Ether Extraction Method of Estimating Degree of Fatness in Carcasses and Cuts

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Because of the sharply selective solvent action of ether toward fat in the presence of other major components of animal tissues, ether extraction methods have long been recognized as standard acceptable procedures. The A.O.A.C. official method for determination of fat in meat and meat products calls for continuous ether extraction of the dried sample for 16 hours. Since ether and water are not miscible, ether cannot penetrate wet tissue to reach fat deposits.

The method sharply defines the manner in which meat should be dried for extraction. Its purpose is to avoid two undesirable reactions. First aim is to avoid development of hard, horn-like, impermeable protein structures surrounding fat deposits. Second aim is to avoid oxidation of unsaturated fats, thereby preventing an increase in their weight. Two methods are approved for drying. One is by use of a vacuum oven (pressure not to exceed 100 mm. Hg) at 95-100° C. Time required is about 5 hours. The second utilizes the moisture absorptive power of concentrated sulfuric acid in an evacuated desiccator. Time required is somewhat longer and may be 5 weeks instead of 5 hours.

One may use a mixture of these two methods to good advantage, drying in a vacuum oven 6 hours at 60° C and finishing in a vacuum desiccator over sulfuric acid. The low temperature helps prevent hardened protein structure, and time required in the desiccator is greatly reduced.

The official method directs that the dried material be ground with asbestos or sand and extracted with anhydrous ether for 16 hours. This step obviously presents an awkward situation, for it involves two quantitative transfers of dry material which may also be brittle enough to have a tendency to jump out of the mortar under impact of the pestle. The ground material is transferred to an extraction thimble.

A common practice to avoid this difficulty is to spread the finely ground wet tissue over the middle third of a thin layer of cotton fiber 3 by 5 inches, the strip running lengthwise. The unoccupied side strips are folded in over the meat strip, and the whole thing is rolled up like a jelly roll. It is well to perform this operation on a glass or porcelain plate, so that material which may strike through the cotton mat can be picked up with the end of the finished roll. The roll is then inserted in an extraction thimble which is placed in a 30-ml beaker for drying as previously described. Some fat may render out of samples with high fat content and be caught in the beaker at 60 degree drying. In that case the beakers are rinsed into the extraction apparatus with the ether to be used in it.

This stage brings us to the extraction procedure, which may employ different means. The equipment is usually some form or modification of a Soxhlet extractor. Some of these forms are shown in the following illustrations.
Figure 1-A and B. Wiley extractor. The sample is contained either in a porous alundum filtering crucible or a Gooch crucible. Or a siphon cup with extraction thimble may be suspended below the condenser in a manner similar to that shown in C.

C. Wiley-Soxhlet, Ford modification. All glass. Vulnerable to breakage.

D. Underwriters' Laboratories pattern. Uses 250 ml Erlenmeyer flask.

E. ASTM. Diameter of siphon cup is 3-1/4 in. Capacity of flask is 3 liters.

F. Bailey-Walker. Large metal condensing surface. Siphon cup is supported on three indentations in flask. Filtering crucibles may be used in place of the siphon cup.

Figure 2-A. A filtering crucible made entirely of Jena fritted glass.

B. Bailey-Walker complete assembly. A 3-heat 660-watt hot plate. The telescopic arrangement of the condenser connections permit raising and lowering of individual condensers to insert or remove flasks during operation. A glass cup or glazed porcelain crucible may be inserted in place of the siphon cup for recovery of solvent when extraction is complete.

Figure 3-A. Pickel Apparatus. Requires little space and small amount of solvent (15 ml).

B. Diagram showing funnel in recovery cup placed so as to direct the condensed solvent into the filtering crucible. When extraction is complete, the glass cylinder is rotated a few degrees, throwing the funnel out of line with the eccentric tip of the condenser. The solvent is thus recovered in the cup. Various views of the recovery cup with funnel are shown at the side of the complete assembly. Note that condenser connections are similar to those on the Bailey-Walker and probably could be mounted on the same support.

C. Glass cylinder indented to support filtering crucible.

D. Porous alundum filtering crucible.

✓ Figure 4. Goldfisch Apparatus. The company claims that "extractions now requiring 16 hours can be attained in 3 to 6 hours." The paramount features are an ether-tight chamber with automatic release valve, the use of high heat rapid condensation, the washing through of a greater amount of solvent in a shorter boiling period, and the reclaiming of approximately 50% of the solvent.

The extraction thimble and contents are put into a glass sleeve which is inserted in the bottom of the condenser and supported by a spring clamp. The beaker containing the ether is affixed to the condenser by a half turn of the metal collar. The tops of the beakers are flanged for the collar and have a ground surface for a tight fit against a cork gasket on the condenser.
Extraction is accomplished by continuous percolation of condensed ether through the thimble contents. When extraction is complete, the sleeve is removed and replaced by a tube in which the solvent is recovered. For final drying off, the beaker is supported in a tilted position on a hinged support which can be flipped over onto the heater.

Figure 5. A typical set of duplicates on ham fat obtained by the Goldfisch apparatus. Duplication is about what may be expected by the Bailey-Walker and similar methods. It all points up to the fact that one may expect an occasional bad actor and repeat performance in fat determinations by ether extraction of meat.

Ether extraction removes some things from meat besides fat, notably cholesterol and phospholipids. Probably some nitrogenous compounds are also removed.

Figure 5. A typical set of duplicates on ham fat obtained by the Goldfisch apparatus. Differences are expressed as percent difference from the mean.

\[ D = \frac{A - B}{A + B} \times 100 \]

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<th>Pct. Fat</th>
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<td>16.03</td>
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MR. BLUMER: Do you have any condensation from the cotton by either one of these methods?

MR. HALL: Yes, that is an objection which has been noted about either one of them, the Goldfisch apparatus or the Bailey-Walker. One of the first objections to the Goldfisch apparatus is the condensation of moisture. In spite of the fact that it is clamped against the cork gasket moisture gets in, and when you release the collar and let it down the moisture goes into your sample. They claimed moisture was getting in, so they wrapped the tops of the condensors with gauze and just taped a bandage of gauze around. There was moisture on the outside of those condensers but that eliminated the difficulty.

A similar sort of thing can be done with the Bailey-Walker. I am sure that it is not quite so devastating there because the sleeve which you will see when you examine it comes down on the outside, and
if moisture does condense on it, it should creep out on the outside. If moisture gets into the condenser, it can condense on the inside, and when that happens, it can be helped a great deal simply by putting a piece of adhesive tape around that skirt that comes down over the outside of the condenser.

MR. KASTELIC: With the Goldfisch apparatus take rubber, cut a hole in it, and slip it up over the body of the condenser, just leaving a tail on it. When you pull it up the rubber will tend to be higher on the side edge than it is against the condenser. Then leave a little over and build a trough and just lead the water down into the sink.

MR. HALL: The company sent the rubber collars. Unless you get it right down where the joint is you will get condensation under that collar anyway.

MR. KASTELIC: We use just an inner tube and cut the hole quite small, so that we have a snug fit.

The glass tube on the bottom of the Goldfisch apparatus weighs something in the neighborhood of 60 grams. You collect fat in it from samples that contain very little fat, and the tare weight of the tube is 60 grams plus. Then you add the fat after extraction and take off the ether, and you have 60 plus a little more. The sensitivity of the balance is greatly decreased as you load the arms, and consequently while you think you are weighing out to four places -- it does not mean a thing. What we have done is weigh the thimbles and subtract the difference between the dry weight of the material before and after extraction.

MR. HALL: You weighed them after they were dry?

MR. KASTELIC: Yes. I don't know if that is the best way, but that is one dodge we have used to attempt to weigh small amounts of fat in view of the very marked decrease in sensitivity of the balance. I wish they could make glass flasks that weigh about a gram or two and make them out of some kind of material that has very low density.

MR. HALL: Their point is that theirs are extremely rugged and the Bailey-Walker flasks are quite fragile. But the man who is in charge of that equipment down there says that that is all very well and good, but the boys are still knocking chips off the flask and they don't maintain their weight too well in spite of their additional rugged construction.

MR. BUTLER: Is it better to use a large apparatus, like a Wiley Soxhlet extractor, and maybe have as many as 15 samples in triplicate rolled in filter paper and weigh by difference?

MR. HALL: All in one extraction. If you will examine those illustrations, there is one which is an ASTM -- American Society of Testing Materials -- model. The siphon cup is three or four inches in diameter. The flask is a 3-D Erlenmeyer flask, so you can put quite a series of samples in there. If you do it as suggested by Mr. Kastelic, extracting all at once and then weighing them individually, I don't see
any reason that wouldn't do it adequately, provided you can see that
the contents don't get mixed up or transferred from one to the other,
and that you don't lose small fractions of cotton fiber, if you use
cotton, in getting them in and out. It is just a matter of manipula-
tion.

MR. KASTELIC. For the procedure I have suggested, I make no
claims because when you water and pestle grind dry material, some of it
is reduced to a fine powder, and I must admit that on examining the
flask below there is at times a little ground material. I wonder how
much of that material filtered in the process of ether washing. The
Carborundum thimbles avoid that difficulty, but you always wonder how
often you can do that without plugging them. We have been using the
regular filter paper, and then carefully examining the receiver below to
see that we don't have any material there. It is a question that you
might well ask about very finely divided materials. Do they wash right
through, so that it cannot be considered a precise method?

MR. HALL: Do you mean you use this paper fiber and do you mix
the sample with it or wrap it in or how?

MR. KASTELIC: We just transfer the material that we weigh out
directly into the thimble and then weigh it. Following extraction dry
and weigh it again. You are never certain that some of the material
might not have gone through. It would add to the error of both methods
of getting the weight of the fat if any material came through that you
would weigh with the fat.

MR. HALL: Do you incorporate the sample with pulp paper?

MR. KASTELIC: No we always put a little cotton on the top to
keep it from boiling out.

MR. HENRICKSON: Mr. W. J. Aunan, of Minnesota will discuss
linear measurements in estimating the degree of fatness in carcasses and
cuts.