Comparative study of methods of TBA-test

Á. JUHÁSZ, V. MIHÁLYI-KENGYEL and L. KÖRMENDY

Hungarian Meat Research Institute, BUDAPEST (HUNGARY)

During processing and storing of meat products a serious problem is caused by rancidity, i.e. the oxidative decomposition of lipids. Unpleasant smell, taste, compounds of undesirable color may rise during this process destroying sensoric properties and exerting toxicity.

Determination of rancidity in meat products is a difficult task for the analysts as many different compounds may be formed during oxidation. For this purpose numerous chemical methods are known e.g. peroxide-test, bensidine-test, aldehyde-index, carbonyl-index, TBA-test etc. (HOLM et al. 1957; CHANG and KUMMEROV, 1955; HENICH et al. 1954; HOFFMANN, 1961.)

TBA-test is based on the fact that thiobarbituric acid composes a red pigment with the dialkenales (e.g. malonaldehyde) in acidic media. This reaction is influenced by many factors e.g. acidity, temperature, metallic ions, peroxides present in the sample.

The acidic TBA-solution has a typical absorption at 45o nm. After heating a new maximum appears at 53o nm that may become more intensive during heating. The molar extinction of the complex depends on pH, temperature and time of heating. Carrying out the reaction without any heating makes the development of color uncertain (TARLAGDIS and WATTS, 196o).

The alkenals which are present together with dialkenals also react with TBA. The absorption maximum is approximately at 53o and 45o nm resp. depending on their structure (MARCUSE and LARS, 1973). Reaction may take place with other materials, too, e.g. carbohydrates, furfurol, epihydrinaldehydes (SINNHUBER and YU, 1977).

The purpose of this paper consists of comparing the most frequent TBA-tests and evaluating their applicability.

MATERIALS AND METHODS

Sampling

TBA-test procedures were proved on randomly selected samples of backfat, pig liver and brain. TBA-tests on backfat were carried out directly after slaughtering, and after 6 and 8 weeks of storage at 6°C after dry curing with salt according to the normal practice in Hungarian meat industry.

TBA-value of brain and liver samples were measured after 24 hours after slaughter storing them at +2°C.

Reagents

Thiobarbituric acid - for the purpose of determinations a crystallic TBA was used.

Standard solution - for the determination of the calibration curves freshly distilled 1, 1', 3, 3' - tetra-etoxy-propane (malonaldehyde-bis-acetal) was used.

loo mg TEP was solved in 5o ml distilled water, 1 ml 1 n HCl was added, then the mixture was hydrolyzed for an hour at 48°C. After cooling distilled water was added unless the volume of this mixture reached loo ml. This solution was later diluted by adding distilled water in the necessary amounts.

Methods

Classical method - TBA-distillation-test published by TARLAGDIS et al. (196o) was chosen as a reference method.

UV-photometric method - The procedure using UV-photometry was carried out according to TAI-KWON and WATTS (1968).

Fluorometric method - Fluorometric method was carried out according to YAGI (1976) and UCHIYAMA and MIHARA (1978) after some modifications in the following way:

1 g of sample was weighed and placed into centrifuge tube, then 2o ml o,9 % NaCl was added and homogenized. The sample was centrifuged for lo mins at 2ooo RPM. 1 ml from the supernatant was put into a test-tube, and 5 ml 1/12 n H_2SO_4 and 1 ml lo % phosphoric acid

were added. After 5 mins standing at room temperature it was filtrated by folded filter. 1 ml TBA-solution (o,67 g TBA solved in loo ml mixture of distilled water and acetic acid 1:1) was added. Sample was heated at 8o°C for an hour, then cooled. The pink complexes developing at heating were extracted by 5 ml n-butanol. The fluorescence of this solution was determined at 565 nm emissionary wave-length (Excitation wave-length was 514 nm.) on EVANS EEL 244 spectrofluorometer. Concentration of malonaldehyde was determined according to the calibration curve set up by the help of TEP-solution.

RESULTS

Calibration curves were set up according to the former three methods. The procedure of establishing the curves was the following: different, but known amounts of TEP-solution were added to a fat-sample. The fat-sample was used as blank. The absorbance and fluoescence values in the pure solutions were determined (Fig. 1.). By comparing the results we could determine the average recovery of the above methods.

By the help of calibration curves the sensitivity of the methods was obtained, too. Results are shown in Table 1.

Table 1.

Method	Standard deviation	Lower limits of detection mg/g	The linear range of measurement mg/g	Average recovery %
Classical	o,833	$1,44 \cdot 10^{-1}$	o,144 - o,75	87,2
UV-photometry	o,o14	$1,5 \cdot 10^{-3}$	$1,5 \cdot 10^{-3} - 1,5 \cdot 10^{-2}$	98,3
Fluorometry	o,o37	$1,5 \cdot 10^{-5}$	$1,5 \cdot 10^{-5} - 1,5 \cdot 10^{-4}$	96,8

Table 1. shows that the standard deviation is the largest with the classical method while it is the smallest with UV-photometry. Fluorometric method is the most sensitive, but concerning reproducibility UV-photometry is considered to be the best. Applicability of methods was tested on samples of pig fat, liver and brain.

First, classical and UV methods were compared on 6o fat samples (Fig. 2.) Fig. 2. shows that methods are highly correlated. Evaluation was carried out on the basis of regression analysis. Regression lines were compared according to analysis of variance by MANDEL.

In this case regression of type y =a+bx can be replaced by type y = x, as difference of a and b from 0 and 1 resp. is not significant. The range of measured TBA-values of fat sample stored for 0 - 8 weeks, i.e. samples have different degrees of rancidity,by both methods were equivalent.

Comparison of UV and fluorometric methods was carried out also on samples of fat, brain and liver (Fig. 3.). Fig. 3. clearly shows that different tissues give different lines. Regression analysis was carried out on each tissue separately. It can be seen that both the regression equations and the correlation coefficients differ at the different tissues. So there is a systematic difference between UV and fluorometric methods giving different TBA-values. Concerning samples of brain the correlation between measured characteristics is loose because of the presence of some disturbing materials. Correlation is looser in the case of liver than of fat samples, though the range was wider in this case.

DISCUSSION

Comparing classical, UV-photometric and fluorometric TBA-tests, classical and UV methods give the same results, so they are equivalent. A systematic difference has been found between the fluorometric and the two other methods.

The problem of selecting a method for the determination of the TBA-value in a given product is settled according to practical reasons (disturbing materials, reproducibility, precision, ease of handling, economic considerations):

- At testing samples of high fat content it is recommended to use the UV-method instead of the classical one. These methods are equivalent but repeatability and recovery by the UV-method is much better. As a disadvantage, both methods are carried out with distillation, so their serial experiments are limited.
- At testing samples of high protein content (e.g. meat products) it is recommended to use the fluorometric method, though the classical one may be applied, too. In such samples the UV-photometric method is disturbed by volatile materials. Concerning reproducibility and recovery, the fluorometric method exceeds the classical one. It is easier to handle as distillation is omitted. As an advantage, it is very sensitive and can be used with small samples, too. It can be easily adapted for serial determinations, and there seems to be a possibility for automation. The disadvantage of the fluorometric method lies in the fact that it gives different results as compared with the other two. But according to practical experiences, in the case of the UV-method the presence of volatile materials during distillation disturb the precise determination, further, in the case of the classical method the acids present can influence the development of color, so the hypothesis that in samples of higher protein content TBA-value determined by the fluorometric method approaches closest to the true value, seems to be highly possible.

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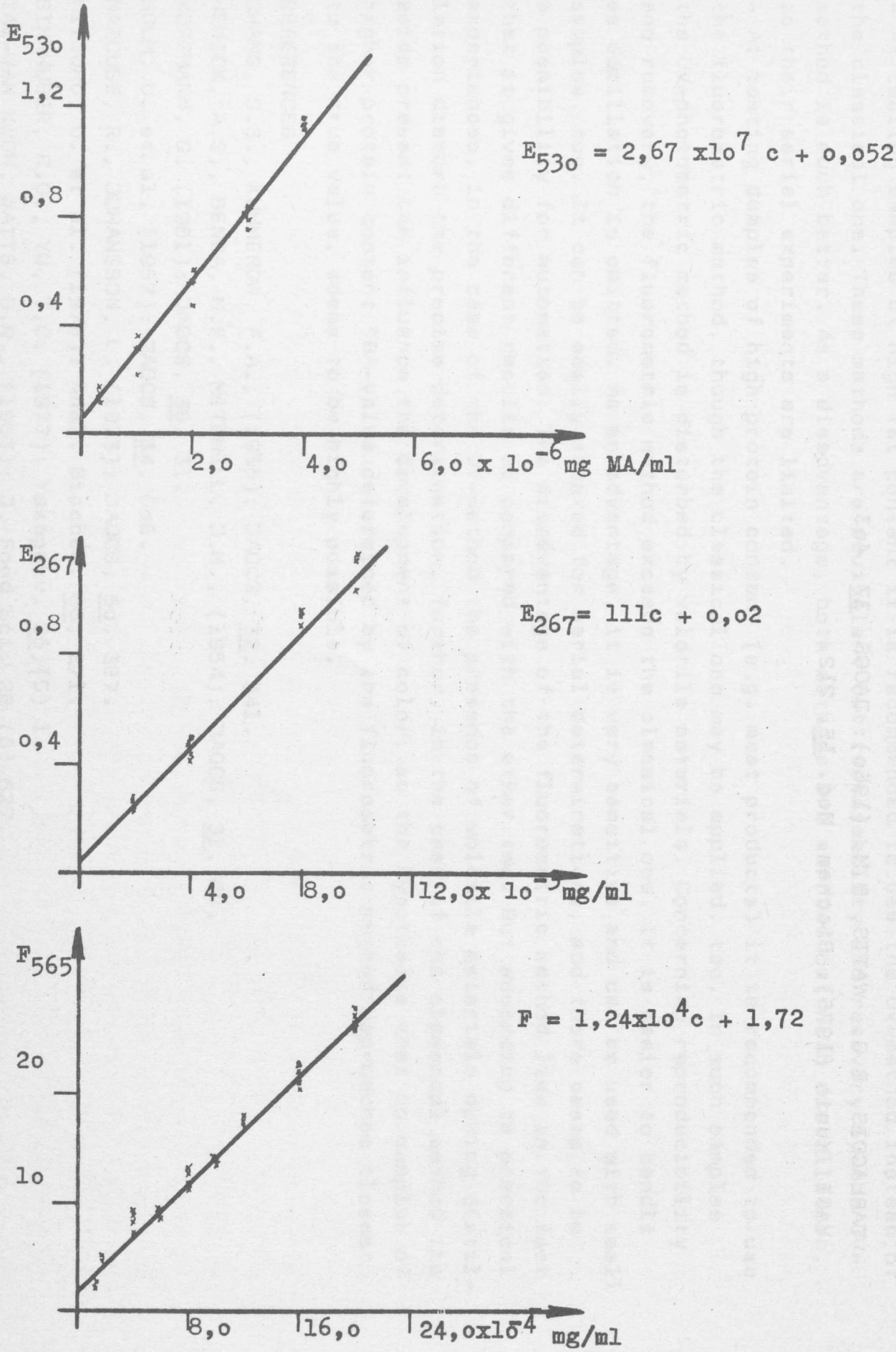
FIG.1. Calibration curves by all the three methods



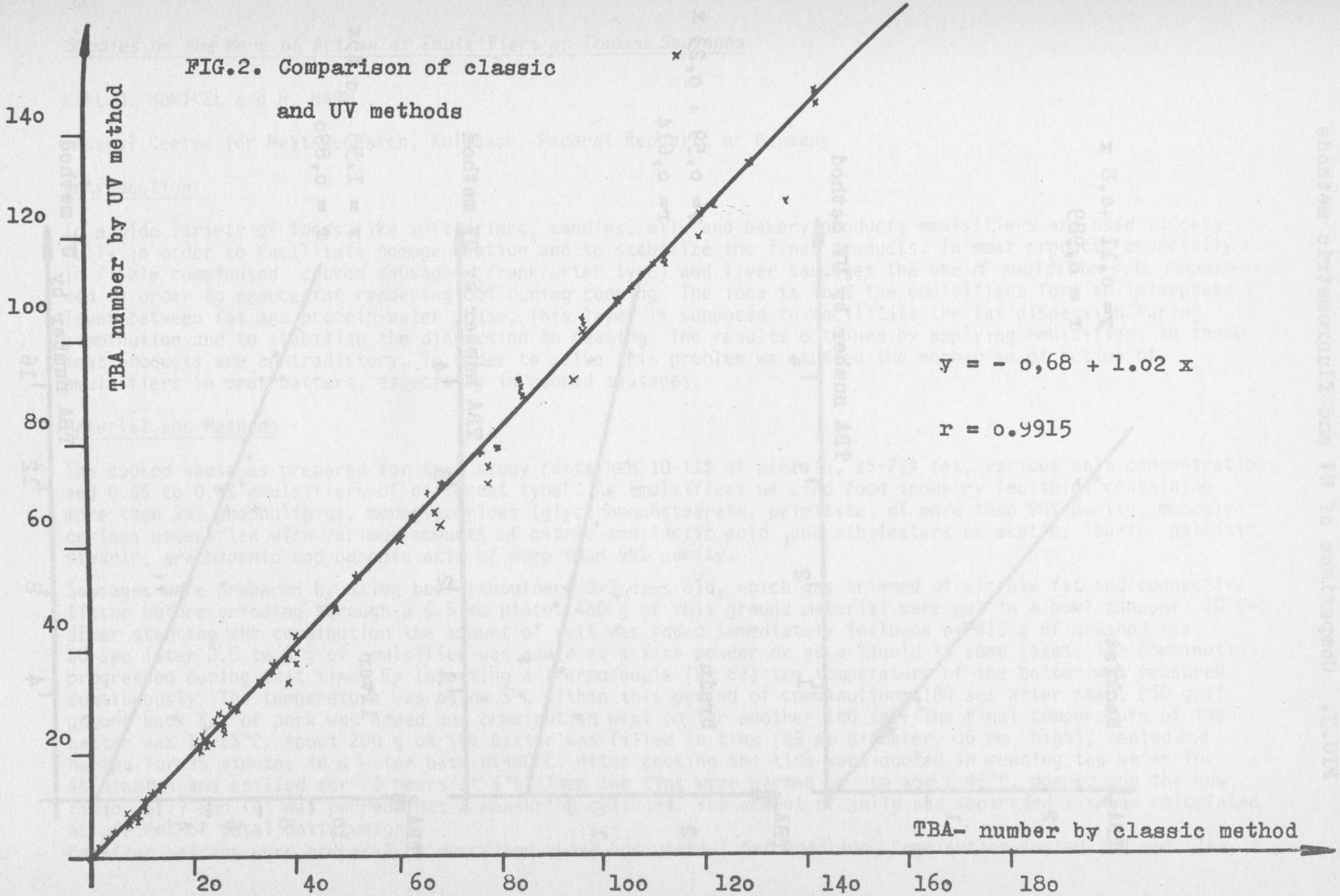


FIG.2. Comparison of classic and UV methods

FIG.3. Comparison of UV and fluorometric methods