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AN IMPROVED TBA TEST FOR RANCIDITY

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The TBA test for oxidative rancidity in foods was introduced some years ago and has proved to be very useful. The method formerly used in this laboratory involved pH adjustment and distillation of a water extract of fish muscle (1). We found it to be time consuming and tedious. A new procedure based on the method outlined by Vynke is now used (2). It has a sensitivity of 0 - 0.05 μ moles malonaldehyde/determination.

Reagents

Extracting solution: 7.5% Trichloroacetic Acid (TCA) in water
0.1% Propyl gallate
0.1% Ethylenediaminetetraacetic Acid (EDTA)

TBA reagent: 0.02M (≃ 2.338 g/ℓ) thiobarbituric acid (TBA) in water.

Mechanical stirring or slight warming with agitation is required to dissolve the TBA. The solution should be stored in a dark bottle in a refrigerator.

Procedure

(1) Blend 15 g of tissue with 30 ml of extracting solution for 30 seconds in a small Waring blendor or similar homogenizer. The amount of sample used is not critical, however the ratio of two parts extracting solution to one part sample should be maintained. Difficulties may occur at a later stage in the procedure if higher TCA/sample ratios are used.

(2) Filter the homogenate using Whatman #1 filter paper, or centrifuge in a small laboratory centrifuge at 2000 rpm for 15 minutes. The clear filtrate is used for the TBA reaction.

(3) Mix 5.00 ml of TBA reagent with 5.00 ml of extract in test tubes having screw caps (Pyrex - 120 x 10 mm). If a high concentration of malonaldehyde is present or if turbidity problems occur later in the procedure it may be necessary to use a smaller volume of extract, perhaps 1 or 2 ml. The volume is brought up to 5 ml with water before addition of the 5 ml of TBA reagent.

(4) Cap the tubes tightly and heat in boiling water for 40 minutes. If larger tubes than those specified are used, the caps should not be fully tightened so as to allow air to escape during heating.

(5) Cool in running tap water.

(6) Measure the optical density at 530 nm against a blank of 5 ml of water and 5 ml of TBA reagent which has been carried through the procedure. The absorption curve over the range from 400 to 700 nm should be checked to make sure that 530 nm is the wave-length of maximum absorption.

(7) The TBA value is calculated from a standard curve determined by using TEP standard (1,1,3,3 tetraethoxypropane). The moisture content of the tissue should be added to the volume of extracting solution in making the calculation. Thus the total malonaldehyde content in each sample is determined in the following manner: (assuming that 30 ml of extracting solution and 5 ml of the resulting extract are used in the determination)

$$\mu \text{ moles malonaldehyde/sample} = \mu \text{ moles malonaldehyde in 5 ml of extract} \times \frac{30 + \text{ml } H_2O \text{ in sample}}{5}$$

The results are then expressed as μ moles of malonaldehyde/100 grams of tissue (wet basis).

Standard Curve Determination

(1) Prepare a 1 μ mole/ml TEP standard solution by dissolving 0.22 g of TEP in enough water to make one liter of solution. Experiments have shown that it is not necessary to prepare the standard in a TCA solution. The acid does not appear to affect the colour produced in the reaction. If the standard is refrigerated it should keep for a few weeks.

(2) A working standard is prepared by diluting the above a hundred-fold. Solutions are then prepared according to the following table:

TBA SOLUTION (ml)	H_2O (ml)	WORKING STANDARD SOLUTION (ml)	TOTAL MALONALDEHYDE CONTENT (μ moles)	APPROXIMATE RESULTING ABSORBANCE
5	0	5	.05	1
5	1	4	.04	.8
5	2	3	.03	.6
5	3	2	.02	.4
5	4	1	.01	.2

(3) These solutions are heated, cooled and the absorbance measured as described in the standard procedure. A straight line relationship between absorbance and μ moles TEP should result.

The relationship between a TBA value and the level of rancidity in a sample, must be determined for each species tested. Organoleptic analysis (taste panels) should be carried out on a number of different samples for each particular species.

REFERENCES

(1) Tarladgis, B.G., Watts, B.M., and Younathan, M.T. J. Am. Oil Chemists' Soc. 37, 44 (1954).

(2) Vynke, W., Fette, Seifen, Anstrichmittel, 72, 12 (1970).