SESSION 8. ANALYTICAL METHODS

REVIEW: RECENT TRENDS AND ASPECTS ON ANALYTICAL MEAT RESEARCH - A CRITICAL REVIEW

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GENERAL ASPECTS OF ANALYTICS

Progress in food research was and is always inseperably connected with the progress of analytical methods. Therefore analytics are also a decisive basis in meat research.

Many of the methods used in analytical disciplines are used in food research and in the analysis of meat and meat products. Thus in many of the papers presented at the European (International) Meetings of Meat Research Workers analytical investigations are included.

More than 100 years ago, J.v.Liebig carried out the first analysis of meat and meat extracts (LIEBIG, 1878). Liebig may be called the first meat scientist. Of course he used analytical methods which nowadays we would call simple or even primitive. However, his results were extremely accurate and comparable with those being found nowadays with modern sophisticated equipment.

We can still learn from that today that it is not always necessary to use very complicated and expensive instruments in order to solve an analytical problem. First of all knowledge, ideas and a good portion of common sense are most important in this field. In this connection another aspect should be mentioned: The application of statistical methods in the interpretation of analytical results. There is no doubt that statistics can be valuable in the evaluation of analytical results, but they are only a tool and they are not able to express what the results do not imply.

In other words statistical methods cannot be used to squeeze from confusing experimental results meanings or tendencies. It is not only an aphorism when E. Rutherford said: If you need statistics for your experiment you should go back and do a better experiment.

In general we have to distinguish between the qualitative "detection" and the quantitative "determination". However, in some publications we can read the term "quantitative detection" which is by no means a grammatical mistake only. It rather shows the lack of familiarity with analytical concepts.

I also want to discuss some aspects that are of general importance in analytical procedures. If a new method is being developed the following points are of special interest: (1) correctness, (2) accuracy, (3) repeatability and (4) comparability. The different points are characterized in Table 1.

Since the reliability of analytical methods is the basis of correct results and conclusions therefrom, we have to take care that in quantitative investigations the criterions given in Table 1 are fulfilled as far as possible.

Table 1: CRITERIONS OF QUANTITATIVE MEASUREMENTS

CORRECTNESS	Agreement between observed and calculated values. Investigation of samples with a known content of the substances analyzed; recovery-test
ACCURACY	Deviation of single values from the mean value; normally expressed by the standard deviation
REPEATABILITY	Repeated determinations in the same laboratory at different times and different conditions
COMPARABILITY	Agreement of results from different laboratories. Investigations with identical samples and the same methods

Furthermore I want to emphasize that "correctness" and "accuracy" do not mean the same: A value being measured very accurately can be incorrect (see Fig. 1,b) whereas a mean value which may be correct can be very inaccurate because of its high standard deviation (Fig. 1,a).

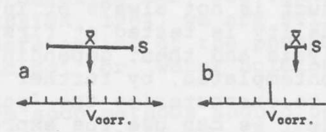


Fig. 1:
Demonstration of "correctness" and "accuracy". In a the mean value $\bar{x}$ is correct ($V_{corr.}$ = correct value) but inaccurate (high standard deviation); in b the mean value is more accurate but incorrect

ANALYTICS AND THE TERM "QUALITY"

For the evaluation of all the properties which are constituent parts of what we call "meat quality" there are more methods necessary than only those usually used for chemical analysis. The term meat quality can be defined as a complex of factors which are relevant for the sensoric impression, the nutritive value and the hygienic and technological properties (HOFMANN, 1987a, see Fig. 2).

In this sense meat quality can be analyzed scientifically and described objectively. Of course, in addition to the methods of analytical chemistry it is necessary to include sensory analysis and others like e.g. physical measurements. For example the measurement of the pH-value by glass electrodes and the velocity of its drop post mortem is an important indicator for the recognition of PSE and DFD meat.

Also ion-sensitive electrodes functioning by the same principle are available for the rapid determination of a series of an- and cations like Na^+, K^+, NH_4^+, Cl^-, NO_3^- and NO_2^-. However, their application on meat and meat products is not always satisfying because of accompanying substances which can interfere in the measurement.

Physical instruments are also used for the objective evaluation of carcasses (grading systems) replacing the former, more subjective, methods of estimation. The tenderness of meat which is an important aspect of meat quality can also be tested objectively by physical instruments.

The sensory analysis of food and of meat and meat products has been developed to a valuable tool which is completely different from the so-called organoleptic tests. The difference between both methods is to be seen in the different levels of training, knowledge and qualification of the taste panel. "Organoleptic testers" are persons with no training in sensory analysis, confusing quality tests with hedonic evaluations whereas a "sensoric analyst" is working like an instrument (JELLINEK, 1981).

The hygienic status of a product is also an important part of the quality assessment. This includes investigations of micro-organisms, toxins and residues as well. However, the latter are determined, again, by analytical methods.

Altogether we can distinguish five kinds of methods of analysis for meat and meat products:

1. Chemical analysis
2. Physical measurements
3. Microbiological analysis
4. Biological tests in toxicology
5. Sensory analysis

In practice, of course, the over-all-quality of a product is not always of interest. Normally, quality is tested at first by the sensory analysis and then, depending on the demand contemplated, by further investigations. In order to characterize the "partial quality" of a product we can use the expressions "sensory quality", "nutritive quality" etc.

We see "meat quality" as a matter of analytical (chemical, physical, microbiological, toxicological, and sensory) research.

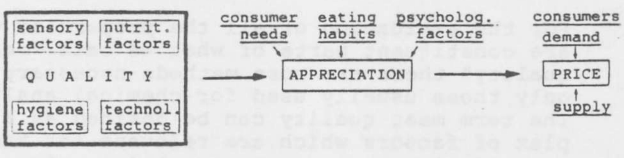


Fig. 2: Relation between the quality of a product, its appreciation and other factors. The arrows mark the main directions of influences. In contrast to the subjective "appreciation", the quality can be characterized objectively and is therefore not a matter of feelings but of analytical findings

Particularly important to me seems to be the distinction between the "quality", which can be measured objectively, and the "appreciation" of a product, which is naturally subjective and depends on a series of influences like the consumer's demand and needs, eating habits, psychological factors etc. (see Fig. 2). In this connection it should be emphasized that the price of a product is by no means a measure of the quality as HAMMOND (1952) postulated. The price depends on the appreciation by the consumers and on several other factors important to the market (Fig. 2).

CURRENT TOPICS IN MEAT ANALYTICS

In order to get an impression of what the situation in the field of analytical meat research is like and in which direction the application of analytical methods is going, I want to give a short review of the development in the last few years. This review will be limited to the papers presented in the Meetings of European Meat Research Workers (beginning with 1980). Although many of the papers presented included analytical methods, only those presentations were taken into consideration in which analytical questions are emphazised or in which the analytical methods themselves were the subject of investigations (Table 2).

The parameters that are important for nutritive value and palatiblity of meat and meat products have been investigated most frequently (21%), followed by the research of proteins including foreign proteins in meat products (15%). However, the development shows that the number of investigations of the nutritive value decreased in the last years.

Also a great deal (14%) of research has been done to determine residues and additives in meat and meat products which may be potentially harmful for human health. However, the boom in this field seems to be over, although actual events can change the situation all of a sudden as the dramatic accident of Chernobyl last year has shown.

Questions about the composition, the properties and the distribution of fats have been investigated as often as questions about meat quality and quality control (12%). It is obvious that the interest in quality aspects is growing.

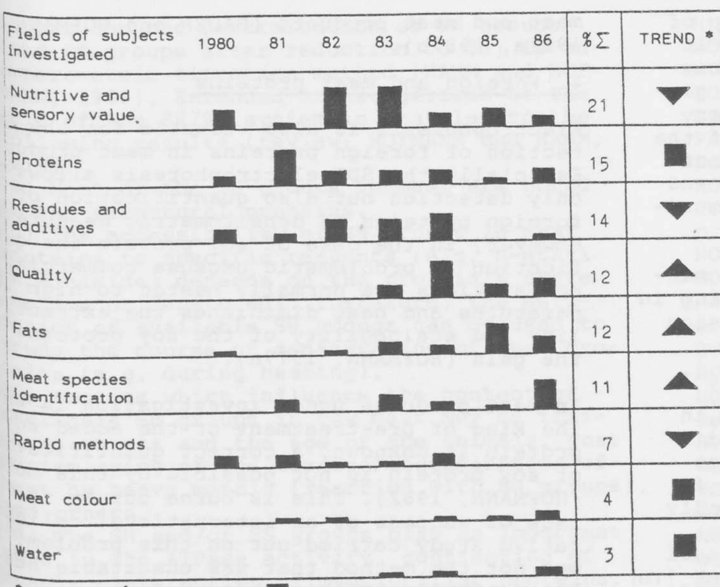
Increasing attention was also given to the development of methods for the identification of meat species (11%). On the other hand, rapid methods for the simultaneous determination of protein/water/fat being of great interest for the meat industry have obviously been developed to a satisfying state (all together 7%). Furthermore, investigations about the colour of meat and their substitution by suitable dyes (4%) and about the water in meat under different aspects (binding, distribution, water activity) (3%) have been carried out (Fig. 3).

In several cases it was difficult to classify the presented papers at the appropriate place. This could also be observed in the proceedings of the different years (some papers located in the analytical sections were not mainly analytical and others being of analytical importance were put in other sections). Therefore the number of papers included in Fig. 3 is not in all cases identical with the number of contributions in the analytical part of the proceedings of the different years.

There are important trends in the development of analytical methods which cannot be discerned from the review given in Fig. 3. The technical revolution in the area of microprocessors and computing systems influences and promotes the development of high resolution instruments.

Furthermore this development is connected with more precision and a better economy of the methods. On the other hand, the analyst who is using these complicated techniques has to be extremely specialised and qualified. He must be able to recognize not only the applications possible but also, or even more, the limits of the methods. Therefore, it is a widespread

Fig. 3: DEVELOPMENT IN ANALYTICAL MEAT RESEARCH (1980-86)



erroneous opinion that modern instruments, even when they work automatically, may be used by everybody who is able to read the application notes. In all cases, the fundamental chemical and physical processes must be understood. Even the best instrument is worthless in the hand of an inexperienced person.

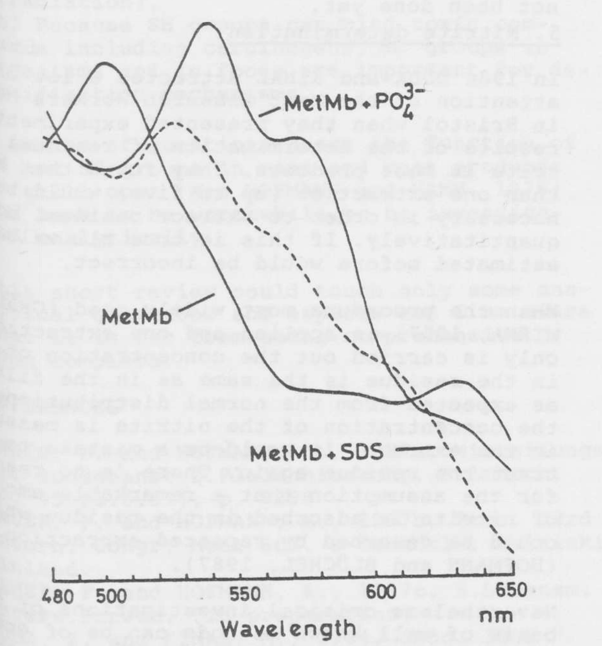


Fig. 4: Absorption spectra of metmyoglobin (MetMb) in absence and in presence of o-phosphate (PO_4^{3-}) and SDS resp. The addition of a phosphate buffer to MetMb causes a visible change of the colour from brown to brownish red and a shift of the absorption maximum of MetMb from 502 to 540

I am sure that in future the application of automatic instruments and computing systems will increase in the field of analysis. They will help to solve questions and problems which are still unsolved or help to carry out analytical work more rapid.

On the other hand there is a need for the practical use of rather simple methods. One example is the estimation of the water-holding capacity in meat with the filter paper-press-method (GRAU and HAMM, 1957; HOFMANN et al., 1982) or, even simpler, by absorption of surface fluids on filter paper (KAUFFMAN et al., 1986).

Curiously, the water-holding capacity (WHC) of meat, being one of the most important and most often investigated properties, is not really defined in an analytical and absolute sense but only in relation to the method applied (HAMM, 1972). However, WHC can in principle be defined as a complex of integrated binding forces (HOFMANN, 1971). Even if there exists very sensitive methods for measuring the physical state of water in meat proteins (e.g. proton-pulse-NMR, see TORNBERG and NERBRINK, 1984) we are still far from a full understanding of the phenomenon of "water binding" (or "water holding").

In practice such methods should be applied for the measurement of WHC in which, during investigation, the meat samples are in the same state (i.e. raw, heated, salted, etc.) as in the products being produced from this material (HONIKEL, 1987).

SPECIAL ANALYTICAL PROCEDURES

1. pH

One of the most important indicators for the evaluation of meat quality is the measurement of the pH value and the velocity of its decrease in meat post mortem. This allows us to differentiate PSE and DFD meat from normal meat which is of great practical interest for the meat industry (review: HOFMANN, 1987; Table 2).

Table 2: pH-VALUES IN MEAT BEFORE AND AFTER GLYCOLYSIS IN RELATION TO THE MEAT QUALITY

Meat Quality ▸	Normal	PSE	DFD
pH ante mortem	7.2	7.2	7.2
time post mortem	24 h	1 h	24 h
pH post mortem	5.5	5.8	6.2

The pH-value can also be used to control the quality of meat products. Using the figures given in Table 2 the initial velocity of the decrease of pH ($\Delta pH/\Delta t$) is as follows (given in pH units per min):

$$PSE \gg 0.03; \quad NORMAL > 0.001; \quad DFD \approx 0.0007;$$

In the case of PSE meat in which the drop of the pH value is higher than 0.03 per min it is possible, theoretically, to monitor this decrease right from the initial phase of glycolysis and thus to recognize PSE meat very early. However, the unavoidable damage of the tissue caused by the insertion of the glass electrode would influence the glycolysis and the fall of the pH (HONIKEL, private communication).

As the kind of presentation of pH values measured in meat post mortem is very confusing in literature, I want to suggest the expression of the time of measurement as an index e.g. pH_{45}, (not pH_1) or pH_{24h} (not pH_2 or pH_{24}).

It is no problem to measure the pH value in solutions or homogenates but it is more difficult in heterogenous materials like meat and meat products. In the latter case it is necessary to measure the pH value repeatedly at different parts of the material and to calculate the mean value. However, the usual manner of calculation $[(pH_1 + pH_2 + pH_3 ... pH_n):n]$ is mathematically not correct. The real average is given by the equation

$$pH = -\log \frac{[H^+]_1 + [H^+]_2 + [H^+]_3 + ... + [H^+]_n}{n}$$

If the difference between two determined pH values is smaller than 0.4 pH units, the error is less than 0.05 pH units and therefore negligible in practice. When the second decimal place of the pH value is given - provided that this is justified at all - the real mean value (pH) has to be calculated (see HOFMANN, 1973).

2. Meat colour

Another factor which is connected with the term meat quality is the colour based on the content of myoglobin and its degree of oxidation.

In this connection, it is interesting that the spectra of metmyoglobin (MetMb) is strongly influenced by the presence of phosphate ions (Fig. 4). This phenomenon has, as far we know, not been described yet in the literature and has obviously not been taken into consideration during the spectrophotometric measurements of the concentration of myoglobin in form of metmyoglobin. Our investigations indicated that a complex between the orthophosphate (but not pyro-phosphate) and the iron of the metmyoglobin is probably formed which is rather stable against the influence of heating (BAUER and HOFMANN, 1987c). In addition, it was found that sodium dodecyl sulfate (SDS) has a remarkable influence on the spectra of MetMb and the MetMb-phosphate mixture (Fig. 4).

3. Meat species identification

Recently the myoglobins have also won a particular significance for the identification of meat species. Each species posesses its own myoglobins with a typical behaviour during isoelectric focusing (IEF) (HOFMANN 1986a, 1986b). Therefore, the species of meat can be identified by the species-specific myoglobin patterns in IEF-gels (HOFMANN and BLÜCHEL, 1986). The sensitivity of this detection can be improved by a special staining procedure making the method able to be applied on heated meat and meat products (BAUER and HOFMANN, 1987a, 1987b).

4. Foreign and meat proteins

Electrophoresis is also valuable for the detection of foreign proteins in meat products. Especially the SDS electrophoresis allows not only detection but also quantification of foreign proteins by densitometric measurements. However, in the case of soy protein the quantification is problematic because commercial soy preparations are normally heated to high temperatures and heat diminishes the extractibility and stainability of the soy proteins in the gels (HOFMANN, 1977a).

In meat products under investigation in which the kind of pre-treatment of the added soy protein is unknown, a correct quantification of soy protein is not possible by this method (HOFMANN, 1982). This is borne out by the lack of success of an international collaborative study carried out on this problem. It was not the method that was unsuitable but the material investigated.

Instead of determining foreign proteins in meat products it seems to be more promising and simpler to determine the meat protein directly, i.e. by densitometric measurement of the actin component in SDS gels after electrophoresis (HOFMANN and PENNY, 1973). In this case the influence of heat can be taken into account because the relation of actin to myosin increases with increasing temperature and may be used to estimate the extent of heat treatment of meat (HOFMANN, 1977b). However, systematic investigations of this possibility to determine the content of meat protein have not been done yet.

5. Nitrite determination

In 1984 DUDA and KINAL attracted a lot of attention at the Meat Research Workers Meeting in Bristol when they presented experimental results of the determination of residual nitrite in meat products. They found that more than one extraction (up to five) would be necessary in order to extract residual nitrite quantitatively. If this is true all values estimated before would be incorrect.

When the procedure most widely used (GRAU and MIRNA, 1957) is applied and one extraction only is carried out the concentration of NO_2^- in the residue is the same as in the filtrate as expected from the normal distribution. As the concentration of the nitrite is measured in the extract, it would be a mistake to extract the residue again. There is no reason for the assumption that a remarkable amount of nitrite is adsorbed in the residue which could be desorbed by repeated extraction (HOFMANN and BLÜCHEL, 1987).

Nevertheless critical investigations of the basis of well known methods can be of great value and are from time to time necessary. Analytical progress needs critical tests.

6. Sulfhydryl and disulfide groups

A field of special interest is the estimation of sulfhydryl (SH) and disulfide (SS) groups. SH groups are the most reactive functional groups in proteins and they can easily be oxidized to SS groups. The SH/SS redox equilibrium is of great biological importance. One of the most convenient and accurate techniques for the determination of SH groups (and SS groups after reduction to SH) is an amperometric titration method (HAMM and HOFMANN, 1966). Extended investigations of the role of the SH/SS system in meat led to the following results (review: HOFMANN and HAMM, 1978):

(a) Most of the SH groups in meat are bound to the structural proteins.

(b) The accessibility of SH groups in meat proteins to specific reagents (e.g. N-ethyl maleinimide) depends on the protein's state (native or denatured). Therefore, the estimation of available SH groups can be used to study the course of denaturation of meat proteins (e.g. during heating).

(c) Factors which influence the content of available SH groups in raw meat may be training, stress and the age of the animals, post mortem aging of the carcase, contamination of meat by heavy metals (reaction with SH groups), and others.

(d) The SH and/or SS groups play an important role in the ATPase activity, contraction of muscle, interaction of myofibrillar proteins, rigor mortis and tenderness of meat.

(e) SH groups are involved in the curing process (blocking of the SH groups prevents reddening during curing).

(f) Smoking decreases the SH content of meat products because smoke contains many components (phenols, aldehydes, ketones) which are able to react with SH groups.

(g) Irradiation of meat causes the formation of hydrogen sulfide and volatil, sulfur-containing compounds, which may be responsible, among others, for the off-flavour of irradiated meat (SH compounds are also effective for the protection of organisms against irradiation).

(h) Because SH groups can bind toxic compounds including carcinogens, SH groups in organisms and in foods are important for detoxification mechanisms.

A series of questions about the function of SH and SS groups in meat and meat products is still open (see HOFMANN and HAMM, 1978) and seem to be worthwhile to be investigated analytically.

This short review could touch only some analytical questions and aspects and I am aware that it is far from being comprehensive or even complete.

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