A Practical Measurement of Water Hydration Capacity of Protein Materials[1]

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ABSTRACT

This method for measuring water hydration capacity of protein materials differs from conventional techniques in that only enough water to saturate the material is used. Unlike conventional techniques that involve excess water, this method is not affected by water solubility of the test material. The method is simple and highly reproducible.

The terms water hydration capacity (WHC), water absorption, water binding, and water holding ability are used interchangeably in the literature to denote the maximum amount of water that a protein material can take up and retain under food formulation conditions. This amount of water was experimentally determined by a variety of techniques as discussed in reviews by Hamm (1960) and Kinsella (1976). Popular techniques emanated from the early work of Yamazaki (1953) and of Janicki and Walczak, as quoted by Hamm (1960). These involved equilibration of the sample with excess water and application of mild stress to separate the retained water from the free water. In practice, the protein sample is mixed with a several-fold excess of water and the dispersion is then centrifuged at low gravity. The supernatant is decanted and the absorbed water is calculated by measuring either weight differences (sediment weight less sample weight) or volume differences (dispersion water less supernatant).

Measurement of swelling is essentially another way of estimating the water absorption of a protein sample (Kinsella 1976). A system to measure swelling was devised by Hermansson (1972). By this method, a small amount of sample is dusted on a wetted filter paper fastened on a glass filter; this is placed on top of a thermostated funnel filled with water and connected to a circular capillary. The amount of water absorbed by the sample can be followed by observing the capillary.

In attempting to relate water binding to protein performance in food products, this laboratory has used and abandoned both techniques. Neither technique accounts for the portion of the protein that is solubilized by the procedure. In the excess water-centrifugation method, soluble proteins are decanted with the supernatant, and in the swelling method, they diffuse into the water reservoir. Samples containing different proportions of soluble to insoluble protein cannot, then, be accurately compared as to WHC by either method. Both methods also suffer from handling difficulties. The excess water-centrifugation method uses low gravity force and, consequently, the supernatant often contains suspended particles. Also, some samples contain components less dense than water and these float on the surface of the supernatant. Procedures to overcome these difficulties add to the measurement error and to the labor involved. The swelling technique, in this laboratory, was not repeatable. This was attributed to the difficulty of applying reproducible thicknesses of sample to the wetted filter paper.

A much simpler method was finally adopted for determining the WHC. In the new technique only enough water is added to saturate the sample. This water is entirely retained upon centrifugation, ie, there is no supernatant. This paper describes the method and compares it with conventional techniques.

MATERIALS AND METHODS

A 5-g sample of material is weighed into a transparent (polycarbonate) 50-ml centrifuge tube and the tube and contents are weighed. Distilled water is added in unmeasured increments and the mixture is vigorously stirred with a spatula after each addition. This process is repeated until the mixture is visibly thoroughly wetted and, by touch with the spatula, somewhat pastelike in consistency. The tube is then centrifuged at 4,000 rpm (2,000 × *g*) for 10 min. The slight amount of supernatant present is discarded and the tube is again weighed. The weight difference per gram of dry sample is taken as the approximate water hydration capacity (approx. WHC). If no supernatant appears on centrifugation, more water is added and stirred into the mixture, and the tube is again centrifuged. This operation is repeated until supernatant appears. Usually, the experienced operator can judge by the consistency of the mix whether the water saturation point has been exceeded. Seldom is more than one centrifugation necessary. The approx. WHC can be found for several different materials at the same time, ie, several samples can be centrifuged together. Because some materials tend to reabsorb part of the supernatant, it is important to examine the tube contents immediately after centrifugation.

The approx. WHC value is used to set water/solids ratios for the final determination. A series of four tubes is prepared. Usually 5 g of material is added to each, but if highly absorbent, lesser amounts are advisable for easier mixing. Measured volumes of water chosen to encompass the approx. WHC value are added and the contents are then vigorously mixed by spatula for 2 min. The tubes are centrifuged for 10 min at 2,000 × *g* and if the proper range of water volumes was used, at least one of the four tubes will contain supernatant and at least one will not. The volumes of water added to the two adjacent tubes, one with and one without supernatant, are divided by the dry sample weight to give the WHC of the material.

An example better explains the procedure. A 5-g sample is found by weight difference, after discarding a slight supernatant, to have bound 15 g of water. Four tubes are prepared to each contain 4 g of the material (which should absorb approximately $15/5 \times 4 = 12$ ml water). Into tubes 1, 2, 3, and 4 are placed 10.5, 11.5, 12.5, and 13.5 ml water, respectively. After mixing and centrifuging, tubes 1, 2, and 3 contain no supernatant but tube 4 contains free liquid. Thus, the 4 g bound 12.5–13.5 ml of water, and the WHC is 3.1–3.4.

To determine the reproducibility of the method, three operators each performed a number of replicate determinations on vital wheat gluten, devitalized wheat gluten (both available from IGP Ltd., Montreal), a textured soy product (Promate 555C from Griffith Laboratories Ltd., Toronto), and rapeseed concentrate (experimentally prepared at the Food Research Institute and containing 10.2% nitrogen).

RESULTS AND DISCUSSION

The data from different trials are presented in Table I. The values are given in ranges. The ranges can be narrowed by using volumes of water differing by smaller amounts in the four tubes; eg, in the example given, 0.5 ml instead of 1.0 ml differences would give a final value of 3.1–3.25 or 3.25–3.4 instead of 3.1–3.4 ml/g. Using volume differences less than 0.5 ml seems meaningless because of the inherent error of a procedure that involves weighing and pipetting operations. It is preferable to increase the weight of sample, in the case of low WHC samples, than to decrease the water volume differences below 0.5 ml.

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The total weight of sample and water to be mixed could be made constant for standardization purposes. That is, the weight of sample to be placed in the four tubes could be made dependent on the approx. WHC found in the first part of the procedure by applying the formula: Sample weight = 15/(approx. WHC + 1). Sample weight is the weight of material to be placed into the tube, approx. WHC is the water hydration capacity in milliliters per gram found in the preliminary test, and 15 is the desired total weight of water and sample. The average volume of water to add would then be 15 minus the sample weight.

Table I records the ranges in which the WHC values occur. The actual WHC could occur anywhere between these values with equal probability, but for purposes of reporting a single point, the midpoint can be used as long as it is accompanied by the range width, ie ± 1/2 the range.

In development and evaluation of this method, the intensity and duration of the mixing were noted to influence the water uptake. That is, before the 2 min limit was set, different operators obtained different WHC values. It was subsequently demonstrated that the sample receiving the greatest agitation (duration or intensity) gave the lowest WHC value. Reproducible values were not obtained by a single operator on replicate samples unless agitated to a certain extent. Vigorous agitation for 2 min proved suitable for consistency among operators and for reproducibility among replicates as demonstrated in Table I.

TABLE I
Water Hydration Capacity Expressed as Milliliter per Gram of Sample

	Operator 1	Operator 2	Operator 3
Vital gluten	1.3–1.4	1.3–1.4	1.3–1.4
	1.3–1.4	1.3–1.4	1.3–1.4
	1.3–1.4	1.3–1.4	1.3–1.4
	1.3–1.4	1.3–1.4	
Divitalized gluten	2.0–2.2	2.0–2.1	2.0–2.1
	2.0–2.2	2.0–2.1	1.9–2.1
	1.9–2.1	2.0–2.1	2.0–2.1
	1.9–2.1	1.9–2.0	
		2.0–2.1	
Textured soy concentrate	2.7–3.0	2.7–2.85	2.7–3.0
	2.3–2.7	2.7–2.85	2.7–3.0
	2.7–3.0	2.7–2.85	2.7–3.0
	2.3–2.7	2.7–2.85	
Rapeseed concentrate	2.5–2.8	2.7–2.85	2.7–3.0
	2.7–3.0	2.7–2.85	2.7–3.0
	2.7–3.0	2.7–2.85	2.7–3.0
	2.7–3.0	2.7–2.85	

TABLE II
Water Hydration Capacity Values of Various Protein Materials

	Excess Water Method[a]	Proposed Method[b]
Pea concentrate	1.05	1.31
Promosoy 100 concentrate	3.10	3.00
Promine D isolate	3.50	3.85
Supro 620 isolate	6.70	5.50
Rapeseed concentrate	4.50	3.29
Caseinate	0	2.33
Egg white	1.30	0.67
Whey concentrate	0	0.97

[a]Method of Fleming et al (1974).
[b]Values were obtained before the technique was standardized. The values are estimates of where the WHC lies within the experimentally determined range.

To date, all materials tested, except sodium caseinate, were easily mixed in the 2 min period. On contact with water, the caseinate formed a clump that resisted water penetration. A smooth paste was achieved only after 15–20 min of hand stirring. Materials that require longer than the 2 min mixing time for uniform wetting should be so noted when reporting the WHC values.

A study in this laboratory[2], involving functionality property comparisons of protein materials, revealed that WHC measurements by the method reported here were quite different from values derived from a modification of a conventional, excess water method (Fleming et al 1974). Comparison of the two techniques is shown in Table II. The relation between the two improves dramatically, however, when the mostly soluble materials (pea, caseinate, egg white, and whey) are eliminated from the comparison. The conventional method appears to be suitable for use on materials that are mainly insoluble. The zero values in Table II represent losses of sample in the supernatant, ie, the supernatant volume is greater than the volume of water initially added.

In the same study[2], the WHC values determined by the proposed method were related to the protein solubility of the eight materials ($r = -0.88$) and to their water adsorption values ($r = -0.88$) as determined by the humidity equilibrium method of Hagenmaier (1972). Other authors also have found an inverse relation between solubility and water-binding ability. Hermansson and Akesson (1975) found solubility of soy, whey, and casein proteins to have a negative influence on the moisture-retaining properties of mixtures of these proteins with meat, and Lin et al (1974) noticed that the water absorption capacity of various sunflower meals generally increased as the solubility decreased because of denaturation treatments.

The proposed technique measures the amount of water absorbed and retained under specific conditions. Whether this measurement applies to a particular food manufacturing application can only be determined by experimentation. The presence of other ingredients in the food product, such as salt and lipid, is expected to change the hydration characteristics of the protein considerably. If a heating step is involved in the manufacture, the effects of temperature on the hydration capacity measurement also should be considered. In fact, if denatured and insoluble protein is to be assayed, the conventional excess water methods may be just as suitable as the method presented and would be much quicker. The proposed technique is preferred, however, for measuring the water hydration capacity of proteins being examined for a potentially wide variety of food product applications.

In summary, the proposed method is totally unsophisticated, being little more than trial-and-error mixing. It is also more time-consuming than the excess water-centrifugation method and more laborious (though faster) than the swelling measurement. It more closely simulates actual food product application conditions, however, because limited, rather than excess, amounts of solvent are used. Results are highly reproducible.

With only slight experience with the method, an operator should be able to measure and confirm the WHC values of four different materials within one working day.

[2]J. R. Quinn, unpublished data.

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