Adaptation of an enzymatic method for the determination of ethanol in meat products

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Introduction

In 1951, Bonnischen and Teorell (1) in Sweden as well as Bucher and Redethky (2) described at the same time a specific process of determining ethanol in blood by means of an enzyme: alcohol dehydrogenase. This method was denoted as "Method ADH". It is based on the enzymatic property of alcohol dehydrogenase (ADH) that in a buffer medium it catalyzes reactions of transferring hydrogen from ethanol to diphosphopyridine dinucleotide coenzyme (DNP[1]). In consequence of the reaction, ethanol converts into acetaldehyde, while DNP converts into a reduced form: DPNH. This lather shows in contrast to DNP maximum absorption at 340 nm. The extent of absorption at 340 nm depends on the quantity of DNPH which is directly proportional to the quantity of ethanol contained in the tested sample. The course of the reaction may be presented as follows:

$$CH_3—CH_2—OH + DPN \xrightarrow{ADH} CH_3—C\begin{matrix}=O\\ \diagdown H\end{matrix} + DPNH + H\,.$$

The presence of semicarbazide in the buffer solution in which a binding reaction of acetaldehyde takes place, causes the reaction to take place only in one direction. The analytic procedure given by the above mentioned two groups of authors differed from each other in details. Bücher and Redetzky (2) used alcohol dehydrogenase isolated from yeast, while Bonnischen and Theorell (1) determined ethanol by means of ADH obtained from horse liver which displays a hundredfold smaller activity than that isilated from yeast.

Own experiments

Isolation of ethanol from raw meat products

Ethanol was isolated from meat products in a glass apparatus by means of steam distillation – constructionally analogous to a set used for the preparation of distillates (Fig. 1.). With this type of isolation, the determination of ethanol content in a distillate creates some methodical difficulties because in consequence

[1] This is actually denoted NHD.

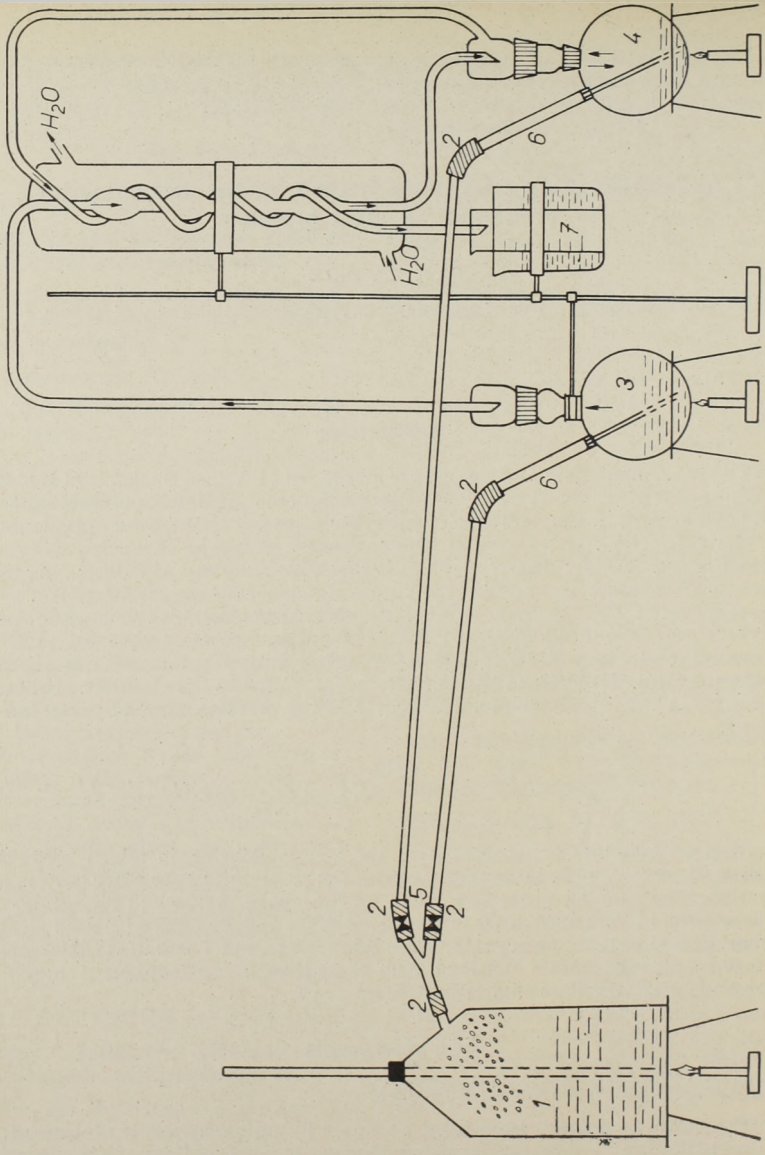

Fig. 1.
Assembly for isolation of ethanol
1. Boiler for steam generation, 2. Rubber sleeves, 3. Distillation flask No. 1, 4. Distillation flask No. 2, 5. Steam inflow control valves, 6. Capillaries, 7. Distillate receiver – graduated measuring cylinder

of steam distillation also other accompanying volatile compounds such as lower volatile fatty acids, aldehydes, ketones, amines, phenols etc are distilled. A considerable number of these compounds have reducing properties which prevent to determine ethanol by chemical (classical) methods. In this connection ethanol must be isolated from raw meat products in a definite medium, i.e. in presence of compounds which eliminate other volatile compounds from the distillate. Such mediums are e.g. a mixture of H_2O distilled with a 10% solution of Na_2WO_4, 1 *N* H_2SO_4 which binds substances having a basic character, for instance amines, and mixtures of saturated solutions of $HgCl_2$ and NaOH binding in turn also volatile chemicals as e.g. phenols, lower fatty acis, aldehydes, ketones, etc. (4).

Technique and method of isolating ethanol

200 – 300 g of processed meat were comminuted twice by grinding in a meat grinder and accurately homogenized. From the sample prepared in this way, 100 g were weighed and quantitatively transferred into a distillation flask (No. 1 in Fig. 1.). Then 90 ml of distilled H_2O, 30 ml of 10% Na_2WO_4 solution and 30 ml of 1 *N* H_2SO_4 were added and accurately mixed by means of an electric stirrer. On adding saturated solutions of $HgCl_2$ and NaOH (25 ml of each) the distillation process was started.

The distillation technique was as follows:

- generation of steam in a closed flask connected with the input of both above flasks,
- connection of the inflow of steam to the distillation flask No. 1 (inflow of steam to the distillation flask No. 2 was at that time closed),
- inflow of steam to the distillation flask. No. 2 was connected, after about 30 minutes of the distilling process, from the flask No. 1,
- termination of the distilling process after obtaining 40 ml of distillate in the receiver.

The receiver was a graduated measuring cylinder. This cylinder was placed in a beaker and cooled with ice water. Total time of distillation was 45 minutes in case of a 100 g sample of processed meat.

The analytical effectiveness of the technique of isolating ethanol was controlled on the basis of results of preliminary investigations which aimed at:

- establishing the volume of distillate in wich 100% of the ethanol content of the examined meat product is present,
- establishing the volume of additions neutralizing the substances wich prevent a quantitative determination of ethanol.

These investigations were made with standard aqueous solutions of a known concentration of ethanol and with samples of meat products enriched with known quantities of ethanol and nonenriched samples.

Results illustrating the analytical effectiveness of the applied method of isolating ethanol were checked by means of three available analytical methods and their effects are shown in *Table 1*.

Method of determining ethanol

Preparation of solutions (reagents).

I – Alcohol dehydrogenase (ADH 30 mg of protein enzyme (simple proteins) – suspended matter

Effectiveness of recovery of ethanol from meat products

Distilled material	Limit values of ethanol content (% by volume) determined by various methods		
	ENZYMATIC	WIDMAR'K	MABON'S
Meat product after addition of 0.5% by volume of ethanol (1)	0.640 – 0.640	0.640 – 0.686	0.670 – 0.674
Meat product without addition of ethanol (2)	0.144 – 0.140	0.170 – 0.180	0.155 – 0.165
Difference: x(1) – x(2)	0.498	0.509	0.512
Recovery – %	99.60	102.00	104.00

II – Diphosphopyridine dinucleotide (DNP).
Dissolve the content of a bottle in 2.85 ml of bisdistilled water
III – Buffer solution

Dissolve 10 g of sodium pyrophospate $Na_4P_2O_7 \cdot 10\ H_2O$, 2.5 g of semicarbazide hydrochloride[1] free of ethanol, and 0.5 g of glycol in 250 ml of distilled water containing 10 ml of 2 *N* NaOH and complete to 300 ml with bisdistilled water. Adjust the pH value 8.7.

Analytical procedure

Transfer the following quantities in the following order into a reaction vessel (test-tube of 5 ml):

4.80 ml of buffer	III
0.10 ml of DNP	II
0.02 ml of ADH	I
0.10 ml of distillate B[2]	

At the same time blank samples are prepared, i.e. samples analogous to the other ones into which bisdistilled water is added instead of distillate. After filling the reaction vessels mix the content so as to avoid foaming wich denatures proteins and may cause inactivation of the enzyme (3). After mixing the sample, allow it to stand for 70 minutes at room temperature (21 – 26°C).

Under such conditions and during this period alcohol contained in the sample undergoes a complete quantitative transformation to acetaldehyde while a portion of added coenzyme reduces DNP to DPNH and then determination of ethanol is made by measuring the optical density in the ultraviolet domain in cells up to 1 cm at 340 nm. In order to determine the quantity of ethanol plot first a working curve.

[1] Carbazide hydrochloride has to be dissolved, before analysis, in 100 – 200 ml of distilled water and heated to 80 °C in order to remove undesired traces of ethanol (3).

[2] Distillate B was prepared so that to 9 ml of bisdistilled water 1 ml of a distillate obtained from meat products was added. If in tested samples the concentration of alcohol is low then greater quantities of distillate B have to be taken while quantities of buffer III must be reduced.

Determination of a working curve

Into a volumetric flask of 1000 ml, 12.65 ml (10 g) of absolute ethanol was transferred. After completing with distilled water up to 1000 ml, the solution will contain 10 mg of ethanol in 1 ml corresponding to 10%. From the stock solution (10%) prepared in this way, solutions of lower concentration namely: 0.5, 1.0; 2.0; 2.5; 3.0; 3.5 and 4% are made by further dilution.

The further procedure is like the tests described above.

Experimental results

Stable raw meat products and aqueous alcoholic solutions in a known concentrations served as experimental material. Ethanol was not determined directly in the tested material, but in a distillate obtained by steam distillation.

As proved by the results of analytical investigations, the adapted ADH method, known in forensic medicine, proved useful in the determination of ethanol in meat products. Maximum error of the method was 0.40 of the obtained values.

The adapted method is simple in application.

LITERATURE

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ENZIMES MÓDSZER ALKALMAZÁSA HÚSIPARI TERMÉKEK ETANOLTARTALMÁNAK MEGHATÁROZÁSÁRA

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Klinikai vizsgálatokra korábbi kutatók által kidolgozott enzimes eljárást alkalmazott húsipari termékek etanoltartalmának egyszerű eszközökkel végrehajtható meghatározására. Az alkohol-dehidrogenáz (ADH) módszer azon alapul, hogy az ADH megfelelő pufferoldatban az etanolt acetaldehiddé alakítja s az így felszabaduló egyik hidrogénatom segítségével a rendszerben jelenlevő difoszfopiridin dinukleotid koenzimet (DNP) redukált állapotba viszi át (DPNH). Utóbbi vegyület abszorpciós maximuma 340 nm-nél mérhető, s minthogy a DPNH mért mennyisége egyenesen arányos az etanol mennyiségével, a reakció analitikailag hasznosítható. A húsmintából az etanolt vízgőzdesztillálással különíti el, és a párlat ismert hányadával végzi el az enzimes meghatározást. Az eljárás maximális hibája 0,40%.

ПРИМЕНЕНИЕ ЭНЗИМАТИЧЕСКИХ МЕТОДОВ ДЛЯ ОПРЕДЕЛЕНИЯ СОДЕРЖАНИЯ ЭТАНОЛА В ПРОДУКТАХ МЯСНОЙ ПРОМЫШЛЕННОСТИ

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Для клинических испытаний исследователями раньше разработанного энзиматического способа автор применял определение содержания этанола в продуктах мясной промышленности. Алькоголь – дегидроназный метод

(ADH) основывается на том, что ADM в соответствующем буфферном растворе превращает этанол в ацетальдегид и с помощью таким образом освобожденного одного атома водорода в системе находящегося коэнзима дифосфопиридин динуклеотида (DNP) превращает в редуцирующее состояние (DPNH). Максимум абсорпции последных соединений измеримых при 340 нм. и так как количество измеренного DPNH прямопропорциональный количеству этанола, реакцию возможно аналитически использовать. Этанол из образца мяса отделяет дистиляцией водяных пар и измерение проводит известной квотой дистилята. Максимальная ошибка этого метода 0,40%.

ADAPTIERUNG EINER ENZYMATISCHEN METHODE ZUR BESTIMMUNG DES ÄTHANOLGEHALTES VON FLEISCHERZEUGNISSEN

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Eine zur klinischen Untersuchung durch frühere Forscher entwickelte enzymatische Methode wurde zur mit einfachen Geräten durchführbaren Bestimmung des Äthanolgehaltes von Fleischprodukten adaptiert. Die sogenannte Alkoholdehydrogenase-Methode (ADH) fusst auf die Umsetzung des Äthanols zu Acetaldehyd durch ADH in einer geeigneten Pufferlösung. Mittels des auf solche Weise freigesetzten einen Wasserstoffatoms wird das in System anwesende Diphosphopyridinukleotid-Koenzym (DNP) in der reduzierten Zustand überführt (DPNH). Das Absorptionsmaximum letzterer Verbindung ist bei 340 nm messbar, und nachdem die gemessene Menge von DPNM mit der Menge des Äthanols direkt proportional ist, kann die Reaktion analytisch verwertet werden. Äthanol wird vom Fleischmuster durch Wasserdampfdestillation abgetrennt, und die enzymatische Bestimmung mit einem bekannten Anteil des Destillats durchgeführt. Der maximale Fehler der Methode beträgt 0,40%.

APPLICATION D'UNE MÉTHODE ENZYMATIQUE AU DOSAGE DE LA TENEUR EN ÉTHANOL DES PRODUITS CARNÉS

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Afin d'effectuer, avec de moyens simples, le dosage de l'éthanol dans les produits carnés, l'auteur fait recours à une méthode enzymatique développée plus tôt par d'autres pour l'analyse clinique. La méthode utilise la déshydrogènase d'alcool (DHA) en tant qu'enzyme et se base sur l'action oxydatrice de l'enzyme, lequel, dans une solution de tampon appropriée, transforme l'éthanol en aldéhyde acétique, tandis qu'un des atomes d'hydrogène libérés réduit en même-temps le coenzyme de nucléotide diphosphopyridine (NDP) en forme NDPH. Ce dernier composé a un maximum d'absorption à 340 nm et, étant donné que la quantité du NDPH ainsi déterminée est en rapport direct avwc l'éthanol, la méthode se prête à l'analyse quantitative. On sépare l'éthanol de l'échantillon de viande par distillation à vapeur d'eau et l'on utilise une part aliquote du distillat pour l'analyse enzymatique. L'erreur maximale du procédé est 0.40 p. c.