## A Simple Method for Determining the Spontaneous Oil Absorption Capacity of Proteins and the Kinetics of Oil Uptake

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A simple method was developed for determining the spontaneous oil uptake and the kinetics of oil uptake by several food protein materials. The amount q of oil taken up by a protein powder during time t was described by the equation q = Qt/(B + t), where Q is the total oil uptake at equilibrium and B is the time required to sorb Q/2. The rate of oil uptake was proportional to the square of the amount of oil that must still be absorbed to reach equilibrium. A specific rate constant for the process was calculated as  $(BQ)^{-1}$  and an initial rate of oil uptake as Q/B.

The capacity of proteins to interact with lipid materials is important in food formulation and processing. Many important properties of foods involve the interaction of proteins and lipids, e.g., emulsion, fat entrapment and flavor absorption (1).

Applications which involve oil or fat absorption could include various meat products in which oil or fat must be held before the protein is fully hydrated, and baked goods and dry blended mixes to which liquid oils and melted fats are added and a uniform dry mixture is desired.

Oil absorption of proteins is usually measured by adding excess oil (or liquid fat) to a protein powder, thoroughly mixing and holding, centrifuging, and determining the amount of absorbed oil [total minus free (2-5)]. Oil absorption values determined by this method depend on the amount of oil and sample, holding and centrifuging conditions. Each condition will lead to the measurement of more or less physically entrapped oil. Voutsinas and Nakai (6) developed a turbidimetric method to measure the amount of oil truly bound to the proteins by eliminating as much entrapped oil as possible.

Both methods, however, measure the oil retained (bound or/and entrapped) by the protein; that is their oilholding capacity.

None of the existing methods allows the measurement of the rate of oil absorption by the protein. Rate of oil absorption has significance in the formulation of foods. For example, the rate of oil absorption could influence the order of addition of dry ingredients into the mixture and it could also be used to determine mixing times to uniformly distribute oil or fats in a dry mixture.

Therefore, the objective of this work was to develop a simple method for determining the spontaneous uptake of oil by proteins, which would reflect their true affinity for interacting with oil and the kinetics of oil uptake.

## **EXPERIMENTAL PROCEDURES**

Materials. The following commercial soy protein isolates were used: Proteinmax 90 NB from Sambra S.A., Sao Paulo, Brazil, and Purina Protein 760, 500 E and 710 from Ralston Purina Co., St. Louis, Missouri. Bovine albumin (AB) was from Sigma Chemical Co., St. Louis, Missouri. Sodium caseinate (SC) was from Lab. Argentinos Farmesa S.A., Argentina. Whey protein concentrate (WPC) had a protein content of 75% and was obtained from New Zealand Milk Products, Inc., California. Bean protein isolate (BPI) was prepared according to Pilosof et al. (7). Pumpkin protein isolate (PUPI) was obtained according to Vigo et al. (8). Meat salt soluble proteins (MSSP) were obtained according to Acton and Saffle (9) and freeze-dried. Gelatin (G) (food grade) was from Stauffer Rioplatense S.A., Argentina. Egg white powder (EW) was obtained by freeze-drying fresh egg white. Commercially available corn oil was from Refinerias de Maiz, SAICF, Argentina.

Spontaneous oil uptake determination. Spontaneous uptake of oil by the proteins was determined on 100-mg samples using the device proposed by Torgersen and Toledo (10) for the measurement of water absorption. The device consisted of a one-ml pipette, graduated in 1/100 ml (Fig. 1A) connected to Tygon tubing ca. 30 cm long (Fig. 1B) and then to a plastic bacteriological field monitor (Millipore Corp.) (Fig. 1C). The field monitor had a diameter of 4 cm. The Millipore filter in the monitor was removed and replaced with a glass Whatman GF/C microfiber filter.

To measure the oil absorption, the pipette was adjusted to a horizontal position at the same level as the glass filter. The pipette and connecting tube were filled with oil through the open field monitor until the meniscus passed the zero mark on the pipette. The glass filter was then placed in the field monitor and allowed to imbibe oil. Excess oil was removed by touching the glass filter with

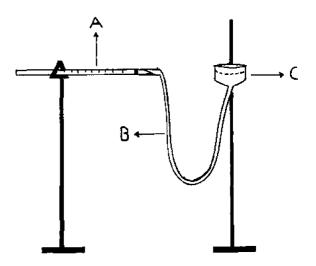


FIG. 1. Device used for measuring spontaneous oil uptake by protein powders.

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an absorbent material (e.g., the same glass filter) until the meniscus moved to the zero mark on the pipette. An exact amount of sample (100 mg), was sprinkled lightly on the glass filter inside the field monitor. At least two replicate experiments were made simultaneously at room temperature.

## **RESULTS AND DISCUSSION**

Spontaneous oil uptake curves by protein powders were all similar to those shown in Figure 2. All proteins showed a restricted oil uptake because the oil uptake curves levelled off. The volume of oil/g sample absorbed at this point represented the oil absorbing capacity (Q) for the samples. The time for reaching equilibrium was different for all the materials and varied from 3.5 min for gelatin and sodium caseinate to 15 min for MSSP and Proteinmax 90NB. However, the rate of oil absorption was rapid initially and slowed down as equilibrium was approached.

Equation for fitting the spontaneous water uptake. In order to describe the oil uptake curves mathematically, the following two-parameter equation was proposed:

$$q = \frac{Qt}{B+t}$$
[1]

where q refers to the total amount of oil taken up to time t; Q is the oil-absorbing capacity, and B is the time needed to absorb half the maximum amount of oil (Q/2).

In order to find the best statistical parameters  $\hat{Q}$  and  $\hat{B}$  which give the best fit of experimental data (q, t<sub>i</sub>), a program for nonlinear least squares analysis (11) was used; data were processed on an IBM computer.

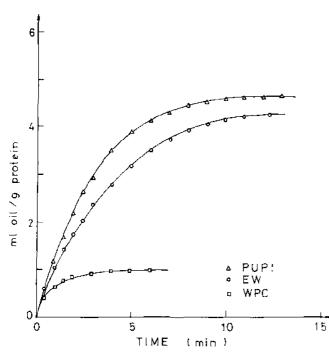


FIG. 2. Spontaneous oil uptake by different protein materials. PUPI, pumpkin protein isolate; EW, egg white; WPC, whey protein concentrate.

In order to evaluate as a whole the goodness of fit of Eq. [1] as applied to the experimental absorption data, the relative error was computed:

$$\epsilon \% = \frac{100}{n} \sum_{n=1}^{n} \frac{|q_i - \frac{Qt_i}{\hat{B} + t_i}|}{q_i}$$
 [2]

In Table 1 are shown the best statistical parameters  $\hat{Q}$  and  $\hat{B}$ , the estimators of their standard deviations and the goodness of fit of Eq. [1] as applied to the different protein materials. Eq. [1] was able to fit the data very well as show the relative error.  $\hat{B}$  was the most uncertain parameter on comparison with  $\hat{o}_B$ ; this result is probably due to the fact that  $\hat{B}$  (the time needed to reach half the maximum amount of oil) is a very short time and it is not possible to obtain experimental data with accuracy during the first two min of the absorption process.

Figures 3-5 compare the experimental and predicted (derived from Eq. [1]) oil absorption curves for all the protein products tested. It can be seen that the agreement is fairly good.

Oil absorption of proteins is affected by the protein source, extent of processing and/or composition of protein (1). As is seen, meat salt soluble proteins, bean protein isolate, egg white and pumpkin protein isolate absorbed much more oil than the other proteins tested. On the other hand, gelatin and whey protein concentrate absorbed the least amount of oil among all the proteins tested.

A linear correlation was found between the oil absorption capacity  $(\hat{Q})$  and the time needed to absorb  $\hat{Q}/2$ . P90NB did not conform to the correlation because it exhibited a higher B value than would be expected according to the Q value. The regression equation was

$$Q = 0.271 + 0.569 \text{ B}$$
 (R = 0.904; P < 0.001) [3]

TABLE 1

Parameters Which Describe Spontaneous Oil Uptake by Different Protein Materials

	Q ± âq	$\hat{\mathbf{B}} \pm \hat{\sigma}_{\mathbf{B}}$	
Protein	ml oil g protein	(min)	٤%
Gelatin	$1.08 \pm 0.02$	$0.57 \pm 0.06$	2.7
WPC	$1.14 \pm 0.01$	$0.92 \pm 0.03$	0.96
PP 710	$1.66 \pm 0.01$	$1.82 \pm 0.05$	1.1
PP 500 E	$1.80 \pm 0.05$	$2.0 \pm 0.2$	3.2
PP 760	$1.97 \pm 0.09$	1.4 $\pm$ 0.2	4.5
SC	$2.0 \pm 0.1$	$1.0 \pm 0.2$	6.0
AB	$2.30 \pm 0.06$	$0.86 \pm 0.08$	2.9
P 90 NB	$2.46 \pm 0.09$	$5.5 \pm 0.5$	6.3
PUPI	$6.0 \pm 0.1$	$3.1 \pm 0.2$	5.8
EW	$6.11 \pm 0.08$	$4.7 \pm 0.2$	1.8
BPI	$6.4 \pm 0.2$	$4.5 \pm 0.3$	5.2
MŠSP	$6.64 \pm 0.08$	$3.2 \pm 0.1$	2.5

WPC, whey protein concentrate; PP 710, Purina soy protein 710; PP 500 E, Purina soy protein 500 E; PP 760, Purina soy protein 760; SC, sodium caseinate; AB, bovine albumin; P 90 NB, Proteinmax soy protein 90 NB; PUPI, pumpkin protein isolate; EW, egg white; BPI, bean protein isolate; MSSP, meat salt soluble proteins.

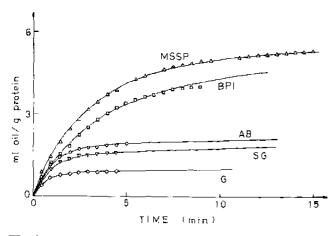


FIG. 3. Comparison of predicted (solid lines) and experimental (single points) oil uptake curves. MSSP, meat salt soluble proteins; BPI, bean protein isolate; AB, albumin bovine; SC, sodium caseinate; G, gelatin.

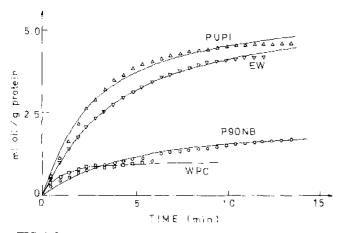


FIG. 4. Comparison of predicted (solid lines) and experimental (single points) oil uptake curves. PUPI, pumpkin protein isolate; EW, egg white; P90NB, proteinmax soy protein 90NB; WPC, whey protein concentrate.

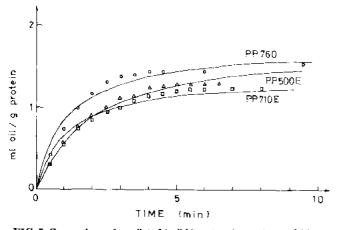


FIG. 5. Comparison of predicted (solid lines) and experimental (single points) oil uptake curves. PP760, Purina soy protein 760; PP500E, Purina soy protein 500 E; PP710, Purina soy protein 710.

and indicates that the greater the oil absorption capacity, the longer the time B.

Kinetics of oil uptake. Rate of oil uptake could be derived by differentiating Eq. [1] with respect to time, which yields:

$$\frac{\mathrm{d}\mathbf{q}}{\mathrm{d}\mathbf{t}} = \frac{1}{\mathrm{BQ}} \left( \mathbf{Q} - \mathbf{q} \right)^2$$
 [4]

where (Q - q) can be termed the "nonsaturation factor" because it represents the amount of oil that must still be absorbed to reach equilibrium and  $(BQ)^{-1}$  the specific rate constant k. Therefore, k could be calculated as

$$\hat{K} = (\hat{Q}\hat{B})^{-1}$$
 [5]

The specific rate constants for the oil uptake of the different protein materials and the estimators of the standard deviation of K are shown in Table 2.

Initial rates of oil uptake, also included in Table 2, were derived from equation (4).

$$R_{o} = \frac{\hat{Q}}{\tilde{B}}$$
 [6]

An acceptable accuracy in the K values was obtained. Proteins which absorbed increasing amounts of oil showed increasingly lower specific rate constants of oil absorption. Thus, the last four proteins in Table 1 which were the most lipophilic (Q values were above 6 ml oil/g protein) showed K values approximately tenfold lower than the other proteins. On the other hand, initial rates of oil uptake were only slightly different between proteins.

The oil uptake values of egg white, bean protein isolate and pumpkin protein concentrate indicate that these proteins would be good protein additives for food systems which must hold high amounts of lipids. However, even if oil absorption can be used as a criterion for selection of protein additives for selected applications, the performance in food systems is the ultimate test of functionality. In addition, generally the protein additive must fulfill other functional requirements. In meat applications in addition to fat absorption, protein additives must possess

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Specific Rate Constant and Initial Rate of Oil Uptake of Different Protein Materials

Protein	σ <sub>QU</sub> ml oil	$\hat{\mathbf{K}} \pm \hat{\sigma}_{\mathbf{K}}$ ml ail min $\hat{\mathbf{L}}$	R <sub>o</sub> ml oil
	g protein	g protein	g protein min
Gelatin	0.001	$1.6 \pm 0.2$	1.89
WPC	0.0002	$0.95 \pm 0.03$	1.24
PP 710	0.0008	$0.33 \pm 0.01$	0.91
PP 500 E	0.008	$0.27 \pm 0.03$	0.9
PP 760	0.01	$0.35 \pm 0.06$	1.41
SC	0.02	$0.5$ $\pm$ $0.1$	2.0
AB	0.004	$0.50 \pm 0.05$	2.7
P 90 NB	0.05	$0.074 \pm 0.008$	0.45
PUPI	0.03	$0.053 \pm 0.004$	1.93
EW	0.01	$0.034 \pm 0.001$	1.3
BPI	0.07	$0.035 \pm 0.003$	1.4
MSSP	0,009	$0.047 \pm 0.002$	2.1

Abbreviations as in Table 1.

water absorption, emulsification and gelling in order to improve binding of the structure and reduce moisture and fat losses.

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