

### **Reagents**

Wijs' Reagent.

Carbon tetrachloride ( $\text{CCl}_4$ ), inert to Wijs' solution.

Potassium iodide solution, 10%, free from iodine and iodates.

Sodium thiosulphate solution, 0.1 N.

Starch solution: mix 5 g of soluble starch and 10 mg of mercuric iodide in 30 ml water, add this mixture to 1 000 ml of boiling water and leave boiling for 3 minutes.

### **Procedure**

The appropriate weight of the sample, in g, is calculated by dividing the number 25 by the expected iodine value. Melt the sample, if necessary, and filter it through a dry filter paper. Transfer the accurately weighed quantity of the sample into a clean, dry, 500-ml glass-stoppered bottle or flask containing 20 ml of carbon tetrachloride, and pipet 25.0 ml of Wijs Solution into the flask. The excess of iodine should be between 50% and 60% of the quantity added, that is, between 100% and 150% of the quantity absorbed. Swirl, and let stand in the dark for 30 min. Add 20 ml of potassium iodide TS and 100 ml of recently boiled and cooled water, and titrate the excess iodine with 0.1 N sodium thiosulfate, adding the titrant gradually and shaking constantly until the yellow color of the solution almost disappears. Add starch TS, and continue the titration until the blue color disappears entirely. Toward the end of the titration, stopper the container and shake it violently so that any iodine remaining in solution in the carbon tetrachloride may be taken up by the potassium iodide solution. Concomitantly, conduct determinations on blanks in the same manner and at the same temperature.

Calculate the iodine value by means of the following formula:

$$(\mathbf{B} - \mathbf{S}) \times 12.69 \text{ N/W,}$$

in which:

(B – S) represents the difference between the volumes of sodium thiosulfate required for the blank and for the sample respectively,  
N is the normality of the sodium thiosulfate,  
W is the weight, in g, of the sample taken.

## **Aroma**

Compare the aromas of your three samples. Note any off aromas. Check the samples of the other groups. Give each sample a rank for intensity for intensity of off aromas.

## **Results**

Compare the control samples with those with the antioxidants and the added copper. Compare the results obtained with different oils to your samples. The data from all of the oils will be furnished by the instructor for use in your laboratory report.

## **Questions**

1. Why was each test performed? What were the typical reactions involved in each test?
2. What was the limitation of each test?
3. Which test compared best to the odor values for each sample? Was this test the same for each of the oil samples?

## **References**

Horwitz, W. editor, 2002. 41.1.16 AOAC Official Method 965.33, Peroxide value of oils and fats. Official Methods of Analysis of AOAC International, 17<sup>th</sup> ed., Gaithersberg , MD: AOAC International.

Narwar, W.W. 1996. Lipids. In: Fennema, O. R., editor. Food chemistry. 3<sup>rd</sup> ed. New York: Marcel Dekker, pp. 225-319.