strumental method by near-IR has been one approach that has resulted in only partial success. Also, the microwave gravimetric method has been used where moisture is determined first and on the residue, fat is determined by rendering. Then, by using a factor, protein is determined. This approach offers some possibilities.

Table 4. Methods of Moisture:Protein:Fat Combination Analysis

1. Instrumental
   a. Infrared
   b. Microwave-Gravimetric (CEM, Hobart)

References

MOISTURE

Addis, P.B.; Chudgar, A.S. 1973. Rapid analysis of moisture in meat by refractometry. J. Food Sci. 38:354.

AOAC. 1980. "Official methods of analysis," 13th ed. 24.001-24.004, Assoc. of Official Analytical Chemists, Washington, DC.

Karmas, E. 1980. Techniques for measurement of moisture content of foods. Food Technol. 34(4):52.

Kropf, D.H. 1984. New rapid methods for moisture and fat analysis: A review. J. Food Quality 6:199.

Pettinati, J.D. 1975. Microwave oven method for rapid determination of moisture in meat. J. Assn. Off. Anal. Chem. 58:1188.

Pettinati, J.D. 1980. Update: Rapid methods for the determination of fat, moisture and protein. Reciprocal Meat Conference Proceedings, 33:156.

PROTEIN

AOAC. 1980. "Official methods of analysis," 13th ed. 24.027-24.036. Assoc. of Official Analytical Chemists, Washington, DC.

Heller, S.N.; Sherbon, J.W. 1976. Interlaboratory study of acid orange 12 dye binding for measuring protein in meat. J. Am. Off. Anal. Chem. 59(1):62.

Pettinati, J.D.; Swift, C.E. 1977. Collaborative study of the rapid determination of protein in meat and meat products by dye binding with acid orange 12. 91st Annual Meeting of the Assn. Off. Anal. Chem., Washington, DC, Oct. 17-20.

Pettinati, J.D. 1980. Update: Rapid methods for the determination of fat, moisture and protein. Reciprocal Meat Conference Proceedings, 33:156.

Revesz, R.N.; Aker, N. 1977. Automated determination of protein-nitrogen in cereals and grains. J. Assn. Off. Anal. Chem. 60:1238.

Seperich, G.J.; Price, J.F. 1979. Dye binding procedure for the estimation of protein content of meat components and sausage emulsions. J. Food Sci. 44(3):643.

FAT

AOAC. 1980. "Official methods of analysis," 13th ed. 24.005-24.008. Assoc. of Official Analytical Chemists, Washington, DC.

Bligh, E.G.; Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37(8):911.

Folch, J.; Lees, M.; Sloane Stanley, G.H. 1957. Simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497.

Kropf, D.H. 1984. New rapid methods for moisture and fat analysis: A review. J. Food Quality 6:199.

Lanza, E. 1983. Determination of moisture, protein, fat, and calories in raw pork and beef by near infrared spectroscopy. J. Food Sci. 48:471.

Maxwell, R.J.; Marmer, W.N.; Zubillaga, M.P.; Dalickas, G.A. 1980. Determination of total fat in meat and meat products by a rapid, dry column method. J. Assn. Off. Anal. Chem. 63(3):600.

Pettinati, J.D.; Swift, C.E.; Cohen, E.H. 1973. Moisture and fat analysis of meat and meat products: A review and comparison of methods. J. Am. Offic. Anal. Chem. 56:544.

Pettinati, J.D. 1980. Update: Rapid methods for the determination of fat, moisture and protein. Reciprocal Meat Conference Proceedings, 33:156.

Discussion

Steve Smith: In biochemical journals, almost everyone determines protein by Lowry, Biuret, or in some cases, dye binding. Why are not Lowry or Biuret methods discussed here?

Katz Ono: The Lowry and Biuret require soluble proteins and many of our samples really are not soluble.

Bob Benedict: There was a report last year from some European laboratories where they did a collaborative study among eight countries. They did moisture fat, protein, hydroxyproline, pH and, I think, one other thing because the European market is more interested in the fat-free protein and in collagen-free protein. They found that the most reliable or reproducible analyses were moisture and pH. The least was hydroxyproline, and if one took the values that were reported there by the various laboratories and calculated out this non-collagen protein, I think the values ranged from 18.8 to 21.2 for the same samples. This indicates that we have a lot of work to do to get reproducible or better analytical methods than we now have.

I think that one of the things that has to be done is to examine other methods and see if we can test them on meat. For example, the Biuret method (as pointed out earlier) measures soluble protein. However there is a method which, if you divide up the Biuret solvents, treat the meat first with alkaline reagent and then add the copper, provides a good test for meat proteins because the high pH dissolves the meat proteins (breaks them up). The Lowry method measures aromatics, so it is not going to be effective at all against connective tissue, which is very low in aromatic amino acids. Thus, one has to know the aromatic amino acid makeup of the sample.

We really need a good test for collagen. Collagen has a different nitrogen value than skeletal muscle proteins, so if you had 100% collagen, then you have 120% protein if the Kjeldhal method is used. If you wanted to obtain a higher protein, you could throw connective tissue in. I would like to comment on Maxwell's procedure for fat (dry column method). I have used it for five years and it is fantastic. You obtain an undenatured sample and you can do all sorts of fatty acid, sterol and cholesterol analyses, and you can obtain a separate phospholipid fraction. It is a test which I think we should get behind and have a collaborative study, if possible.

Ono: I agree with you. There is great difficulty in getting people interested in developing analytical methods. It is not a very glamourous type of research to develop methods, and this is reflected in the attitude in both academic and govern-

ment labs. In recent years, efforts to get some of these methods approved by AOAC have taken a back seat. I think it requires a little push from somebody to get this work back in motion so that some of these methods, like the dry column method, can be pushed ahead and finally accepted by the AOAC.

Bob Henrickson: Does the digesting unit for the Tecator have to be in a hood?

Ono: Theoretically, it can be operated outside a hood. It has a cover on it, and it is hooked up to a water aspirator so that any fumes that come out are flushed down the sink. In the old units, we tried to operate the unit outside the hood, but there were some leaks so we put it under the hood and it worked well. I understand the newer units are sealed fairly tight, and work with the water aspirator.

A.M.S. Alam: What is the reliability or dependability of the Tecator unit, mechanically speaking?

Ono: Let me state, first, that I do not endorse any equipment by brand. As a public servant, I cannot do this, but I can speak from experience. The unit which I have was inherited from one of my colleagues because he could not make it work satisfactorily. It was brand new and cost about $14,000. I was happy to get it, and we finally got it to work.

Alam: Sometimes this equipment is very frustrating.

Ono: Yes it is. The same with the Kjel-Foss. People have bought brand new units which have sat idle because people cannot master them, but once it becomes workable, it functions well.

Henrickson: Did you say it is reliable?

Ono: It depends on how much patience you have. For all of these systems, I do not think you can expect to put a graduate student on it for one year and expect that it will continue to run. It requires continuous attention. You need someone who knows a little bit about machines so that they can trouble-shoot and not have to call the service man every time something goes wrong.

Kaj Foget: I was wondering if you would be willing to talk about the near-IR reflectance versus transmission, and how much work has been done in terms of sample preparation, and its effect on accuracy of the method.

Ono: I will go with the sample preparation first. People have tried different sample preparation procedures and find that they must recalibrate every time they change the matrix or the size of the grind. This calibration procedure is not easy since you have to calibrate against known procedures. Every time you change the size of the grind, a new calibration is needed. It really is troublesome. With respect to transmission versus reflectance, people have tried this. The reference by Lanza (1983) goes into this. Basically, they get into the same type of problem.

Dennis Olson: We have been working with near-IR reflectance, and what you said about sample preparation is probably the most critical, going from coarse grinds to very fine grinds, to finding that those are not even acceptable. We are now putting samples in a kind of impact machine. It is a Vitamix, in which you can also make soups, malts, etc. There, the sample preparation seems to be quite good. We are using an open cup in that instance and the surface preparation is probably the most critical, but we are developing some quite good calibrations at this point.

Ono: The Europeans have been using some of these emulsion techniques and they too have had some good results.

Benedict: Also, pulsed NMR is being touted now by IBM as a means of fat and moisture, and you can select your relaxation time. If you wanted to take the time, supposedly you could select the relaxation times, and either get the water protons or fat protons. We have been doing some work with it during the past year, and it is very susceptible to outside interference, such as salt level and ice crystals. It is being sold and you will see advertisements for it, but don't rush out and buy one.

Ono: How much does it cost? Is it not quite expensive?

Benedict: I think it is around $10,000. It is a desk-top pulsed system.

Paul Lewis: Could you go over sample size? What sample size do you need for most of these analyses?

Ono: The near-IR analyses could be conducted using a 6 g sample or even larger. It depends on your reflectance eye. Most of the equipment we have discussed could be done with a 5 to 10 g sample, except the Anal-Ray, which requires a 13-pound sample.

Glenn Schmidt: Regarding sample preparation, we are using a Sunbeam food processor to homogenize our sample. Does anybody know why we should not be doing this? It really works well with small sample sizes, and washes up well.

Ono: Do you end up with a paste?

Schmidt: Right.

Ono: We do something like this and it is quite acceptable.

Schmidt: The Sunbeam food processor and a Kitchen Aid mixer are very nice lab instruments. They are readily available and work well. We have recently installed a Tecator protein analyzer. We have used it daily and it seems to be working fine.

Alam: The only problem you may have is in breaking the particles to a homogeneous size when you have connective tissue or tendon, or something like that. The blade clogs and you have to check it periodically for connective tissue.

Schmidt: We have fewer problems with the food processor than the grinder.

Ono: With the food processor, most of the connective tissue is shredded, and it becomes part of the mix.

Olson: We do have some problem with connective tissue unless we freeze the tissue in liquid nitrogen and pulverize it into a powder. If we want to be most accurate, this is the way we do it.

Alam: What is the temperature of liquid nitrogen?

Ono: $-196°C$.

Harold Herring: One of the problems with the CEM equipment, using the microwave procedures, is the error involved after microwave drying of a processed meat sample that contains salt and sugar. This leads to errors in the analysis of fat. Has anyone had a similar experience, and how do they get around it?

Benedict: Joe Pettinati has used the multiple analyses system and the Hobart. Actually, with high fat samples, there is a tendency for holdup of the fat. This was a collaborative study and some other people here may have been involved with it. There was a problem with high fat where the moisture

came off, and then the fat was allowed to melt into a separate container. You do not get all of the fat coming off; consequently, when you do the subtractive measurements, there is a tendency for you to be too low in fat and too high in protein. For normal samples, it should not be a problem. Joe Pettinati is preparing a publication on the results of the collaborative study.

Olson: On near infra-red reflectance, we are looking at developing calibration curves for collagen and, in just the last month and a half, we have started that. It's looking so good that we are encouraged to go on and do a little bit more, but it is yet not good enough to use as a determination. Being a bit facetious with that, I think there is promise there, but it is not good enough at this point. It seems to me that it is one of the techniques which does offer possibilities for future use if development will continue, because you do not need a trained operator and it could be applied to production. When we look at all rapid analysis techniques, we are talking about 15 minutes or less. The other question is the training of the technician. We must be concerned about the complication and safety of the method. I think these are important considerations when you evaluate all rapid techniques.

Ono: Dennis Olson, I think the audience might be interested in knowing the price range of the equipment that you use.

Olson: We are using a Tecnicon machine right now, but it is difficult to quote a price because the last time I inquired was two years ago. It also depends on the different kinds of options obtained. I suspect that the machine that we have, with top-of-the-line options, would cost between $40,000 and $60,000.

Ono: Can you get a stripped-down model for about $20,000?

Olson: Yes.

Schmidt: When you are on a tight budget, is there anything cheaper with similar accuracy than a Babcock-Paley bottle and a hot plate?

Ono: Probably not.

Person not identified: We have done a lot of work with the automated methods and we have always had to go back to ether extraction and the normal Kjeldahl method. What we do with CEM and Hobart is to compare 50 samples of a certain type of material against the ether extraction, and we will actually (unfortunately, I hate to admit) put in a fudge factor. This is because of the reasons that Harold Herring mentioned, that sometimes you do not get the total amount of fat; consequently, your moisture reading is a little bit higher. But for pure accuracy, I don't think you can beat ether extraction and the Kjeldahl. We have always had to go back to those methods.

Ono: By Kjeldahl, do you mean automated or traditional?

Person not identified: Traditional.

Ono: By the way, the automated methods (the Kel-Foss and the Tecator) have received final AOAC actions. The Technicon has received one action from AOAC but it is not final. I do not see why it should not be an accepted AOAC method before too long.

Acton: Would anyone comment on rapid methods for salt analysis? There seems to be some newer techniques other than the dip stick and the traditional methods for chloride titration.

Ono: I do not really have any information.

Benedict: We have been doing that in conjunction with the NMR to see if we could use microwave for moisture and a rapid method for salt and another method for water activity. Using the NMR, we could get some indication. We found, comparing atomic absorption to the chloride, Hobart and to the sodium electrode, that the sodium electrode was the most rapid. You get a four-decade range so you do not have to dilute. Any time you have to dilute out for the atomic absorption, you run the risk of error. I would recommend a pH meter, calibrated with a sodium electrode, and check it frequently. In 15 minutes, you can determine moisture and salt.

Herring: The Orion company has a good pH meter called the 901. You can purchase electrodes for sodium, chloride, or floride or others to go with the meter. It works quite well for chloride.

Olson: We use both sodium and chloride specific ion electrodes. The chloride electrode is the easiest and most reproducible. We have had a few problems with sodium, but we are quite happy with the results and it is quite rapid. We have found that we have to boil the sample for a brief period in acid, though, to get best results.

Henrickson: When do you prefer to use ether and when do you prefer to use chloroform?

Ono: The chloroform-methanol extraction method is rather slow. In our hands, we have done approximately 12 per day. That is awfully slow, but the advantage is that if you want to measure fatty acids on the extract, you need a chloroform-methanol extract. The ether extracting method is faster but more dangerous in terms of safety. I would say that if you really are not concerned about the phospholipid fraction, go ahead and use the ether extraction.

Alam: How would you compare the dye-binding method with regular methods?

Ono: For well-defined systems, if you are not bothered by connective tissue, they compare very well. Pettinati at the USDA, Philadelphia, has done some work with this and was impressed with dye-binding, but if you have any connective tissue, you do need a convection factor.

Alam: Which dye-binding method do you use?

Ono: Udy dye-binding, using acid orange dye.

Mike Dikeman: What are the recommended rapid methods for calories and cholesterol?

Ono: By determining protein and fat, one can calculate calories by using the Atwater factor of 4.27 kcalories per gram protein and 9.02 kcalories per gram of fat. There is no fast method for cholesterol.

Schmidt: In the lipid extraction method, is there any specific advantage to using petroleum ether versus diethyl ether?

Ono: No, but I recommend petroleum ether because it is a little safer in that it has a slightly higher boiling point.

Benedict: There are several references here by Pettinati and Maxwell of the USDA Eastern Regional Laboratories. Those of you who would like reprints of this work, please send me your requests.

Ono: Six labs must cooperate to promote new methods for AOAC approval.

Foget: From Oscar Mayer's standpoint, we are quite

interested in the near-IR and other physical instrumental methods of analyses because rapid results are of tremendous importance to us. However, until we can get AOAC approval for these methods, we are still trapped by the chemical methods because our regulations are based on them, and it will take an enormous effort to persuade AOAC to accept the IR methods. I think an organization like this one could spearhead that effort.

Ono: I don't foresee that near-IR will be approved by the AOAC any time soon. There are too many problems with this method.

Schmidt: It seems that this would be an excellent project for the USDA.

Ono: In earlier days, it would have been, but recently we have been discouraged from doing this type of work. Many people do not consider this as good science. I am not sure how we can reverse this. As you know, the USDA has an evaluation system through which every scientist is evaluated by a peer committee, and people who have been doing this type of work have really taken a beating because the work is not considered original or exciting.

Schmidt: Maybe the FSIS should be the funding agency to insure that this work is done to facilitate standardization and enforcement of composition.

Dikeman: I feel that AMSA could be effective in getting some of these methods approved. I am not sure what the mechanism should be; perhaps a committee could be established to determine how this could best be accomplished.

Benedict: There is an organization, the American Society of Testing of Materials, that could do this work. It is designed for this type of work. The AMSA does have a representative on this committee.

Ono: Bob Benedict, do you think the ASTM is really capable of pushing a method such as the dry-column method for lipid determination?

Benedict: I don't know. Joe Pettinati is the chairman of the ASTM committee and Bob Rust is the AMSA representative.

Ono: I will contact Joe and see what we can do about getting something organized.

Jim Price: I see an interest from industry to be able to determine animal protein versus protein from other sources. Does anyone in the audience know of any rapid methods to determine meat protein where some plant protein is present?

Ono: FSIS is very concerned about additives. Are you aware that titanium dioxide is not now required in soy proteins because soy was the only one that had to have it added? Soy products are permitted, milk products are permitted, there is a push to have fish products allowed and poultry is already allowed (as additives to meat products). We have two people working on obtaining background information. Using gel electrophroesis, you can get certain patterns to pick out these various proteins. However, you cannot pick out all of the different types of meat such as pork vs beef. We are now trying HPLC, and so far, it isn't working. Therefore, we are trying immunological or some other method where we will be able to pick up some of these additives, but it is not going to be rapid. Once the product is heat processed, the immunological determinants are altered or denatured. You would need a procedure for a low-temperature process and another for a high-temperature process, as well as immunological sera, for anything that you might suspect to be in the sample.

Ono: Jim Price was one of the first users of the dye-binding method. Do you care to make any comments, Jim?

Price: George Seperich, now at Arizona State, completed his M.S. Degree using the dye-binding method for protein determination. He wanted very much for this method to work for raw sausage as well as finished sausage. He worked out some very good regression equations using stepwise elimination. Using elements of those regression equations, you can determine protein from dye-binding very accurately, if you can get a very close estimate of how much protein is in there to begin with (laughter).

Foget: One final comment on the physical methods of measurement. I think we have to recognize that establishing a correlation is going to be very difficult. Let us say with microwave determination of moisture, we are not really determining water, we are determining volatiles. With Kjeldahl, we are not determining protein, we are determining nitrogen. In ether extraction, we are determining everything that is extractable by ether, not fat, whereas with physical methods, at least in theory, you are determining water, protein and fat. Therefore, correlations can never be perfect.

Kotula: There seems to be some interest in the IR methods. Karl Norris of the USDA has done some work on this for a number of years. The problem that he has encountered is that he had a very high correlation of fat and moisture content, but he had a problem in developing standards. Historically, what happened was that if we were to give him a sample of meat on which the composition had been chemically determined, he could standardize as long as we gave him a high and a low, and he could determine everything in between quite well. But then NeoTech took the concept and tried to develop a machine that would be used in every supermarket so that every consumer could take their ground beef and put it on the machine and know specifically what the fat content was. It fell by the wayside because the standards were not that good. Karl Norris was never able to develop standards. NeoTech worked on it and they were a little more successful so that now there are some instruments out by NeoTech. The other point, on the EMME, is that the EMME has had a pretty poor reputation in the industry as far as its capabilities. But of interest to this group is that Dickey-John has bought out EMME and they have a good engineering staff, so watch for progress in that area. If the problems encountered are associated with engineering, then the new engineers can probably solve them without too much difficulty. One last point that I would like to mention is that there are two methods that we have not discussed, and those are the Hobart extraction and the Univex. The Univex is probably as inexpensive as the Babcock because all you need is a small cylinder of meat and an extraction procedure. If you have a need for critical determination of composition, for example, fat or moisture, because of a court case or something like this, only AOAC procedures are going to be accepted. Some of these rapid methods are used by the industry so that you are not giving product away. We were working in one plant where they had an old man who used to mix the ground beef. He eyeballed it. That was the way they had been doing it for 10 years until we had to run a chemical determination

anyway. It turned out that he had been giving away about 4% lean.

Darrell Bartholomew: Would you comment on the problems of IR analysis of protein?

Ono: Yes. The basic problem is trying to get a uniform sample. This applies to moisture and fat as well. Any time the matrix of your sample changes, whether it is particle size or geometry of the layout, the machinery requires recalibration and this calibration is quite tedious. You have to calibrate against known methods. Often you are not comfortable about whether your geometry has been reproducible each time you do it.

Bob Kauffman: I would like to hear your comments on the ammonia electrode. Have you ever used it?

Ono: I have never used it.

Darl Swartz: It is a function of pH. What you are doing is changing the pH to get ammonium ions, and the electrode is attached to a pH meter. But you have a problem of getting the exact pH. Temperature and ionic variations mess it up.

Ono: Do you digest the sample first, like Kjeldahl, but then your analytical portion is different?

Swartz: Yes, you do everything like the Kjeldahl. You do the digestion, then do a dilution. Therefore, you get a high ionic strength to begin with. Then you have to dilute it more to get far down on the curve.

Don Kinsman: Can you tell us what the accuracy is on the microwave method?

Benedict: I do not think it has been accepted as an AOAC method yet. I don't have the exact figures on accuracy.

Ono: It has not been accepted. It would be acceptable. It is very comparable to the oven method.

Benedict: In some cases, Pettinati recommends adding an iron oxide salt to increase the heating.

Ono: When Joe Pettinati first originated this method, he added iron oxide because iron oxide absorbs some of the microwave energy so you can heat up the sample a little better. Later modifications indicated you could probably use filter paper and put your sample in a "sandwich," place it in the microwave oven and dry it this way in about three minutes.

Kinsman: Is sample size critical?

Ono: Not necessarily. I think Joe has used about a 4 or 5 g sample in a little dish.

Don Beerman: How do you avoid the problem of fat mobilization when the fat heats up and starts spreading through the filter paper?

Ono: Don't heat the sample too much. Be careful not to melt the fatty samples.

Beerman: How do you decide on the optimum heating time?

Ono: This is something you must determine for whatever oven you use. If you use a household oven, it is quite variable since the microwave oven does not heat uniformly. You have to know your oven. Some of the commercial ovens designed specifically for moisture are more reliable, but it can be done with a household microwave oven.

Trout: One thing they are doing in Australia for fat analysis is to use the microwave oven to do the moisture determination and then do a correlation between moisture and fat. They get a coefficient of determination between moisture and fat of 98% to 99%, so for most cuts of meat they can just do the moisture and use the regression equation to estimate the fat content. It is reliable within 0.5% to 1.0%.

Ono: Does this method satisfy regulatory requirements?

Trout: This was for fresh meats, so it is selling on a fat level basis. Therefore, it is not really a problem of meeting specifications. It is better than what they had before.

Benedict: Can you distinguish between official methods that must be used in court cases, and rapid industry methods and research lab methods to obtain undenatured fractions?

Ono: For moisture, the vacuum or convection oven is AOAC-approved. For protein, the Kjeldahl method is accepted and the Kjel-Foss, which is an automated Kjeldahl method, has received AOAC approval with final action in 1981. The Tecator unit was approved in 1983. The Technicon unit is basically similar. Its first action is not quite final. For fat, the soxhlet and Kjel-Foss procedures are official. The CEM has not been approved yet.

Benedict: Pettinati has a writeup on it now. For high fat samples, there is some deviation because it is a subtractive method. You remove the moisture, and the fat is rendered off. When you start with 100% you can have an error on one of the two. Then it causes backup errors on the other two. So, for high fat samples, check it with another method.

Maryama: Could you comment on the dry-column method vs the soxhlet methods for fat analysis?

Ono: The results are very comparable. Maxwell developed this dry-column method in 1975. Someone must push it to obtain AOAC approval.

Maryama: The technicians I have had trouble when they changed to a new type of celite. They get all kinds of funny results.

Benedict: There are two types of celite. Do not use acid-washed celite. Use regular celite. I have used this method for five years and I am an advocate of it because you can get a total lipid fraction or polar and nonpolar fractions. If you want to do a phospholipid analysis, the unsaturated fatty acids, which will be in the polar fraction, can be recovered. The sterols are in the nonpolar fraction. If you wish to measure cured meat pigments that will come off as a separate fraction after the nonpolar fraction (for research), it is very useful. This method is also good for oxidation products because you can take the solvent and run peroxide values on it. For sample preparation, first grind the sample with sodium sulfate (this takes out the moisture and denatures the protein lipid matrix). Then add celite as an absorbant, pack it in a column, elute first with dichloromethane or methylene chloride (to remove the nonpolar fraction and sterols), then add 10% methanol to the dichloromethane. Elute with that, and it takes out the polar fraction. If you want total lipids, you just use the 90:10 mixture and it takes everything out in one fraction. This method is not hazardous and you can have several going at the same time. Since it is gravimetric, just evaporate off the solvent and then get the weights. You also have the fractions if you need them.

Trout: What is the time involved in the analysis?

Benedict: You can get the whole sample done within 2 hours. Weighing and grinding the sample takes 15 minutes, and elution takes less than 1 hour. Then evaporate off the solvent. Within 3 hours, you can do a number of samples

because you can have multiple columns.

Ono: As you elute, you can also evaporate this sample with a heater under the sample flask because the solvents are nonflammable.

Norm Marriott: When I worked in industry, we had the opportunity to use a K-40 method for determination of fat and protein. We declined because of costs. Are you aware of any work being done in this area?

Kotula: Years ago, work was done at Beltsville. We still have the K-40 counter that was brought from Great Britain. The work was done with hams and beef rounds. We worked on the procedure for three to five years and concluded that it has no value for use in meats applications.

Clair Terrill: The fallacy was that it was based upon the theory that the potassium and hence the potassium 40 (isotope) is randomly and equally distributed through the water of the body. In fact, this is not the case. It turns out that there is more in some tissues than in others. This casts doubts on the theoretical basis of the system. With Dr. Kolrip's work, the longer he counted the more accurate were his results. He was leaving samples in the counter for 40 or 50 minutes; however, the object was to develop some kind of on-line device. Obviously it would not work for that.

Kotula: This was quite a sophisticated piece of equipment. It had to measure the K-40 level over the normal background. It was insulated with about 8 inches of lead. We still have this counter which is available. It doesn't work, but it might be of historical interest.

Trout: We are using the EMME system on line and have found it to be extremely unreliable. We conducted thousands of fat analyses on samples and we could not get a consistent result.

Kotula: If you go into the meat industry, you can probably find an unused EMME in about every meat establishment's back room.

Trout: Tecator had a method at one time which is similar to the Kjeldahl, using the Kjeldahl equipment. It was a straight alkali distillation of the protein in which you put your sample of meat along with a certain amount of sodium hydroxide. Then you start distilling it, and the protein starts to degrade and release ammonia. They were citing it as a rapid method for determining meat protein. We found this method to be incredibly unreliable.

Ono: They have gone back to the block digestion method, which is basically back to square one.

Bartholomew: How well do quantabs work for salt?

Ono: I never have used that, has anyone used it?

Maryama: As far as I am concerned, it has only qualitative usefulness, not quantitative.

Terrill: For rapid measurement of sodium chloride in cured meats that are not aged, extraction with hot water and titration with mercuric chloride works well for brines. It is accurate and rapid. It does not work for aged meat because of protein-salt interactions. You must digest the sample.

Steve Seideman: For fresh meat, such as beef longissimus, you can determine the amount of moisture by oven drying. Then, if you calculate the protein based upon the constant relation of protein to water, the fat can be calculated by difference from 100%. The only analysis that must be done is for moisture. Work was done at Clay Center on over 1,000 samples and it works reasonably well.

Ono: What principle is this based on?

Seideman: Protein exists in a constant ratio with moisture, so if you determine moisture, you can estimate protein. If you assume about 1% for mineral and carbohydrates, the rest must be fat. The animal breeders found that this ratio of protein-moisture-fat exists all of the time (in fresh muscle).

Kauffman: However, this is true only for mature muscle. The protein-moisture ratio is lower for very young, immature muscle.

Trout: Also, in dehydrated cattle, the protein-moisture ratio is altered.

Kropf: If you were building a new lab, which technique would you use for protein?

Ono: Forget the classical Kjeldahl. Buy a new automated unit. They are much faster and more economical.

Kropf: Which one would you buy?

Ono: I would consider the Technicon, the Kel-Foss and the Tecator units. Pricewise, they are very similar – about $20,000. Computer units cost more, but are not necessary.

Reynolds: Larger sausage plants are using the Kel-Foss units for accurate results, and they use a fairly large sample of about 20 to 25 g. You can complete a sample within 12 to 18 minutes. You can run samples while production is on the line, and obtain an AOAC result within 15 minutes. That is pretty hard to beat. The only problem is when you are running batches of 10 to 12 thousand pounds, sample sizes are still very small.

Person not identified: Has anyone had any experience with the Baltimore Spice Analyzer?

Ono: This is a Udy method. Here again, people are working on this method at the USDA Eastern Regional Lab. Without too much connective tissue, this method works quite well for protein. It is very fast, and economical in that you can run a sample for about 8¢ per analysis. A correction factor must be used if the sample has connective tissue. The principle is that the dye binds with the basic amino acids. In connective tissues, these amino acids are lacking, so you do not get the true reaction.

Benedict: Baltimore Spice has a separate unit for measurement of collagen of connective tissue using the Woessner method for hydroxyproline. Instead of an 18-hour acid hydrolysis, they found that if you do a 2-hour 6-Normal HCL hydrolysis plus stannous chloride, you get a percentage of the total protein that is broken down. Standards are prepared under similar conditions. You carry out the standard oxidation with Chloramine T, and the color reaction with para-dimethylaminobenzaldehyde to get the color. They had it set up so that within 2½ hours you can get a value for collagen. Has anyone used it here? I think collagen content may be important because it interferes with the true protein value.

Trout: Has there been any work using pulsed NMR for moisture in meat?

Ono: There has been quite a bit of work, but it's not very practical because of expense.

Trout: The units are small and only cost about $5,000 to $6,000. They are mainly used on drier samples such as grains.

Kropf: Which protein method is fastest?

Person not identified: The Kel-Foss can do about 20 per hour, whereas Technicon can do about 40 per hour, theoretically.

Benedict: We have a Tecator unit which works well. It takes about 80 to 90 minutes per digestion, and about 3 minutes for distillation.

Ono: We have had the same experience.

Trout: Has there been much work on using different catalysts in protein digestion?

Ono: Yes, you can use copper or selenium rather than mercuric chloride.

Trout: How about titanium?

Ono: I'm not aware of its use.