1972). The immobilized lactase described in this paper would be most applicable in special cases such as with acid wheys and deproteinized acid whey. Hydrolysis of lactose in these materials might make them more nutritionally acceptable for certain applications.

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Screening Method Based on Electric Hygrometer for Obtaining Water Sorption Isotherms

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A discontinuous gravimetric screening method with high precision has been developed for the determination of water sorption isotherms. The equipment needed is cheap and simple. A detailed analysis of the precision shows that the water activity can be determined within ±0.016 a_w units and the water content with an accuracy of ±0.1% if the temperature is kept constant. The water activity is determined by an electric hygrometer. By a strict calibration and measuring routine, the accuracy of the electric hygrometer has been improved about 30-fold. Using 20 electric hygrometers, one person can determine 160 isotherms in 15 days.

Obtaining water sorption isotherms has long been a rather elaborate method for characterizing a material and its relation to water. This is mainly due to the methods available being either expensive, laborious, time-wasting, or not accurate enough (Gál, 1967). The existing methods are based on the determination of the water content, the water activity, or a combination of both. Those determining the water content are mainly gravimetric and imply that the sample is completely equilibrated with its surroundings; *i.e.*, has attained a known water activity. The methods determining water activity are either manometric or hygrometric, depending on whether the partial pressure of the water or the relative humidity above the sample is measured. Each sample is equilibrated with a known amount of water.

Both continuous and discontinuous methods have been worked out on these principles. Continuous registrations involve complex and expensive instrumentation, compared to the discontinuous registrations, and are characterized by high precision. Their capacity is, however, very low.

The approach presented in this paper is a discontinuous screening method based on the determination of both the water activity and content. It is characterized by high precision, as well as high capacity, and is suitable for small quantities of sample. The method has been developed with special attention to the characterization of protein-rich products such as meals, concentrates, and isolates. Until now little has been known about the relation of these products to water. It is of greatest importance for the functional properties of the final product to know how this relation is affected by chemical composition and molecular structure, as well as by heat and organic solvents. It is our hypothesis that isotherms could be a useful, analytical tool in such work. Investigations in this line are now being worked out using the method described in this paper.

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MATERIALS

Chemicals and Protein Preparations. Salts of highest purity have been used to obtain well-defined atmospheres of different water activities (Robinson and Stokes, 1959) (Table I). All amino acids were of CHR quality and obtained from Fluka AG. The proteins used as application samples are: casein (nach Hammarsten), Merck AG; gelatin (Difco certified), Difco Laboratories; egg albumen (grade V, salt-free, lyophilized and recrystallized), Sigma Chem. Co.; keratin (pract.), Fluka AG.

Hygrometer (Lion, 1959; Wexler, 1957, 1965). A Pope hygrometer (Pope, 1955) numbered PCRC-55 and pur-

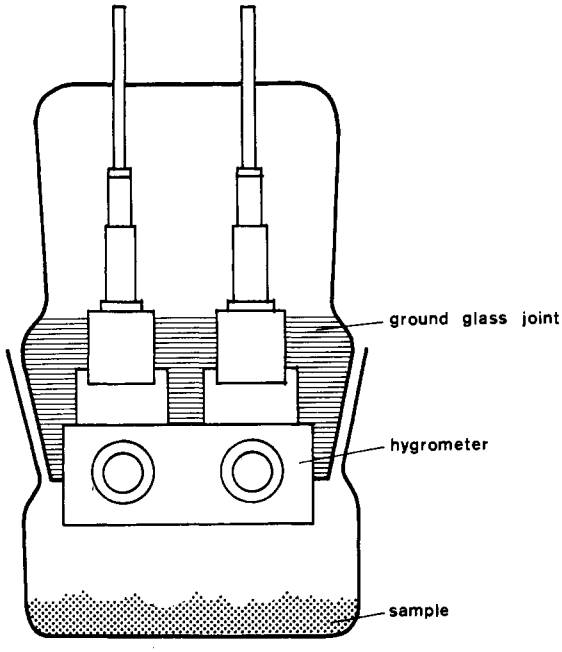

Figure 1. Sample container with hygrometer.

chased from Phys-Chemical Research Corporation, New York, USA, was used for the determination of the water activity. The hygrometer consists of a small piece of polystyrene (6 × 12 × 2 mm) which has been sulfonated on the surface in order to obtain ion-exchange properties. There is a semilog relationship between the resistance over the hygrometer and the relative humidity in the surrounding atmosphere. The water activity can easily be determined through a standard curve. Gold-plated connections are mounted in direct contact with the ion-exchanging surface.

Sample Containers. Weighing bottles made of borosilicate glass and provided with ground glass joints (19/12) were selected as suitable for measurements of a_w (Figure 1). Gold-plated clips were mounted through a number of extra lids and these are supplied with hygrometers. The lids are designed to protect the hygrometers against contamination during handling and measurements. A volume of 4 ml was found to be suitable for the containers, allowing the distance between the sample and the hygrometer to be minimized to 2–5 mm. In this design the containers will hold a sample of about 1 ml (equivalent to 0.3–0.8 g).

Sample containers partly made of plastic materials adsorbed water to such an extent that accurate gravimetric determination of the water content in the samples could not be made.

Instrumentation for Resistance Measurement. A high impedance AC Wheatstone bridge was built, which measures resistances up to 200 Mohm. The instrument is fed with maximum 20-V AC at a maximum current of 1 mA. In order to avoid any possible electric polarization of the hygrometer and transient DC current through the hygrometer, special care has been taken to use only AC voltages of not less than 20 Hz with zero DC component. The resistance of a sample is indicated by a minimum on a sound generator.

Table I. Salts Used in Reference Solutions in Different Desiccators

Desiccator no.	a_w of sat salt soln at 25°	Salt	Quality purchased from
0	5.7×10^{-5}	P_2O_5	p.a. Merck
1	0.11	$LiCl \cdot H_2O$	p.a. BDH
2	0.22	$CH_3COOK \cdot 1.5H_2O$	p.a. Merck
3	0.33	$MgCl_2 \cdot 6H_2O$	p.a. Riede de Hoën
4	0.43	$K_2CO_3 \cdot 2H_2O$	p.a. BDH
5	0.53	$Mg(NO_3)_2 \cdot 6H_2O$	p.a. Merck
6	0.62	NH_4NO_3	p.a. BDH
7	0.75	NaCl	p.a. Merck
8	0.84	KCl	p.a. Merck
9	0.92	KNO_3	p.a. Merck

METHODS

A fundamental principle throughout the design of the method has been to measure *both* the water content and the water activity of a sample at a number of points along the isotherm. As the determination of an isotherm by itself is a water treatment of the sample, special care has been taken to minimize interference from the measurement routine in the actual conditions of the sample. The water activity of the sample is determined as the relative humidity above the sample when equilibrium is established. There is a very close correspondence between the water activity and the relative humidity/100 (Gál, 1967). The discrepancy has been compensated for in the standard curves of the hygrometers. Every measurement has to be carried out under thermostated conditions.

Procedure for Obtaining Standard Curves for the Hygrometers. There are considerable dissimilarities between the hygrometers, and it is advisable to determine an individual standard curve for each hygrometer. Since the hygrometers also show hysteresis effects, it is necessary to obtain both desorption and adsorption standard curves. For this purpose, 1-ml samples from five different saturated salt solutions (see Table I, a_w = 0.11, 0.33, 0.53, 0.75, and 0.92) are pipetted into each of the five containers (see above). An adsorption standard curve is obtained by consecutive registration of the hygrometer response to the standard solutions, starting at the lowest a_w. The hygrometer is allowed to equilibrate for 1 hr between each determination. The measured values of resistance are plotted against the water activities in the containers to give a standard adsorption curve. A standard desorption curve for the hygrometer is obtained by registration of the hygrometer response of the standard solutions, starting at the highest a_w.

Very little is known about the behavior of the hygrometers, and it is therefore advisable to recheck the standard

Table II. Variables Influencing the Accuracy of the a_w Measurements

Variable	Error a_w units
Amount of sample	*a*
Particle size	*a*
Equilibration time	
For the hygrometer	*a*
For the atmosphere in the container	*a*
In the desiccator	*b*
Temperature coefficients	
For the sample	*b*
For the hygrometer	0.0036/°C[c]
For the reference solution	−0.003/°C[c]
Accuracy of the Wheatstone bridge	±0.002
Individuality of the hygrometer	*a*
Aging of the hygrometer	±0.014
Hysteresis of the hygrometer	*a*
Total maximum error	±0.016 a_w units plus a correction factor varying from 0.0006 to 0.0036 a_w units/°C

[a] The standard conditions and the measuring routine have been selected and worked out, respectively, to make these errors neglectable. [b] This error is included in the error in water content. [c] This is a correction factor rather than an error.

Table III. Maximum Errors Introduced in the a_w Measurements after 6 Months' Use of PCRC-55 Hygrometers Compared to the Error after Proper Calibration

	Error in relation to original standard curve $\pm a_w$ units	Error in relation to individual standard curves $\pm a_w$ units	Error after compensation for aging $\pm a_w$ units	Error after compensation for hysteresis $\pm a_w$ units
Individual deviation	0.11			
Aging	0.175	0.175	0.014	0.014
Hysteresis	0.17	0.17	0.17	
Temp coeff	0.0018	0.0018	0.0018	0.0018
Wheatstone bridge	0.002	0.002	0.002	0.002
Total maximum error	0.4588	0.3488	0.1878	0.0178

The figures are presented as maximum values of ten hygrometers obtained at 25 ± 0.5°. The figures for individual deviation and hysteresis refer to a_w = 0.53 and the figures for aging refer to a_w = 0.33.

curves of a hygrometer at appropriate intervals. It is not possible to use sulfuric acid of different concentrations as reference solutions, since sulfur oxides contaminate the hygrometer even at very low concentrations.

Procedure for Obtaining Adsorption Isotherms. Nine desiccators are supplied with saturated salt solutions holding water activities from 0.11 to 0.92, according to Table I. In addition, one desiccator is supplied with P_2O_5. Each measuring container is weighed on an H16 Mettler balance, giving an accuracy of ±0.05 mg. The sample is transferred to the container to a volume of about 1 ml (0.3–0.8 g) and dried over P_2O_5 for 72 hr. The container with the sample is then equilibrated in desiccator 1 for 24 hr, weighed, and provided with a hygrometer. The resistance of the hygrometer is read after 1 hr equilibration. The sample is passed over to the next desiccator and so on. In desiccators 7, 8, and 9, a prolonged equilibration time of 48 hr has been found necessary. The pressure in the desiccators during equilibration is always taken down to the vapor pressure of its salt solution. When opening the desiccator, the incoming air is always humidified in a gas washing flask containing the same humidity as the desiccator. The values of resistance are converted to a_w units through the standard curves. If the *hygrometer,* during the measurement, has been submitted to a desorption process, the desorption standard curve should be used and *vice versa.*

Desorption isotherms are obtained in the same manner as described for adsorption isotherms, except that the sample is passed from desiccator 9 to desiccator 1 in decreasing order. When not used for measurements, the hygrometers are stored in desiccator 5 (a_w = 0.53), according to the recommendations of the supplier (Wexler, 1965).

RESULTS

Accuracy of a_w Measurements. When using the described measuring routine, we have found a maximum total error of ±0.016 a_w units at constant temperature. The maximum temperature coefficient is found to be 0.0036 a_w units per °C. The different variables of the a_w measurements have been listed in Table II. They are divided into two groups, the first referring to the direct measuring routine and the second referring to the appropriate calibration of the hygrometer. Navy beans (*Primus* variety) have been chosen as a suitable test material for investigating the accuracy of the method. The components include low molecular weight materials such as salts and lipids, as well as high molecular weight components such as proteins and carbohydrates, and these are inhomogeneously distributed throughout the beans.

Errors Referring to the Direct Measuring Routine. Several series of tests showed that the water activity measured in the container was independent of sample and particle size (Table II). This was true even for very coarse samples and indicates that the relative humidity in the sample container established during the 1-hr equilibration time before measurement is only affected by the water activity of the surface layer of the sample particles and also that this layer is equilibrated to the relative humidity of the used desiccator independent of sample and particle size.

Regarding errors introduced into the a_w measurements through inadequate equilibration (Table II), three different steps have to be considered. These are: the equilibration of the hygrometer to the atmosphere of the sample container; the equilibration of the atmosphere in the sample container to the water activity of the sample; and the equilibration of the sample to the atmosphere of the desiccator.

The equilibration of the hygrometer to the atmosphere over the sample has been investigated by Pope and others (Wexler, 1965) by the dewpoint method. They tested both the adsorption and desorption characteristics of the hygrometer and found that 0.5 and 1 hr, respectively, were sufficient for equilibration. We have confirmed their results using saturated salt solutions as references and chosen 1 hr as a suitable equilibration time for the method. The error introduced through this standardization is found to be below the detection limit of the Wheatstone bridge.

For the measurement of water activity, each container has to be provided with a lid equipped with a hygrometer. This causes a disturbance in the equilibrated atmosphere of the container. It was, however, found that no extra time, beyond the 1 hr mentioned above, was needed to restore the equilibrated atmosphere in the container if this were made sufficiently small and designed as in Figure 1.

The sample surface reaches a water activity very close to the a_w of the desiccator within 6 hr. As the equilibration of the atmosphere inside the containers mainly depends on the water activity in the sample surface, the completeness of equilibration in this case only can be determined from the constancy in water content. Therefore (see Table II) this variable has no influence on the determination of the a_w (see water content determination).

The equilibrium of the protein-water complex at a fixed a_w is temperature-dependent. It is not possible to give any general figure for the temperature coefficient of the sample. This, however, is not necessary, since errors due to inadequate thermostating of the sample will automatically be included in the error of the determination of water content.

The temperature coefficient for the hygrometer is 0.0036 a_w units/°C, according to the supplier, while the temperature coefficients for the salt solutions in Table I vary widely but are in no case greater than −0.003 a_w units/°C (Wexler and Hasegawa, 1954).

The accuracy of the Wheatstone bridge equipped with

Table IV. Variables Influencing the Accuracy of the Water Content Determinations

Variable	Error % water content
Particle size, 70% <20 mesh	*a*
Equilibration time, 24 and 48 hr, respectively	*a*
Amount of sample, 0.3–0.8 g	*a*
Weighing procedure	±0.10%
Equilibration temperature	−0.25%/°C[b]
Total maximum error	±0.1%–0.25%/°C

[a] The standard conditions and the measuring routine have been selected and worked out, respectively, to make these errors neglectable. [b] This is a correction factor rather than an error.

Table V. Comparison Between Different Methods of Water Content Determination

Sample	% water content found by determination procedure I[a]	II[a]	III[a]
Gelatin	7.3	7.1	7.4
Chitin	4.6	4.7	4.7
Navy beans	7.0	6.8 (72 hr) 6.7 (48 hr)	7.0

[a] Further details concerning procedures, see text.

the tone generator has been determined to be better than ±0.002 a_w units.

Errors Due to Inadequate Calibration. The individual deviations in hygrometer response for ten hygrometers at the time of delivery, compared to the accompanying standard curve, varied from 0.03 to 0.11 a_w units. It is therefore necessary to use individual standard curves for each hygrometer to obtain higher precision (see Methods).

The aging effect on the hygrometers after 6 months varied between 0.005 and 0.175 a_w units. It is obvious that the aging is more pronounced for some hygrometers than for others. At low levels of water activity, however, all the hygrometers showed a marked aging effect (*e.g.*, not less than 0.120 a_w units at a_w = 0.33). This error is reduced to 0.014 a_w units by obtaining new individual standard curves twice a month.

The hysteresis should be about ±0.015 a_w units, according to the supplier. We found, however, an error of ±0.085 a_w units in the region $a_w \approx 0.5$, where it is most pronounced. Again, there is a considerable variation between the hygrometers. But this can be completely eliminated by proper use of adsorption and desorption calibration curves, as described under the Methods section.

Summation of the errors in Table II leads to a maximum total error of ±0.016 a_w units and a maximum temperature coefficient of 0.0036 a_w units/°C. The rather low value of the temperature coefficient does not demand a thermostating better than ±0.5°.

Table III illustrates how the error in water activity is influenced by improving the methodological procedure. Provided the thermostating conditions are ±0.5°, the maximum error in a_w measurements is ±0.459, calculated by using the standard curve of the supplier 6 months after delivery. With the proposed calibration routine, it can be limited to ±0.019 a_w units.

Accuracy in Water Content Measurements. The percentage of water in the sample can be determined to within ±0.1% with a temperature coefficient of −0.25%/°C. The main factors contributing to this error have been listed in Table IV. They are due to direct measurements of the weight increase or decrease of the sample.

In a series of parallel tests, navy beans were ground to different particle sizes and equilibrated for 96 hr at different constant relative humidity. From these tests it transpired that with a flour with 70% of the particles less than 20 mesh and an equilibration time of 24 hr in desiccators 1–6 and 48 hr in desiccators 7–9, the deviation due to inadequate equilibration is measured as a deviation in the water activity, and thus follows the isotherm of the sample.

A variation in sample amount within 0.3–0.8 g did not influence the accuracy of the water content determination. Below 0.3 g, errors from the weighing procedures could be detected (see below).

The upper limit was restricted to about 0.8 g by the available volume of the measuring container.

For sample weights of 0.3 g or more, a maximum error of 0.1% water has been derived from the performance of the H 16 Mettler balance used.

The maximum temperature coefficient for the water content of navy beans equilibrated to a fixed water activity has been determined to be −0.11%/°C at a_w = 0.75 and −0.25%/°C at a_w = 0.92. With the specified restriction [*i.e.*, particle size 70% less than 20 mesh, equilibration time 24 hr (48 hr), and a sample amount of 0.3–0.8 g], a maximum total error of ±0.1% water and a maximum temperature coefficient of −0.25% water/°C is found. Thermostating is thus of greatest importance in order to minimize the error in the determinations of water content. Extremely inhomogeneous materials or materials with very high temperature coefficients may give an error greater than this. In order to assure high accuracy, it is therefore advisable to check these conditions when applying the method to new or poorly characterized materials.

The errors in the determination of water content discussed above are of absolute character and independent of effectiveness of the applied drying procedure. For complex materials such as enriched protein products, a relative error due to the drying method applied is always introduced. We have therefore investigated three different drying methods: drying in a thermostated oven at 105° for 24 hr to a_w = 0.011 and 760 mm; drying in a desiccator supplied with P_2O_5 and continuously evacuated with a two-stage pump to 0.2 mm ($a_w = 5.7 \times 10^{-5}$) for 72 hr; and a combination of these two procedures; *i.e.*, the second procedure for 24 hr followed by the first procedure.

In the first method the water is boiled away by increasing the pressure of the saturated water vapor and keeping the partial pressure of the water vapor constant. In the second method the partial pressure of the water vapor is lowered by evacuating the desiccator, while the pressure of the saturated water vapor is kept constant. In other words, in the second method, water is mainly removed from the sample through diffusion. The diffusion process is very slow but may be accelerated by applying high vacuum. As can be seen from Table V, there is a considerable difference between the amount of water removed by the three methods. Most water is removed by the third method, which was used in our application examples.

This type of error is of course only of interest when isotherms obtained by different drying procedures have to be compared. It is, however, more important to note that since water is a potent protein denaturant at higher temperature (Barker, 1933a,b), all samples in an investigation should, if dried in an oven, be preequilibrated to the same and preferably low water activity in order to get comparable values of water content.

APPLICATIONS

The purpose of this investigation is to develop a tool for characterization of food proteins and their functional properties. With the aid of this method, work is now in progress aimed at better understanding of physicochemical properties and reactions of the proteins in relation to

treatments such as solvent extraction, dehydration, and deodorization, as well as optimal storage conditions.

In order to illustrate the applicability of the method to this kind of investigations, some examples are given in Figure 2.

Part A shows the isotherms of four different proteins. At

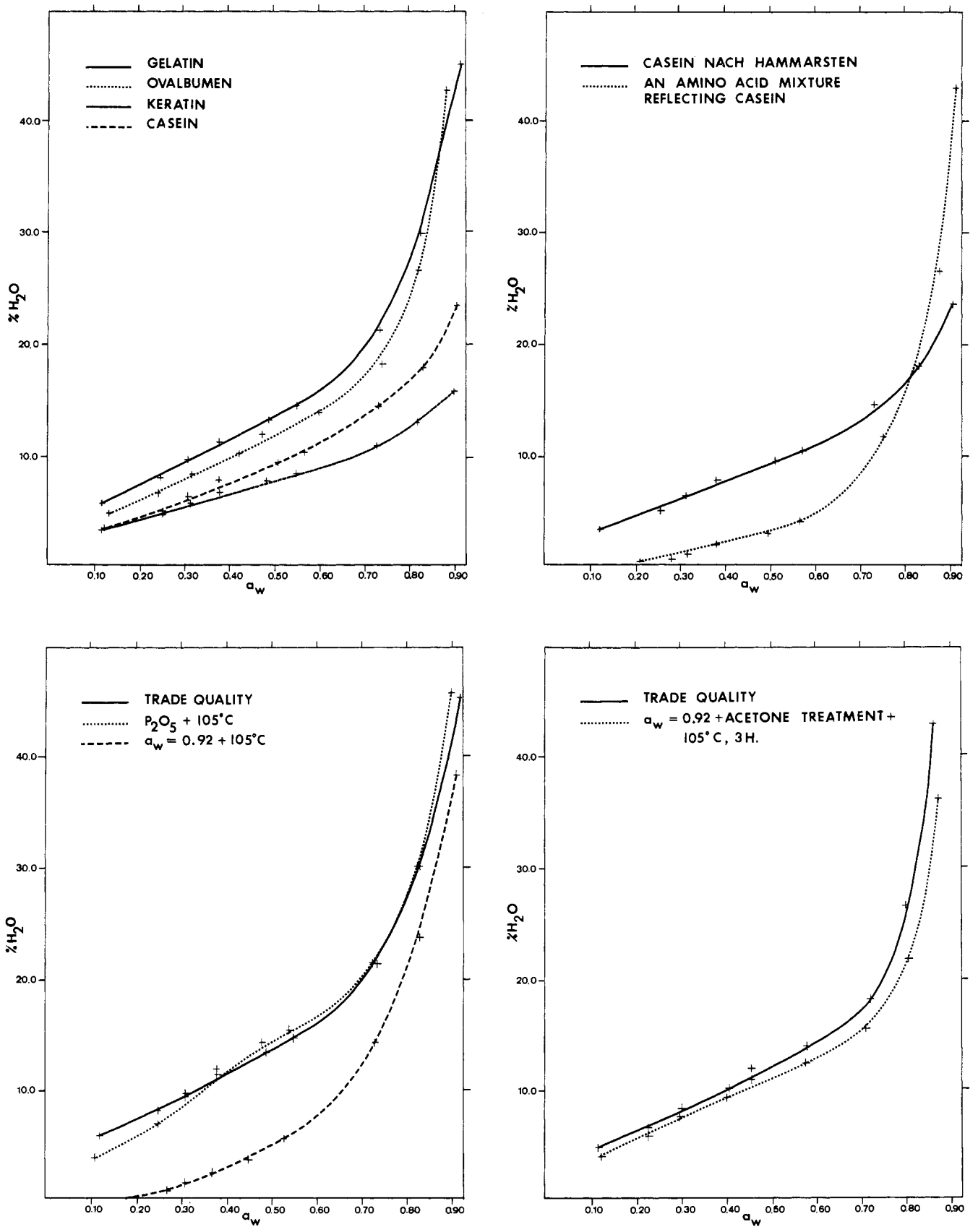

Figure 2. Application examples of the method described showing adsorption isotherms (25 ± 1°) for various proteins applying drying procedure III. A. Four different proteins. B. Casein (nach Hammarsten) and a corresponding amino acid mixture. C. Gelatin, trade quality, and heat-treated. D. Ovalbumen, trade quality, and acetone heat-treated.

this stage it is enough to note that there are great differences between the isotherms. Further investigations are needed for a discussion of the relationship between the shape of the isotherms, protein structure, amino acid composition, and other physicochemical data.

In connection with this it has to be stressed that isotherms from samples of different composition cannot be compared as long as the shape of the isotherm cannot be deduced from the composition of the sample.

Part B shows the difference between the isotherms of casein and an amino acid mixture reflecting casein. Here the two samples differ not only in structure but also in functional groups, mainly due to peptide linkages in casein. As can be seen, this causes a radial shift in the shape of the isotherm over the whole water activity range.

Heat treatment also causes a change in the shape of the isotherms, as seen from Part C. The main effect is a lower water-binding capacity for the heat-treated sample. The same is valid for ovalbumen treated with an excess of acetone at a_w = 0.92 and then dried at 105° for 3 hr, as shown in Part D.

DISCUSSION

The intention of this investigation has been to develop a method for obtaining water adsorption isotherms which was simple, inexpensive, and not time-consuming, and had a great capacity, needed small sample amounts, and gave reasonable accuracy. That the simplicity has obviously been achieved can be judged from the description under "Procedure for Obtaining Adsorption Isotherms." All the steps and manipulations are well known. The different parts of the measuring equipment are also cheap. From the description above it is clear that an adsorption isotherm can be obtained within 15 days and that one investigator provided with 20 hygrometers can obtain 160 isotherms simultaneously during this time, which is a remarkable capacity. Furthermore, by proper choice of a small electric hygrometer, it has been possible to bring down the amount of sample to as little as 0.3 g.

These five characteristics of the method are important in order to render the method useful for characterization of the protein–water relationship in new protein-rich products and the way this relationship is influenced by different processing steps. It is easy to understand that only a method with a great capacity can be used. In this connection it can also be useful to investigate model systems based on pure proteins, which stresses the fact that the method should allow measurements on small amounts of sample.

The accuracy of the method has been thoroughly discussed and a total maximum error as low as ±0.016 a_w units has been documented. The maximum temperature coefficient has been determined to 0.0036 a_w units/°C. This is a high level of accuracy for an electric hygrometer of the Pope-type and is only possible to obtain with the proposed strict calibration routine which improves the level of accuracy about 30-fold.

The estimated error is the maximum error. In most determinations the level of accuracy has been significantly higher. For example, a determination of the isotherm for casein using ten hygrometers simultaneously gave an error in water activity of only ±0.007 a_w units. It is even more important, however, to note that a deviation as little as ±0.016 a_w units is of small significance compared to the deviations the method is designed to measure. This also means that ±0.5° are quite sufficient thermostating conditions for the method.

The errors discussed in "Accuracy in Water Content Determinations" are also maximum errors, which means that the total absolute error in most investigations is much lower. This was clearly demonstrated in the experiment referred to above, where the isotherm for casein was obtained. That is, it was found that the total maximum error in water content at any a_w level was not greater than ±0.06%. This again is an accuracy which only marginally effects the shape of the isotherm.

The relative error introduced in the determination of the water content is due to the fact that there does not exist any simple method for direct water determination *in situ* and that therefore all applicable methods are based on removing the water from the sample and determining the weight loss or the amount of water adsorbed by some convenient reagent. This adds an additional error to the determination of the water content, due to the applied drying procedure.

The way of removing the water from complex organic materials often causes structural damage and decomposition. Therefore every drying method will be a compromise between complete removal of the water and no structural changes. Table V shows that the third procedure gives the highest value of water content. It must, however, be considered that the value obtained by this method may include small amounts of volatile decomposition products. This of course also refers to the values obtained by the first method. From this point of view it is advisable to use the second method, even if it is the most time-consuming method. It is often overlooked that obtaining an isotherm in itself is a treatment of the sample; *i.e.*, it is not possible to reproduce the isotherm for a sample by using the same sample a second time. This was clearly demonstrated by Berlin *et al.* (1970). For this reason it is important that the sample dry weight is determined *before* obtaining both adsorption and desorption isotherms.

The principle of measuring both a_w and water content gives the method certain very important advantages. At high and low water activities, the state of equilibration relative to the desiccator is not reached for all samples. These deviations from the water activity of the equilibration desiccator are then measured by the hygrometer. Moreover in complex materials hygroscopic components may result in failure to reach a state of equilibration. These deviations are again measured by the hygrometers. Furthermore the double measurement of both water activity and water content has brought the equilibration time down to 24 and 48 hr, respectively. The time needed for obtaining an adsorption isotherm is not more than 15 days. During this period less than 1 hr per isotherm is spent for direct measurements. Measuring both a_w and water content also gives the advantage of neglecting the temperature coefficient of the water activity so long as the temperature coefficient for the water content is known and the shape of the isotherm is not supposed to change with temperature.

It is our opinion that the described method possesses all the demanded qualities (*i.e.*, it is simple, cheap, and quick, has great capacity, needs small sample amounts) and that the accuracy still is great enough to make very valuable conclusions from it.

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Quantitative Determination of Dimethyl- and Trimethylamine in Fish Protein Concentrate

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Dimethylamine (DMA), 25 to 150 ppm, and trimethylamine (TMA), 5 to 10 ppm, were detected in samples of fish protein concentrate (FPC) prepared from frozen red hake (*Urophycis chuss*) and Pacific hake (*Merluccius productus*) by isopropyl alcohol extraction. The following compounds were also identified by combined gas–liquid chromatography and mass spectrometry: methyl mercaptan, acetaldehyde, propionaldehyde, methylene chloride, acetone, chloroform, isopropyl alcohol, ethyl alcohol, butanone, toluene, dimethyl sulfide, and dimethyl disulfide. The probable presence of ethylamine and a butylamine was indicated.

The occurrence of secondary amines (Kröller, 1950; Lerenkov *et al.*, 1960; Miyahara, 1960; Preusser, 1966; Wick *et al.*, 1967) and the presence of nitrite (Hanni, 1953; Kamm *et al.*, 1965; Phillips, 1968) in various foods are well established. Significant levels of dimethylamine (DMA), rather than trimethylamine (TMA), are formed in several species of hake currently utilized in the preparation of fish protein concentrate (FPC). Since some dialkylnitrosamines are potent carcinogens (Afkham *et al.*, 1967; Magee and Barnes, 1967), the presence of DMA in FPC prepared from hake may have significant toxicological implications. In the presence of nitrite, DMA can be nitrosated under acidic conditions to form dimethylnitrosamine (DMN). The formation of DMN has been demonstrated in herring meal (Ender *et al.*, 1964; Sakshaug *et al.*, 1965) and in smoke-processed marine fish such as sable, salmon, and shad (Fazio *et al.*, 1971). Although DMN, and possibly other nitrosamines, could be formed in the gastric contents during digestion (Sander, 1967; Sander *et al.*, 1968; Sen *et al.*, 1969), it should be emphasized that the lowest level of DMN which could elicit a carcinogenic response has not, at present, been established.

Wick *et al.* (1967) identified DMA, TMA, and several other volatile amines in FPC prepared from red hake (*Urophycis chuss*); however, no quantitative data were presented. This investigation was initiated to quantitatively determine the DMA and TMA contents of FPC prepared from red hake (*U. chuss*) and Pacific hake (*Merluccius productus*).

EXPERIMENTAL SECTION

Sample Preparation. Regular fish protein concentrate (R-FPC), prepared from frozen red hake (*Urophycis chuss*) by extraction with isopropyl alcohol, was obtained from the National Center for FPC, National Marine Fisheries Service, College Park, Md. In attempts to increase protein functionality (Anglemier and Petropakis, 1972), FPC was modified essentially as follows. R-FPC was hydrolyzed with 0.2 *N* NaOH at 95–100° for 14 min in a closed system. The mixture was centrifuged and the protein in the supernatant fraction was precipitated at pH 4.5 with 6 *N* HCl. The resultant precipitate, redissolved and adjusted to pH 7.0 with NH_4OH, was then spray dried. HmPh-FPC was prepared from the supernatant remaining after isoelectric precipitation at pH 4.5 by treatment with sodium hexametaphosphate.

Gas–Liquid Chromatography. A stainless steel column (5.5 m × 3 mm o.d.), containing 40–60 mesh Graphon (Cabot Corp., Billerica, Mass.) coated with 2% tetraethylenepentamine (TEP), was used in conjunction with an alkali flame ionization detector (AFID) for the selective separation and quantitative determination of DMA and TMA. Approximately 30 ml of 10% NaOH was added to each FPC sample (5 g) contained in a screw-capped bottle (250 ml), and the amine contents were determined by equilibrium vapor analysis after heating at 60° for 25–30 min (Miller *et al.*, 1972).

A column (3.7 m × 3 mm o.d.), containing acid/base washed Celite 545 (60–80 mesh) coated with 20% 1,2,3-*tris*(2-cyanoethoxy)propane (TCEP), was used with a gas entrainment, on-column trapping procedure (Morgan and Day, 1965) for the identification of relatively low-boiling compounds. Approximately 25 ml of double, glass-distilled water was added to each 5-g portion of FPC contained in screw-capped bottles (250 ml). Each sample, containing a few milligrams of 1-tetradecanol to control foaming, was tempered at 60° for 30 min and the volatiles were condensed on the column packing using a nitrogen gas purge rate of 12 ml/min for 10 min. The nitrogen purge time was increased when more concentrated samples were needed for mass spectral analysis.

The TCEP and Graphon plus TEP columns, installed in Varian Aerograph series 1200 and 1400 gas chromatographs, respectively, were operated isothermally at 60° with a nitrogen gas flow rate of 30 ml/min. The detector and injector port temperatures were 210° and 190°, respectively. The series 1400 instrument was equipped with an AFID and the series 1200 instrument had a flame ionization detector (FID).

Mass Spectral Analysis. An F&M Model 810 gas chromatograph was used in conjunction with an Atlas CH-4

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