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A PROTOCOL FOR THE RAPID EVALUATION OF FRESH AND PROCESSED MEAT PRODUCTS

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SUMMARY

We present here a protocol for the Rapid Evaluation of Fresh and Processed Meat Products, which we hope will find widespread use in industrial and regulatory laboratories for evaluation of meat, raw materials for meat food products, meat products in process and finished processed meat products, for compliance with specifications and applicable regulations. The sample is subjected to simultaneous drying and fat extraction with boiling toleune as the extractant. In the case of samples of meat products containing additives, a determination of sugar, chloride, nitrate, nitrite and calcium is made on separate aliquots.

INTRODUCTION

Moisture, fat and protein determinations are routine analyses in meat product quality control. Specific samples may require additional determinations such as:

Estimates of the amount of salt, sugar, salt peter and/or non-fat dry milk.

One of the most serious disadvantages of the presently used methods is that they are very time consuming. Modern management methods and automation techniques give a high value to analytical methods which can be obtained more quickly than is possible by the conventional analytical methods. Often, the last iota of precision is unnecessary.

A second disadvantage to the use of conventional or "Official" methods is that they are not integrated into an organized protocol for convenience in the conduct of routine work. If conventional methods were used on routine meat samples, a separate aliquot would be required for each different determination and replication. We have sought to combine procedures to the greatest extent practical and have realized a considerable saving in material and investigators' time.

Most methods for moisture determination involved heating the sample in an oven to remove moisture (AOAC 1960; AMI 1954). Oven drying at moderate temperatures such as 101°C require 16 to 18 hours (AMI Method A2b54). We have found difficulties applying the higher temperature methods such as 125°C for two to four hours (AMI Method A2c54). The major difficulties encountered here are:

Sputtering in the oven and case hardening of the sample. Ironically, weight loss due to sputtering and moisture retained due to case hardening sometimes gives results misleadingly similar to the results obtained from more precise methods. Drying with infra red radiation has given difficulty due to case hardening and localized thermal decomposition. The principle of azeotropic distillation has been studied by (Everson et al. 1955) and in our laboratory by Wistreich et al. 1960). We have found that it is possible to simultaneously determine moisture and fat content of samples by azeotropic distillation. Further, it is often practical to estimate protein content by calculation from the weight of the dried toleune residue (DTR).

DETERMINATION OF MOISTURE, FAT AND PROTEIN

In order to determine fat and moisture simultaneously, a special modification of the toleune distillation apparatus is required. This has been described elsewhere (Wistreich 1960). This special flask has subsequently been improved and simplified. Figure I shows the assembled apparatus and Figure II the simplified special flask. Collection and preparation of the sample is well described in AMI Method A1a54. Procedures are given for fresh meats, sausage and other meat products. Space here will not permit more than the reminder of the complete dependence

Fig. I Assembled Apparatus

A. Drying Tube
B. Reflux Condenser
C. Bidwell-Sterling Type
Receiver Arm with Drip Tip
D. Adapter
E. Special Flask

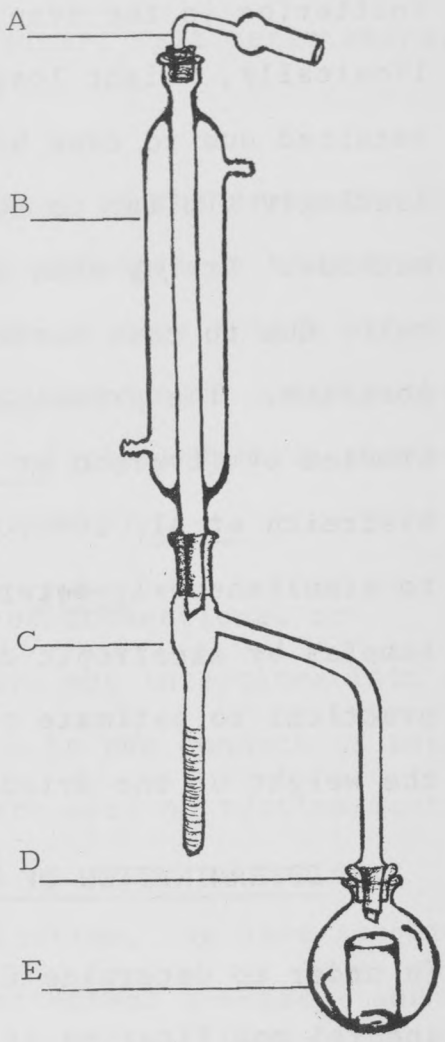
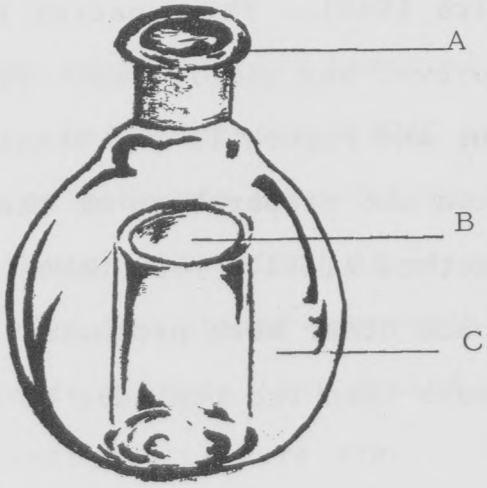


Fig. II Special Flask

A. Joint size $\overline{\$}$ 34/45
B. Center well-
Dia. 40 mm
Length - 88 mm
C. 500 ml. Flask



of all the analytical results on an adequate sampling and preparation job.

Approximately 10 grams of the prepared sample is spread on a 9 cm disc of Whatman No. 2 filter paper and folded to completely contain the meat. The weight of sample used is best determined by difference in weight of a quantity of the material in a stoppered weighing bottle, weighed before and after removing the aliquot used. The wrapped sample is inserted in a 19 x 90 mm extraction thimble and topped with the tuft of glass wool. The thimble, filter paper and wool were previously oven dried and tare weighed. This assembly is placed in the center well of the special flask where it will stand approximately erect. The flask should be manipulated so the drip tip of the solvent return tube is above the open top of the extraction thimble. In this position the toluene will drip continuously into the sample thimble. Approximately 250 ml of pure dry toluene is added and the apparatus assembled. Current is applied to the electric heating mantle to bring the toluene to a smooth boil. Note that pure toluene boils at 110.7°C. This is the highest temperature to which the sample is exposed. The toluene and moisture in the sample form an azeotrope boiling at 84.1°C. (1) Consequently, a high driving force is available to remove moisture from the sample and send it to the condenser.

Since liquid toluene is immiscible with liquid water (Solubility: 0.047g toluene/100g. water @ 16°C) and toluene is less dense (0.866 @20°/4°C) than water, the water drops separate from the toluene and fall into the collecting tube of the apparatus as the azeotrope condenses.

Essentially, pure toluene is automatically decanted back to the flask where it falls into the sample thimble in the center well. The hot toluene continues to form toluene-water azeotrope so long as water is present in the sample. The toluene serves to extract lipid from the sample at the same time. This extract fills and overflows the center well into outer compartment of the flask. Thus the lipid is extracted from the sample and carried into the outer compartment of the flask.

Water is evolved for a half hour to as much as one and a half hours depending on the nature of the sample. Processed meats give up their moisture more readily than fresh lean meat. It is our standard laboratory practice to run all extractions for two hours. We have found that this is also sufficient time to extract all the lipid.

The thimble assembly containing the toleune extracted residue is removed from the apparatus and dried. Our procedure is to dry it at room temperature in the laboratory hood for a half hour or until there is no odor of toluene. It is then dried in the air oven at 110°C to constant weight,

about an hour.

The condensed water is measured directly by volume in the collecting tube of the apparatus. This measured volume of water is related to the sample weight to calculate the moisture content of the sample.

For the routine estimation of fat in meat product, it is sufficient to combine the weight of the water measured with the weight of residue recovered and subtract this total from the original sample weight. This determination of fat by difference can and should be checked occasionally by rinsing the extract from the special flask into a tare weighed vessel from which the toluene is evaporated and the weight of the lipid measured. If more convenient, the toluene extract can be washed into a volumetric flask and an aliquot evaporated for determination of the lipid.

In routine work where it is deemed sufficient to estimate fat by difference, several samples may be extracted in the apparatus, one after the other with the same batch of solvent. Table 1 shows a comparison of the moisture and lipid determined on several samples by this technique and the results by the much longer official methods of the AOAC.

For the estimation of protein, it is necessary to calibrate the method for various types of sample material. The procedure is run on a number of different samples of the same

Table I. COMPARISON OF ANALYSIS

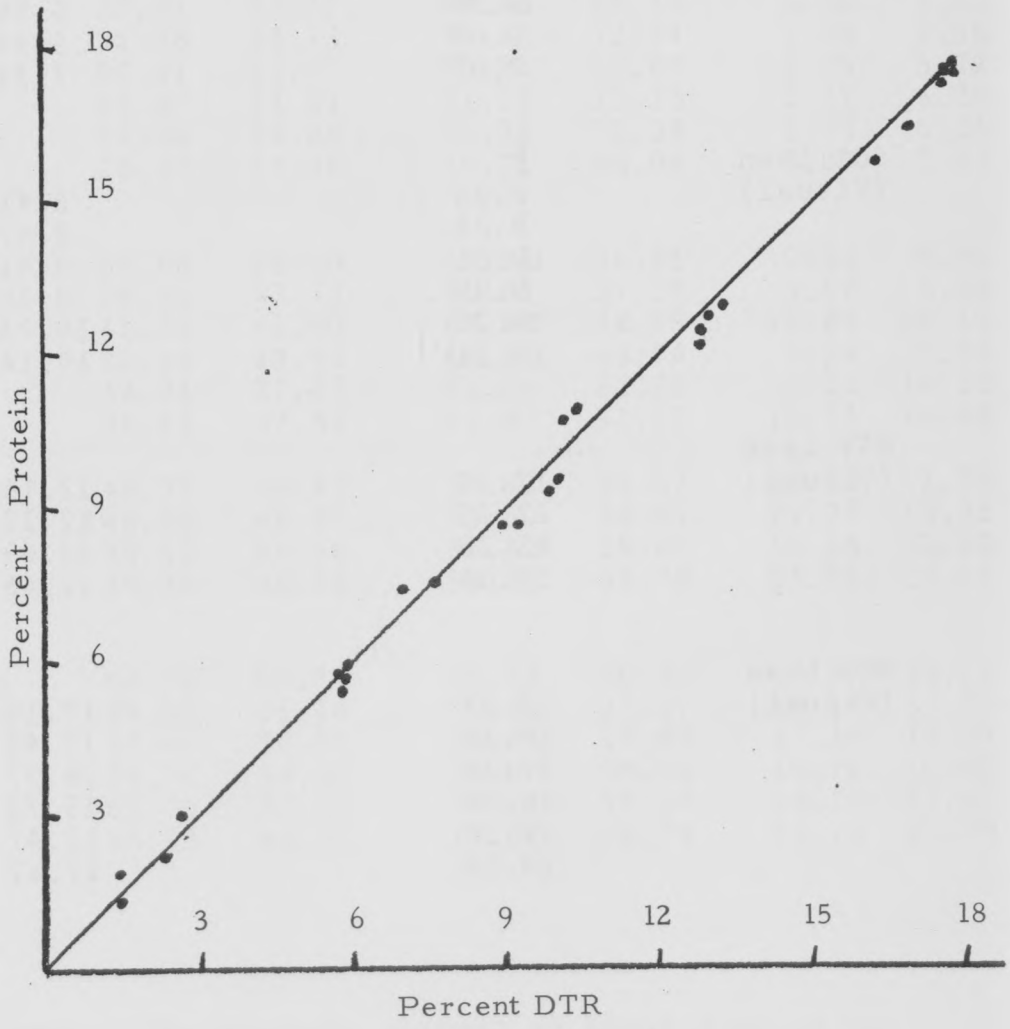
	% Moisture		% Fat		% Residue	
Produce	Toulene	AOAC	Toulene	AOAC	Toulene	AOAC
Fat Back	6.17	5.68	92.46	93.10	1.37	1.13
	7.08	5.65	91.62	93.10	1.30	1.37
	11.60	11.03	85.74	86.22	2.66	2.60
	12.19	11.27	85.34	86.04	2.47	2.50
30% Lean (Visual)	27.51	27.37	64.93	65.32	7.56	7.62
	22.88	22.15	71.40	72.74	5.72	5.56
	23.31	22.67	70.94	72.13	5.75	5.72
	22.97	21.81	71.25	72.35	5.77	6.29
	22.53	21.68	71.78	72.58	5.69	6.14
	28.22	27.26	64.71	65.06	7.07	7.67
50% Lean (Visual)	34.86	33.90	55.86	56.81	9.28	8.93
	34.91	33.72	56.01	57.25	9.08	8.96
	41.15	41.00	48.84	48.85	10.81	10.10
	41.18	40.92	48.83	48.74	9.99	9.99
	38.24	37.47	51.54	52.29	10.22	10.52
	38.42	37.32	51.47	52.17	10.11	10.49
65% Lean (Visual)	48.37	48.47	38.90	38.57	12.73	12.79
	48.95	48.49	38.30	38.41	12.75	13.05
	48.42	48.35	38.32	38.65	13.26	12.99
	48.36	48.29	38.58	38.79	13.06	13.01
80% Lean (Visual)	60.79	61.34	21.34	20.30	17.87	18.01
	60.65	61.16	21.21	20.37	18.14	18.05
	58.91	58.35	23.74	23.93	17.36	17.04
	54.34	54.24	28.97	28.64	16.70	16.42
	62.79	62.45	19.11	19.13	18.10	17.97
	63.93	62.92	17.90	18.74	18.18	17.98

kind of meat. The protein content of these samples is determined by the Kjeldahl-Wilfarth-Gunning method (AMI method A4a54). The protein values found are plotted against residue values to give a chart such as Fig. 3,

Table II. PROTEIN BY KJELDAHL - DTR CORRELATION

Product	% Residue	% Protein by Kjeldahl
Fat Back	1.37	1.12
	1.30	1.50
	2.66	2.92
	2.47	2.31
30% Lean (Visual)	7.56	7.34
	5.72	5.24
	5.75	5.49
	5.77	5.76
	5.69	5.58
	7.07	7.21
50% Lean (Visual)	9.28	8.41
	9.08	8.47
	10.01	9.41
	9.99	9.30
	10.22	10.23
	10.11	10.14
65% Lean (Visual)	12.73	12.16
	12.75	12.22
	13.26	12.69
	13.06	12.43
80% Lean (Visual)	17.87	17.19
	18.14	17.45
	17.36	16.37
	16.70	15.72
	18.10	17.47
	18.18	17.57

Fig. III Protein - DTR Correlation
(Pork Trimmings)



plotted from the data of Table 2. In subsequent routine testing it is only necessary to read the protein value from the chart. A separate chart is needed for each general type of meat product.

For the calculation of "Added substance" as prescribed by the Meat Inspection Division, it is necessary to estimate the salt content as well as protein and moisture. The formula used by this control agency is

Added Substance = (Moisture + Salt) - (Factor* x Protein).

DETERMINATION OF SALT, PHOSPHATE, NON-FAT DRY MILK AND SUGAR.

If salt is the only additional determination required, a satisfactory method is the AMI method A6a54. This is a Volhard titration on a nitric acid digest of the meat sample. Digestion is performed on a separate aliquot of the meat being tested so that the salt determination can be made concurrently with the determination of moisture, fat and protein. This method assumes that there are no unusual sources of chloride ions in the sample other than from the salt.

In the United States, phosphates are commonly used in a number of meat products. The amount of phosphate which may be used

*Protein moisture relationship factor prescribed in the MID regulations.

is limited by Meat Inspection Division regulations. A portion of the acid digest may be used for this purpose, applying one of the photometric methods. We prefer a variation on the Berenblum and Chain method and intend to publish our specific technique at a later date.

For a more complete set of determinations, it is desirable to make an acid extract of a sample of the product. This extract is used for the determination of salt by sodium measurement, added non-fat dry milk by calcium measurement, potassium, sugar and phosphate.

A 30 gram portion is homogenized for two minutes in a Waring Blendor or equivalent machine with 100 ml of cold 0.1 normal hydrochloric acid. The mixture is filtered through Whatman No. 1 or similar paper. The filter, residue and paper are returned to the Blendor and rehomogenized with a fresh 100 ml portion of cold 0.1N Hydrochloric acid for two minutes. This mixture is filtered, the filtrates are combined in a 200 ml volumetric flask and make up to the mark.

Sodium, Potassium, and Calcium may be determined by flame photometery on aliquots of the acid extract without further modification except the adjustment of dilution to meet the requirements of the particular flame photometer used.

For use of the Beckman Model 105 Flame photometer it is necessary for the sodium sample presented to the instrument

to be in the range of 0.5 to 1.0 meg/liter or between 0.003% to 0.006% salt. If, for example, the sample is of ham expected to contain 3.0% salt, the acid extract will contain 0.45% salt and must be diluted 1:100 to bring it into the working range of the instrument.

In the case of calcium, the Beckman instrument range is 0.0 to 0.4 meg/liter of calcium or less than 0.0008% calcium. After taking natural calcium in meat and the calcium content of non-fat dry milk into consideration, the acid extract must be diluted 1:10 to measure the added non-fat dry milk by the formula:

$$\% \text{ added NFD Milk} + \frac{\% \text{ Ca} - 0.017}{0.0132}$$

The total sugar in the sample is determined on another part of the extract by the anthrone method of Seifter, et. al. (7). As in the case of most photometric methods a calibration chart is required which has been prepared from standard sugar solutions.

Phosphorus may be conveniently determined on another aliquot of the extract by the photometric method of Berenblum and Chain (4) or alternately by the traditional Fiske and Subbarow (6).

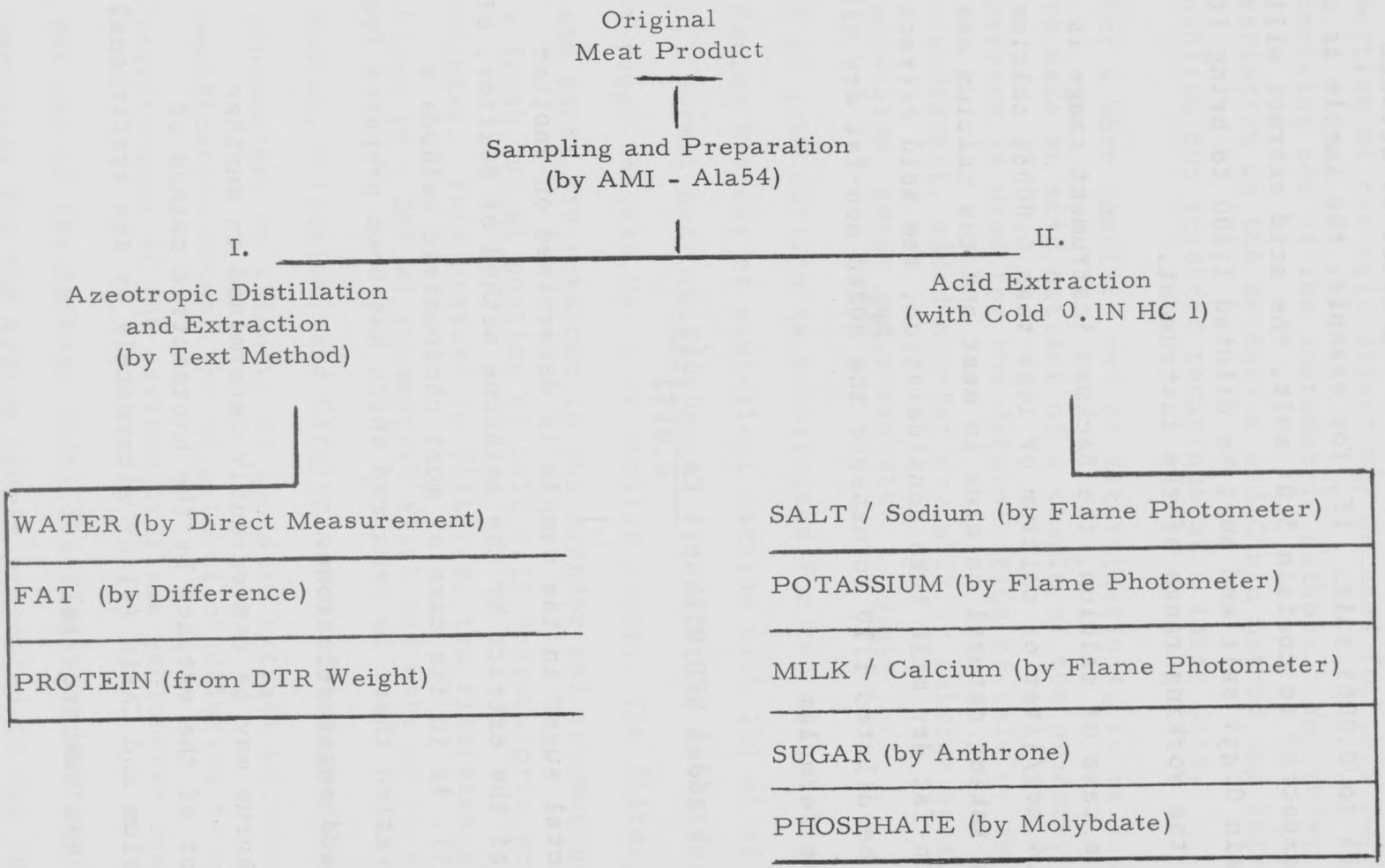


Fig. IV. Schematic Diagram of Protocol for Meat and Meat Product Evaluation

DISCUSSION

Fig. 4 presents a schema for the conduct of the entire protocol. For control laboratory evaluation of raw materials for sausage manufacture, it is quite likely that only the azeotropic distillation and consequent determination of protein, fat and moisture will be needed. Thus the work of such a laboratory can be handled routinely by this one simple method.

In American meat processing plants, careful attention must be given to adequately controlling the amount of moisture contained in smoked meat products. The use of too little moisture adversely affects the cost while too much moisture may result in the product being retained by the meat inspectors.

For the control of "added substance" or moisture content, it is necessary to determine salt content in addition to the factors available from the azeotropic distillation. For this purpose the determination of salt may be the only additional determination required. If Phosphate is used in the preparation of these products, it may be desirable to determine the phosphate content as well. Fortunately, both salt and phosphate may be determined on one acid extract of a sample of product. For such a laboratory, the combination of the azeotropic distillation method and the acid extract determinations will be sufficient to meet the needs.

If in addition to the determination of salt and phosphate, it is also desirable to know the amount of added sugar and the amount of added non-fat dry milk, the acid extract may also be used for these. In this case, the phosphate is determined from the acid extract and the salt from sodium as part of the flame photometric determination of sodium, potassium and calcium. The calcium determination permits calculation of the amount of non-fat dried milk added.

ACKNOWLEDGMENT

Acknowledgment is made to Mr. Endel Karmas for his valued assistance.

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ADDENDUM TO: A PROTOCOL FOR THE RAPID EVALUATION OF FRESH AND PROCESSED MEAT PRODUCTS

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ZUSAMMENFASSUNG

Wir unterbreiten hiermit ein Verfahren für die rasche Bewertung von frischen und verarbeiteten Fleischerzeugnissen und hoffen, dass es auf breiter Basis Eingang in industrielle und Überwachungslaboratorien zwecks Bewertung von Fleisch, Rohstoffen für Fleischnahrungsmittel, Fleischerzeugnisse in der Verarbeitung und fertiggestellte verarbeitete Fleischerzeugnisse zwecks Befolgung der anwendbaren Spezifikationen und Verordnungen findet. Die Probe wird einer gleichzeitigen Trocknung und einem Fettentzug mit kochendem Toluol als Entzugsmittel unterzogen. Falls Fleischerzeugnisproben Zusätze enthalten, wird der Zucker-, Salz-, Phosphat- und Kalziumgehalt auf separaten aliquoten Proben bestimmt.

BESPRECHUNG

Abbildung 4 stellt ein Schema zur Durchführung der gesamten Methode dar. Um in Überwachungslaboratorien die Rohstoffe für die Wurstherstellung zu bestimmen, dürfte wahrscheinlich die azeotropische Destillierung und daraus folgende Eiweiss-, Fett- und Feuchtigkeitsbestimmung allein genügen. Daher kann die von einem derartigen Labor durchzuführende Arbeit routinemässig mittels dieser einen einfachen Methode gehandhabt werden.

In den amerikanischen Fleischverarbeitungsbetrieben muss die in geräucherten Fleischerzeugnissen enthaltene Feuchtigkeitsmenge mit grösster Sorgfalt kontrolliert werden. Zu wenig Feuchtigkeit wirkt sich nachteilig auf die Erzeugungskosten aus, während infolge zu viel Feuchtigkeit die Fleischinspektoren das Produkt eventuell nicht zum Verkauf freigeben.

Zwecks Kontrolle der Zusätze muss man ausser den durch die azeotropische Distillierung erhaltenen Faktoren auch den Salzgehalt bestimmen. Zu diesem Zweck ist die Salzbestimmung unter Umständen die einzige zusätzlich benötigte Bewertung. Wenn bei der Herstellung dieser Erzeugnisse Phosphat verwendet wird, ist es eventuell wünschenswert, auch den Phosphatgehalt zu bestimmen. Glücklicherweise kann man sowohl den Salz-als auch den Phosphatgehalt mittels eines einzigen Säureentzuges an der Erzeugnisprobe bestimmen. Für ein derartiges Laboratorium dürfte die Vereinigung der azeotropischen Distillierung mit dem Säureentzug genügen, um die gegebenen Bedürfnisse zu erfüllen.

Falls es wünschenswert ist, ausser dem Salz- und Phosphatgehalt auch die Mengen des zugefügten Zuckers und der mageren Trockenmilch zu bestimmen, kann man für diese Bestimmungen ebenfalls den Säureentzug einsetzen. In diesem Fall wird der Phosphatgehalt vom Säureentzug und das Salz vom Natron als Teil der flammenphotometrischen Bestimmung von Natrium, Kalium und Kalzium bestimmt. Die Kalziumbestimmung ermöglicht die Berechnung der Menge der zugefügten mageren Trockenmilch.

SOMMAIRE

Nous présentons ici un procédé pour l'évaluation rapide des produits de viande fraîche et préparée, qui nous l'espérons, trouvera une large acceptation dans les laboratoires régulateurs et industriels pour l'évaluation des viandes, des matériaux bruts pour viandes de consommation, produits de viandes en traitement et produits de viandes préparées, tendant à confirmer les exigeances et règles applicables. L'échantillon est soumis à un séchage simultané avec l'extraction du gras, à l'aide de toluène en ébulition comme agent extracteur. Dans le cas d'échantillons de viandes contenant des additifs, la détermination du sucre, du sel, du phosphate et du calcium est faite à l'aide d'aliquotes séparées.

DISCUSSION

La figure 4 montre un schéma sur la conduite du procédé entier. Pour l'évaluation sous controle de laboratoire des matériaux bruts pour la fabrication de saucisses, il est très probable que seule la distillation azéotropique et la détermination en conséquence des protéines, du gras et des hydratants soient nécessaires. Ainsi le travail d'un tel laboratoire peut être mené sous routine par cette simple méthode.

Dans les complexes américains pour le traitement des viandes, une attention soigneuse doit être accordée au controle éfficace des hydratants contenus dans les produits de viande fumée. L'emploi de trop peu d'hydratants affecte malheureusement le coût, tandis-que celui de trop d'hydratants peut résulter en un produit refusé par les inspecteurs des viandes.

Pour le controle de la "substance ajoutée" il est nécessaire de déterminer la proportion de sel en addition aux facteurs résultant de la distillation azéotropique. A cet effet, la détermination du sel peut etre la seule détermination additionnelle requise. Si du phosphate est employé dans la préparation de ces produits, il peut être souhaitable de déterminer aussi la proportion en phosphate. Heureusement, le sel et le phosphate tous deux peuvent etre déterminés à partir d'un seul extrait acide de l'échantillon du produit. Pour un tel laboratoire, la combinaison de la méthode de distillation azéotropique et les déterminations des extraits acides seront suffisants pour répondre à la demande.

Si, en addition à la détermination du sel et du phosphate, il est aussi désirable de connaître le taux de sucre ajouté et le taux ajouté de lait en poudre non-gras, l'extrait acide peut aussi etre employé pour ceux-cis. Dans ce cas, le phosphate est déterminé à partir de l'extrait acide et le sel, à partir du sodium comme facteur constituant dans la détermination par flamme photométrique du sodium, du potassium et du calcium. La détermination du calcium permet le calcul de la proportion de lait en poudre non-gras ajoutée.

Приложение к трактату по скорому анализу свежих и обработанных мясных продуктов. Метод анализа для представления на 12-м Европейском СЬезде Работников Исследователей Мясной Промышленности. Сандфорд, Норвегия, 14-19 Августа. 1966.

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КРАТКОЕ СОДЕРЖАНИЕ

Представляя краткое описание скорого анализа свежих и перерабатываемых мясных продуктов мы надеемся, что этот способ анализа сможет найти обширное применение в производствах мясных продуктов так и в регулирующих и контролирующих их лабораториях, для выпуска продукции в соответствии с установленными пищевой промышленностью правилами. Применяя этот метод анализа образец мяса или мясного продукта подвергается одновременно сушке и обезжириванию с помощью кипящего толюина как экстрактанта. В случаях проб мясных продуктов содержащих добавочные примеси, определение содержания сахара, соли, фосфата и кальция производится каждый раз отдельно на отдельно взятых пробах.

ДИСКУССИЯ

Рис. 4 показывает схему всего процесса этого анализа. Для контрольной лаборатории по анализу мясного сырья для колбасной промышленности, возможно, что единственно небходимым явится только подвержение образца изотропической дистилляции и последовательное определение протеина, жира и влаги. Таким образом работа такой лаборатории может быть сведена к одному простому методу исследования.

В мясных фабриках Америки особое внимание уделяется точному контролю содержания влаги в копченых продуктах. Недостаточное содержание влаги может отрицательно отразиться на цене продукта, в то время как преизбыток влаги может привести к забраковке продукта инспектором.

Для контролирования количеств "добавочных примесей" необходимо кроме данных полученных изотропической дистолляцией определить количества соли в примесях. Вполне возможно что этот анализ будет достаточен.

При употреблении фосфата во время обработки продукта, желательно сделать определение количественного содержания фосфора.

К счастью, как определение содержания соли, так и содержания фосфата может быть сделано действием кислотного экстракта на образец продукта.

Как мы видим, для такой лаборатории комбинация методов изотропической дистилляции и кислотного анализа могут быть вполне достаточными.

Если к определению содержания соли и фосфата желательно узнать количество содержания сахара или добавленного обезжиренного молока (сухого), кислотный метод также может быть применен и тут.

В таком случае фосфат определяется кислотным способом, а соль - содовым, как часть огневого фотометрического метода по определению соды, потассия и кальция.

Полученное в результата содержание кальция может нам позволить регулировать по желанию добавление обезжиренного сухого молока в данный продукт.

ERRATA

PAGE	LINE	FROM	CHANGE	TO READ
1	7	Bottom	toleune	toluene
1	5	Bottom	chloride	salt
1	5	Bottom	nitrate	phosphate
1	4	Bottom	delete nitrite	
2	1	Top	salt/peter	phosphate
3	13	Top	toleune	toluene
3	9	Bottom	toleune	toluene
5	5	Bottom	toleune	toluene
7	Table I		Toulene	Toluene
7	18	Bottom	10.81	10.01
11	1	Top	meg/liter	meq/liter
11	7	Top	meg/liter	meq/liter
11	Formula		+	=
12	12	Bottom	delete or moisture content	