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Study of the Hydration Process in Tehina

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ABSTRACT

The physical changes occurring during the hydration of tehina (sesame butter) were investigated. The hydration process transforms tehina from a suspension of hydrophilic insoluble solids in the anhydrous state, to an oil-in-water emulsion wherein the solids become dispersed in the aqueous continuous phase when the water content exceeds 30%. The sequences of physical changes that take place during hydration with water or 1M NaCl are similar and include a significant increase in viscosity upon addition of small amounts of water (< 12%), and the semi-solid highly viscous behaviour at 14–24% water content followed by oil separation from the hydrated solids at a water content of 24–30%. These changes are determined by the capacity of the hydrophilic solids to absorb water and flocculate. The oil/water emulsion formed, at water contents exceeding 35%, is stabilized by the adsorption of the swollen insoluble solids onto the interfacial film around the oil droplets. Processing of sesame into tehina did not alter the solubility of the meal proteins, change the amino acid composition nor the electrophoretic pattern of the subunits of the salt-soluble α-globulin fraction, the main protein fraction of sesame. The mode of aggregation of the α-globulin subunits changed to form units of half the molecular weight reported for sesame globulins.

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INTRODUCTION

Tehina consists of a paste with high content of oil (56–62%) and protein (25–27%) obtained by grinding dehulled and toasted sesame seeds (Sawaya *et al.*, 1985). In mid-eastern countries tehina is served as an appetizer or dressing, after hydration with 1–2 volumes of water to form a thin oil-in-water emulsion. Tehina is also the base for production of the middle-eastern candy halavah (Damir, 1984). During the process of hydration the material undergoes a series of rheological transformations. However, very little is known about the nature of these changes. The purpose of this study is to describe the physical changes taking place during the hydration of tehina and to relate them to the chemical composition of anhydrous tehina and to its nature as a dispersion of hydrophilic solids in oil. Tehina has a high content of protein and this seems to determine to a great extent the physical behaviour of the material on hydration; hence special attention was given to studying the properties of the meal proteins and their relationship to sesame proteins (reviewed by Kinsella and Mohit, 1985).

MATERIALS AND METHODS

Preparation of tehina

Tehina was prepared from Sudanese sesame seeds obtained from a local factory practicing a traditional but fully mechanized process. The seeds were soaked in water for 6 h, dehulled by mechanical abrasion and separated from the hulls by flotation on brine. After washing with water to remove the salt, the seeds were dried and toasted to reduce their water content below 1% and ground in a stone mill. The paste leaving the mill at 65°C was packed without additives. This preparation is referred to as anhydrous tehina.

Extraction of oil and preparation of meal

Tehina was dispersed in 5 volumes of hexane and stirred for 1 h. The solvent was removed by centrifugation for 20 min at $2500 \times g$ and the meal was extracted again 4 times with a similar amount of hexane. The solvent was removed from the combined extracts by evaporation under reduced pressure in a rotavapor and the oil was weighed. The meal was allowed to dry at room temperature in a hood to remove the solvent, then dried over silica gel in a desiccator and weighed.

Particle size distribution

The distribution of sizes of the solid particles of the meal was obtained from free sedimentation in hexane and is expressed as weight per cent distribution with respect to equivalent Stokes diameters. Thus, 5 g of tehina dispersed in 90 ml hexane were poured into a cylinder to form a 30 cm high column of liquid. Sediments from the same sample were collected after decantation at various time intervals, determined by the velocity of sedimentation, calculated by the equation:

$$v = \frac{d^2 g(\rho_p - \rho_l)}{18\eta} \tag{1}$$

where v is the sedimentation velocity, d is the particle's equivalent Stokes diameter, ρ_p and ρ_l the densities of the particles and liquid respectively, η the viscosity of the liquid and g the acceleration due to gravity (980 cm/s^2). Assuming a density of 1·3 g/cm^3 for most of the solids and values of 0·660 g/cm^3 for the density and $3{\cdot}258 \times 10^{-3}$ poise for the viscosity of hexane (Lange, 1967), fractionation by successive decantations was carried out according to the time schedule given in Table 1. Fractions I–VI were purified by repeating the fractionation with fresh hexane twice; the supernatant of each purification was used to resuspend and purify the subsequent fraction. The last fraction was collected by centrifugation for 20 min at 2500 g. Each fraction was washed with 20 ml of hexane, then dried as described above. The amount of material of size larger than 500 μm was determined by sieving fraction I. The dried fractions were weighed and

TABLE 1
Fractionation Schedule of Tehina Solids[a]

Fraction	*Equivalent Stokes diameter* (μm)	*Sedimentation time*	*Height of liquid column* (*cm*)
I	>100	0·46 min	30
II	100–50	1·45 min	30
III	50–20	11·67 min	30
IV	20–10	46·68 min	30
V	10–2	19·5 h	30
VI	2–1	26 h	10
VII	1>	particles remaining suspended in liquid	

[a] Calculated by eqn (1) with $\rho_p = 1{\cdot}3$ g/cm^3, $\rho_l = 0{\cdot}660$ g/cm^3 and $\eta = 3{\cdot}258 \times 10^{-3}$ poise (Lange, 1967).

samples of 25–40 mg were taken for determination of nitrogen by the Kjeldahl method. Samples of 250 mg were then taken for determination of nitrogen extractability (see below).

Chemical assays

Nitrogen was determined by the Kjeldahl method. Phospholipids were determined colorimetrically as dipalmitoyl lecithin by the ammonium ferrothiocyanate method of Stewart (1980). Carbohydrates soluble in aqueous extracts of meal and the carbohydrate content of salt-soluble proteins, were determined as glucose by the phenol sulfuric assay of Dubois *et al.* (1956). SH groups in salt-soluble proteins were determined with the Ellman reagent using a molar absorption coefficient of 13 600 for the chromophore at 412 nm (Ellman, 1959). A solution of 5 mg/ml of lyophilized salt-soluble protein was prepared in 8M urea containing 0·005M EDTA and 0·1M Tris. HCl buffer at pH 8·5. To an aliquot of 2 ml, 2 moles of Ellmans reagent were added. A stable reading at 412 nm was obtained after 30 min. Phytic acid was determined by the method of Latta and Eskin (1980) using a solution of 0·03% $FeCl_3$ and 0·3% sulfosalicylic acid to assay the amount of phytate extracted from the meal. Protein was determined in protein extractability experiments by the biuret method (Gornal *et al.*, 1949) with bovine serum albumin (BSA) as standard. The content of neutral detergent fibre was determined by the method of van Soest and Wine (1968).

Protein extractability

250 mg of meal were suspended in 20 ml of extracting solutions of 0·02M NaCl titrated to various pH values or in NaCl solutions of various concentrations containing 0·02M phosphate buffer, at pH 6·8. After adjusting the pH to the desired value, stirring was continued for 1 h at room temperature. The suspensions were centrifuged for 15 min at 27 000 *g* and the pellet was extracted again with 10 ml of extracting solution. Protein in the supernatant solutions was assayed colorimetrically by the biuret method (Gornal *et al.*, 1949) with BSA as standard.

Preparation of water-soluble and salt-soluble protein fractions

Tehina meal was successively extracted twice with 10 volumes of distilled water (pH of slurry 6·4) and three times with 10 volumes of 1M NaCl. The aqueous extract was dialysed for 48 h in a cold room against distilled water, in a dialysis membrane tubing with a molecular weight cut-off value of 3600, and lyophilized. The combined salt extracts were dialysed overnight against

distilled water containing 0·02% NaN_3 at room temperature in dialysis tubing of molecular weight cut-off of 10 000. The protein which precipitated was purified twice by dissolving in a minimum volume of 1 M NaCl and reprecipitating by dilution with 6 volumes of distilled water. Before lyophilization, the precipitated protein was washed twice with distilled water.

Gel filtration

Proteins were fractionated by gel filtration at room temperature using agarose 0·5 M (Biorad) in a column with bed dimensions of 700 × 15 mm. The column was eluted at a rate of 10 ml/h with a solution at pH 7·4, containing 1 M NaCl, 0·02 M phosphate buffer and 0·02% NaN_3. Fractions of 2 ml were collected.

Gel electrophoresis

SDS-PAGE was carried out according to the method of Laemmli (1970). Lyophilized samples were dissolved (2 mg/ml) in a sample buffer at pH 6·8 containing 0·1 M Tris. HCl, 3% SDS, 10% sucrose and bromophenol blue as a marker, with or without 1% mercaptoethanol (ME). The samples were incubated for 3 min at 100°C and then applied to the gels which were run, stained and destained as described in detail by Weber and Osborn (1975).

Amino acid analysis

0·010 ml of a solution of lyophilized salt-soluble protein (0·6 mg/ml) in 6 M HCl were subjected to gas phase hydrolysis for 70 min at 150°C. The amino acid composition was determined as described by Rector *et al.* (1989).

Hydration of tehina

Tehina was mixed with water or a solution of 1 M NaCl in a blender (Krups 3Mix, maximum speed: 1300 rpm). Before the water content reached 40%, water was added in small increments. After each addition, mixing was continued at least for 3 min and a sample was removed to measure conductivity and to test whether the sample was dispersible in hexane. The specific conductivity was measured at a frequency of 1000 Hz with a conductometer (El Hama Instruments, Israel). Emulsification of the oil which separated at 24–30% water content was assessed visually and by inspection of samples under a light microscope. Emulsification activity (EA) was measured on tehina hydrated with 1·5 volumes of water or 1 M NaCl by

the method of Yasumatsu *et al.* (1972). EA was measured on samples prepared as described above and on samples subjected to additional blending in a Waring blender (100 ml emulsion in a cup of 250 ml) for 60 s at low speed (16 300 rpm) and 30 s at high speed (19 400 rpm). Samples were centrifuged at 1200*g* for 5 or 10 min in a graduated tube and the volume of the cream layer was measured. The standard centrifugation time in this test (5 min) would not cause separation of a cream layer in all the samples of hydrated tehina tested. Protein content of the aqueous phase was measured after centrifugation of the emulsion for 15 min at 17 000*g*. Samples of liquid below the cream layer were removed with a syringe and analysed by the biuret method (Gornal *et al.*, 1949) with BSA as standard.

Viscosity measurements

Viscosity was measured with a Haake Rotovisco-100 viscometer, equipped with a M500 torque sensing unit. Samples at various degrees of hydration were loaded in a suitable sensor (cup + rotor) and equilibrated for 1 h at 24°C in the gap between the cup and the rotor. The torque was recorded at constant shear until a constant value was obtained. The yield stress was determined, by extrapolation of the measured stress to zero shear rate.

RESULTS AND DISCUSSION

Chemical composition

Anhydrous tehina is essentially a dispersion of solids in oil. The composition of the tehina used in this work is shown in Table 2. It contained 59% oil with phospholipid content of $<0{\cdot}3\%$. The defatted meal contained 62% crude protein (N × 6·25) with 5% of the total nitrogen being non-protein nitrogen, 9% soluble carbohydrates, 8·3% crude fibre and 4·5% phytic acid.

Particle size distribution

The distribution of the sizes (equivalent Stokes diameters) of the solid particles is summarized in Fig. 1 and their content of crude protein and its extractability in 1M NaCl are shown in Table 3. The particles showed a general distribution among two main size ranges; particles in the 100–500 μm range and particles smaller than 10 μm amounted to 14% and 76%, respectively, of the total weight of the solids, with 6% of the solids being smaller than 1 μm. Less than 1% of the particles were larger than 500 μm. Inspection using a microscope with polarized light revealed that the

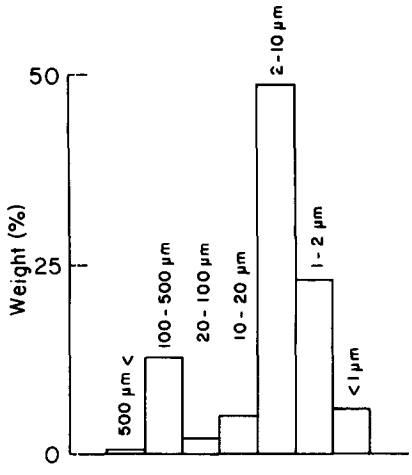

Fig. 1. Particle size distribution of tehina solids separated by sedimentation in hexane (equivalent Stokes diameters).

TABLE 2
Chemical Composition of Tehina

Component	*Amount*[a]
Oil	59
Phospholipids	<0·18
Crude protein (N × 6·25)	25
NPN[b]	0·20 (5% of total N)
Soluble carbohydrate	3·7
Fibers[c]	3·4
Phytic acid	1·8
Na	0·37
K	0·29

[a] % by weight, of whole tehina.
[b] Non-protein nitrogen.
[c] Neutral detergent fibers.

TABLE 3
Distribution of Total and Salt-Soluble Protein in the Major Particle Fractions of Tehina

Particle size[a] (*µm*)	*% by weight of the meal*	*Crude protein* (*g/1g*[b])		
		Total	*Extracted by 1M NaCl*[c]	*% N extracted by 1M NaCl*[b]
>100	13	0·39	0·27	69
20–10	5	0·58	0·46	79
<10	76	0·73	0·54	74

[a] Equivalent Stokes diameter.
[b] N × 6·25.
[c] pH 6·4.

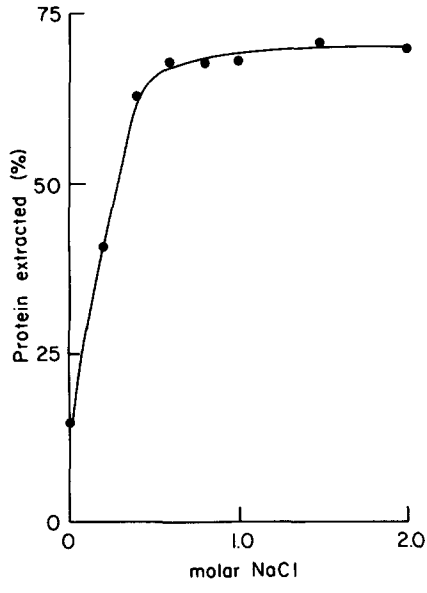

Fig. 2. Extractability of protein from tehina meal by NaCl solutions at pH 6·4.

large particles were mainly tissue fragments in which intact cell walls were clearly visible. These particles, relatively rich in cellulose, had a crude protein content of 39%, of which 70% was extractable in 1 M NaCl. The small size particles were not birefringent; they contained 73% protein of which 74% was salt-soluble (see below).

Solubility of tehina meal proteins

The effects of salt concentration and pH at low ionic strength on the solubility of defatted tehina proteins, are shown in Figs 2 and 3, respectively. At neutral pH, 70% of the total protein was extracted at NaCl concentrations of 1·0–2·0 M. Drastic reduction in solubility was observed at NaCl concentrations below 0·4 M. At low ionic strength (0·02 M NaCl) the solubility was minimum in the pH range 3·0–8·0, where only about 10% of the total protein was then soluble.

Gel filtration and electrophoresis

The elution profiles of the salt-soluble proteins from an agarose 0·5 M column are shown in Fig. 4. The protein precipitated from the 1 M saline extract, by dialysis against water, consisted of three peaks. The main peak corresponds to protein with a molecular weight of 170–180 000 daltons with a minor peak at 260–270 000 daltons. This minor peak did not appear in the chromatograms of the supernatant remaining in the dialysis tubing or in the

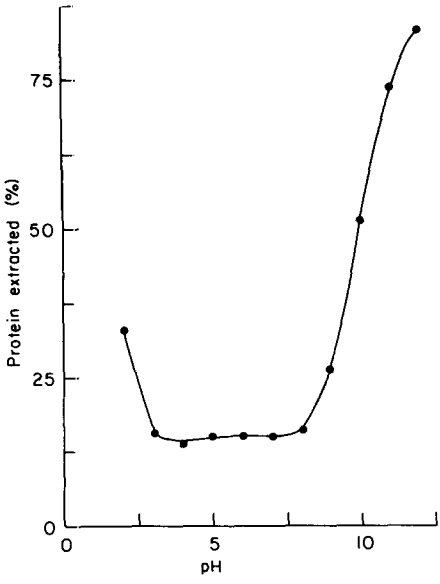

Fig. 3. The effect of pH on protein extractability from tehina meal at low ionic strength (0·02M NaCl).

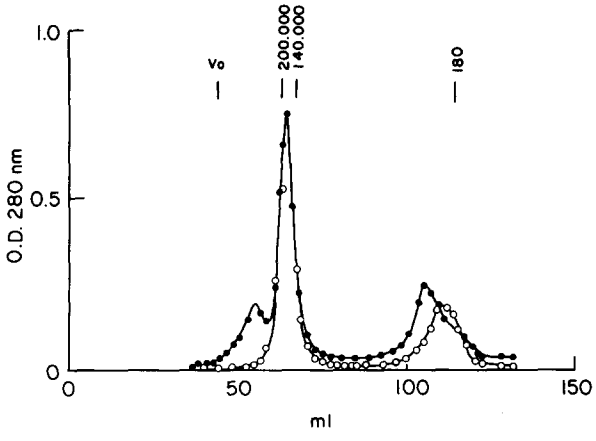

Fig. 4. Gel filtration of salt-soluble tehina meal proteins on agarose 0·5 M. Bed dimensions: 680 mm × 15 mm; elution buffer: 1M NaCl, 0·05M phosphate buffer and 0·02% NaN_3 at pH 7·4; flow rate: 12 ml/h; 2 ml fractions. ●——●, material precipitated from 1M NaCl extract by dialysis against water; ○——○, supernatant remaining in the dialysis bag. Void volume V_0 and elution volumes for potato β-amylase (MW 200 000) yeast alcohol dehydrogenase (MW 150 000) and tyrosine (MW 150) are indicated.

saline crude extract. The low molecular weight material was removed by purification of the material by repeated precipitations from 1 M NaCl. The electrophoretic patterns of the water-soluble and salt-soluble protein fractions are shown in Fig. 5 and the molecular weights of the subunits are listed in Table 4. The subunit pattern of the salt-soluble fraction is typical of sesame α-globulins as reported by Yuno *et al.* (1986). In presence of ME in the sample buffer, it consisted of two bands in the 20 000–22 000 daltons range and three bands in the 30 000–34 000 daltons range. These two groups of subunits correspond, respectively, to the basic and acidic subunits reported by Okubo *et al.* (1979*a*) and Yuno *et al.* (1986). A similar subunit pattern was obtained when the meal residue, after extraction with 1 M NaCl, is extracted with sample buffer containing ME (Fig. 5(a) and 5(b), lanes 4). In the absence of ME in the sample buffer, the salt-soluble fraction yielded a pattern consisting mainly of two bands (Fig. 5(c)) at 47 000 and 52 000

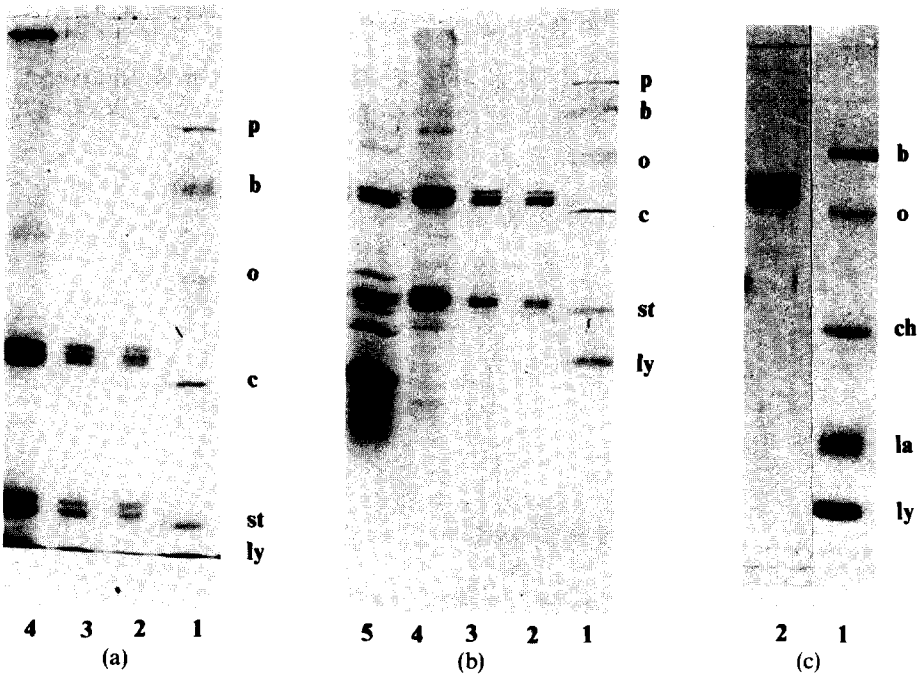

Fig. 5. Separation of tehina proteins by SDS-PAGE. (a) 10% polyacrylamide, ME in sample buffer. Lane 1: molecular weight markers, (p) phosphorylase B 92 500, (b) BSA 66 200, (o) ovoalbumin 44 000, (c) carbonic anhydrase 29 000, (st) soy trypsin inhibitor 20 100 and (ly) lysozyme, 14 400. Lanes 2 and 3: lyophilized salt-soluble proteins, 25 μg. Lane 4: protein not extracted from meal by 1 M NaCl, extracted by 3% SDS + 1% ME (5 min at 100°C). (b) 15% polyacrylamide, ME in sample buffer. Lanes loaded similarly to the lanes in (a). Lane 5: water-soluble proteins, 35 μg. (c) 12·5% polyacrylamide. Lane 1: molecular weight markers, BSA 66 200, ovoalbumin 44 000, chymotrypsinogen 25 700, β-lactoglobulin 18 400, lysozyme 14 400. Lane 2: salt-soluble proteins, no ME in sample buffer.

TABLE 4
Molecular Weight of Main Subunits of Tehina Proteins, Resolved by SDS-PAGE

	Polyacrylamide (%)		
	10%	*15%*	*12·5%*
Mercaptoethanol sample:	*+salt soluble proteins*	*+water soluble proteins*	*—salt soluble proteins*
			52 000 s
			47 000 s
		44 700 w	
	32 400 s[a]	33 500 s	
	31 300 s[a]		
	30 600 s[a]	31 000 s[c]	
		22 100 s	
	20 900 s[b]	20 700 s	
	20 300 s[b]	20 200 s	
		17 200 s	
		13 500 vs	
		< 11 000 m	

Intensity of bands staining: w: weak; m: medium; s: strong; vs: very strong.
[a] Corresponding to the acidic subunits reported by Yuno *et al.* (1986), ±300.
[b] Corresponding to the basic subunits reported by Yuno *et al.* (1986), ±200.
[c] The two lower bands which are clearly resolved in the pattern of the salt-soluble fraction (Fig. 5(b), lanes 2 and 3) are not resolved, but are resolved in the pattern of this fraction obtained on a 10% polyacrylamide gel (not shown in Fig. 5).

daltons, respectively, which are disulfide-linked pairs of acidic and basic polypeptides. The water-soluble fraction contained, besides low molecular weight components of 22 000, 17 200 and 13 500 daltons and one component below 11 000 daltons, subunits typical of the salt-soluble fraction (Fig. 5(b), lane 5). The intense band at 13 500 daltons is probably consistent with the β-globulin fraction, soluble at low ionic strength, which was recently described by Rajendran and Prakash (1988).

Amino acid analysis

The results of the amino acid analysis of the salt-soluble protein fraction are given in Table 5. The amino acid profile is similar to that published by Prakash and Nandi (1978) for sesame α-globulins. The carbohydrate content, in the salt-soluble protein fraction, is similar to the value reported by these authors (Prakash & Nandi, 1978). The carbohydrate and free SH contents were 7·6 and 2·4 moles, respectively, per 1 mole of protein of mol.wt 180 000.

TABLE 5
Amino Acid Analysis of Tehina Salt Soluble Proteins

Residue	*No. Residues/ 1000 residues*	*g/16 g N*	
		Tehina	*Sesame*[a]
Asp.	92	9·7	9·6
Glut.	168	19·8	20·0
Ser.	66	5·6	5·1
Gly.	91	5·5	5·2
Bis.	21	2·7	2·8
Arg.	120	16·8	14·2
Thr.	45	4·3	4·2
Ala.	76	5·4	5·0
Prol.	44	4·0	2·0
Tyr.	30	4·3	3·9
Val.	56	5·4	4·5
Met.	19	2·3	2·3
1/2 Cys.[b]	5	2·2	0·7
Ileu.	41	4·3	3·6
Leu.	71	7·5	7·1
Phe.	40	5·3	4·9
Lys.	16	1·9	2·0
Mean residue weight = 114			
Carbohydrate	4·8[c]	0·8	0·8
Free SH	1·5[d]		

[a] Prakash & Nandi, 1978.
[b] Under the experimental conditions of this method of analysis, cystine and cysteine are reported as the sum of half cystine residues.
[c] Determined as glucose, corresponds to 7·6 moles/1 unit of 180 000 daltons.
[d] Corresponds to 2·4 moles/1 unit of 180 000 daltons.

Hydration characteristics

When tehina is hydrated by addition of water or salt solution, all water is first absorbed by the hydrophilic solids dispersed in the oily phase and these partially hydrated particles progressively flocculate. This is demonstrated in Fig. 6 which shows the progressive sedimentation of solids from anhydrous and partially hydrated tehina dispersed in hexane. While a dispersion of anhydrous tehina in hexane did not clarify after standing for 24 h, similar dispersions of tehina hydrated to water contents of 12% and 17% clarified within 60 and 30 min, respectively (Fig. 6). Using the formerly specified parameters (see experimental section), the particle size in the material to which 17% of water was added was estimated to exceed 40 μm.

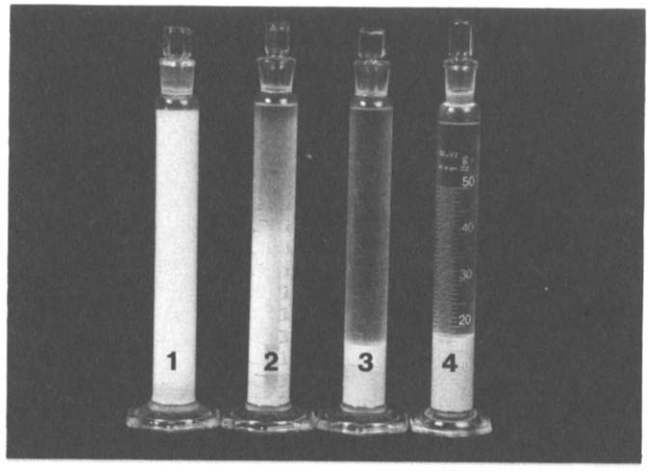

Fig. 6. Sedimentation of partially hydrated tehina solids in hexane. 10 g tehina dispersed in 60 ml hexane. Water added to tehina samples:

Sample:	*1*	*2*	*3*	*4*
% added water	—	6	12	17
Water/meal	0	0·16	0·33	0·50

Time after mixing: 60 min. Sample 4 clarified after 30 min.

In the range of 14–20% water content, tehina is a semi-solid in which oil is retained. The changes in specific conductivity of tehina with hydration are shown in Fig. 7. Although the specific conductivity increased when water was added to tehina at low water content, its value remained below 100 micromhos/cm up to a water content of 17%. Up to this water content, the hydrated material is wetted by hexane and readily disperses in this solvent. At a water content of 18% the hydrated mass dispersed no further in hexane and a sharp increase in conductivity was observed. At a water content of 24% the material separated into two phases, a slightly turbid oil phase containing a small amount of solids and water droplets and a sticky mass of hydrated solids in which some oil was trapped. At a water content above 30% an oil-in-water emulsion started to form, with the solids dispersed in the aqueous continuous phase. This process was completed at a water content of 35–40%. The conductivity increased as the volume of the continuous aqueous phase increased. Similar behaviour was observed when hydration was carried out with 1 M NaCl instead of water. Tehina can be served only after the emulsification process is completed and a thin dispersion of oil and solids in water is formed. Usually 1–1·5 volumes of water are added to the anhydrous material for consumption.

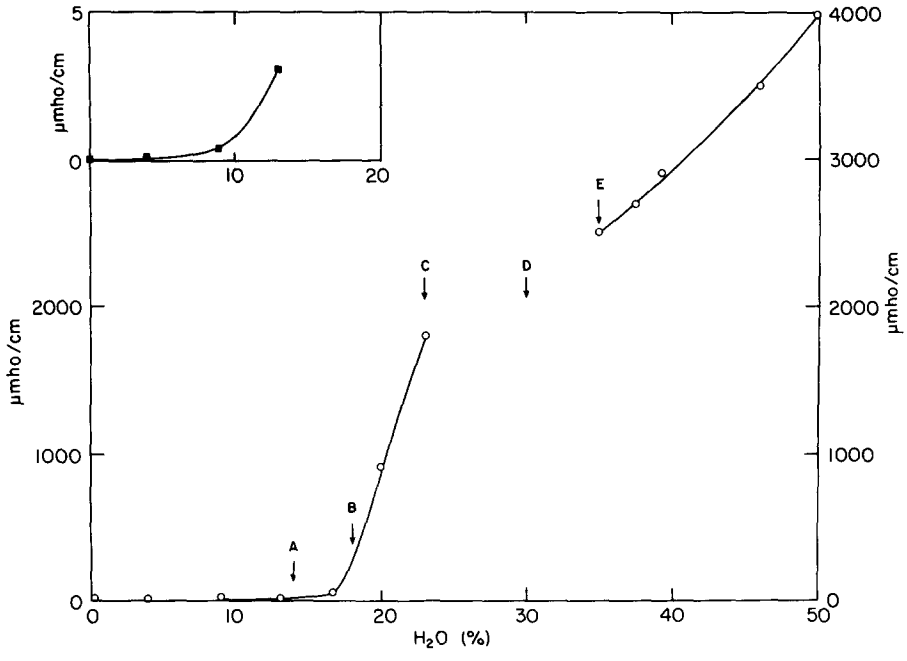

Fig. 7. The effect of water content on the electrical conductivity (at 1000 Hz) of tehina. Hydration with 0·02M NaCl. A: material becomes semi-solid; B: material does not disperse in hexane; C: oil separation; D: emulsification of oil begins; E: emulsification completed.

Viscosity measurements

The apparent viscosities and yield stresses of tehina hydrated with water and with 1M NaCl are shown in Table 6. Following the addition of small amounts of water, the yield stress and viscosity increased. At water contents of 14–35% the viscosity could not be measured because the material does not flow. In the 14–24% range the rotor slips in the semisolid material and in the range of water content of 24–35% the material is not homogeneous. Slippage of the rotor already occurs at water content below 14%; this explains the lower yield stress at a water content of 12% as compared to a water content of 6%, and the similar apparent viscosity measured at both water contents (Table 6). Measurements of viscosity could be resumed at a water content of 40% when an oil/water emulsion with solids dispersed in the continuous aqueous phase was formed. On further addition of water the viscosity continuously decreased.

Tehina as an emulsion

Ready-to-serve tehina is prepared by hydration of the anhydrous suspension with 1–1·5 volumes of water; mixing is carried out manually or in

TABLE 6
Viscosity[a] of Tehina at Various Water Contents

Hydration with water			*Hydration with 1 M NaCl*		
% Added water	*Apparent viscosity (Pa s)*	*Yield stress (Pa)*	*% added 1 M NaCl*	*Apparent viscosity (Pa s)*	*Yield stress (Pa)*
—	3·75	45			
6	10·4	175	6	9·6	145
12	9·8	145	12	12·0	120
			45	7·3	126
50	1·8	23			
			55	1·1	15
60	0·9	12			

[a] Apparent viscosity at a shear rate of $19\,s^{-1}$.

a slow blender. Tehina hydrated with 1·5 volumes of water or 1 M NaCl (60% water, 24% oil and 16% of solids) in a slow blender (Krup 3Mix), was found by inspection under a light microscope to contain a wide distribution of oil droplet sizes (1–20 μm). In the case of samples hydrated with water an upper layer of water, but not oil, slowly separates. The material hydrated with 1 M NaCl showed no visual signs of creaming or oil separation, probably reflecting the availability of soluble protein which stabilized the droplets. The presence of insoluble solids, which were dispersed in the aqueous phase, complicated the interpretation of the results obtained by the turbidimetric method of Pearce and Kinsella (1978) for assessing the emulsifying activity (EA) of tehina. The centrifugation method (Yasumatsu *et al.*, 1972) was therefore employed. The EAs of tehina hydrated with water or 1 M NaCl in two types of blender, slow and high-speed, are compared in Table 7. Tehina hydrated with 1 M NaCl had a higher EA and a 5-fold higher protein content in the aqueous phase than tehina hydrated with water. The difference in EAs was not large and was more pronounced in samples prepared by additional blending in a Waring blender (high-speed blender). For samples hydrated with 1 M NaCl and subjected to additional blending in a Waring blender, a longer centrifugation time (10 min) was necessary to obtain clear separation between sediment, aqueous and cream layers. The EA of tehina hydrated with water and blended in a Waring blender was higher than the corresponding soy-oil emulsion in BSA solution (24% oil, 1·76% BSA in 40 mM phosphate buffer at pH 6·4). The EA of this emulsion as well as the EAs of samples of hydrated tehina which were not blended in a Waring blender, are below the minimum value expected for closely packed spherical globules for an emulsion of 24% oil. For an oil density of 0·85, and a volume

TABLE 7
Emulsification Activity of Tehina Hydrated with 1·5 Volumes of Water[a] or 1 M NaCl and Homogenized with Two Mixers

Hydration solution	*Krups 3MIX*[b]			*Waring blender*[c]		
	Emulsification activity[d]		*% protein in aqueous phase*	*Emulsification activity*[d]		*% protein in aqueous phase*
	5 min	*10 min*		*5 min*	*10 min*	
Water	0·35	0·34	1·33	0·52	0·47	1·72
1 M NaCl	0·40	0·36	8·15	[e]	0·53	8·80

Emulsification activity of 23·6% soy-oil emulsion in a solution of 1·76% BSA in 0·04 M buffer phosphate, pH 6·4, prepared in a Waring blender: 0·35 for 5 and 10 min centrifugation times.
[a] Composition by weight: 60% water, 23·6% oil and 16·4% insoluble solids, pH 6·4.
[b] Slow blender, 1300 rpm maximum speed.
[c] Fast blender, 16 300 rpm low speed, 19 400, high speed.
[d] Centrifugation at 1200*g* for times specified.
[e] No clear separation between sediment, aqueous layer and cream.

fraction of 0·74 for closely packed spheres, the minimum EA should be 0·38. Apparently coalescence and flattening of globules took place during centrifugation.

Adhesion between the oil droplets and the suspended solids in hydrated tehina is demonstrated in Fig. 8. When tehina hydrated with 1·5 volumes of water was further diluted 7-fold with water, the solids and the oil droplets precipitated together. This was also observed in non-diluted tehina hydrated with water, after prolonged holding at room temperature. In samples hydrated with 1 M NaCl and diluted with 1 M NaCl, a layer of cream was formed on top of the liquid, as is the usual case in diluted oil in water emulsions.

Tehina is a concentrated suspension of hydrophilic particles in oil. Despite the high concentration of suspended solids, a layer of oil separates above a dense cake of solids after prolonged standing. This undesirable phenomenon is caused by the predominance of the small particle fraction of soilids (Fig. 1) which slowly sediment and pack efficiently at the bottom of the container.

The low content of phospholipids in the oil suggests that the hydration characteristics of tehina are not determined by a component of the oil which can interact with water. The characteristics of tehina at various hydration stages are determined by the ability of the solid particles to absorb water and flocculate when water is added, and by the oil-holding capacity of the partially hydrated solids. Most of the insoluble solids are comprised of small particles ($< 10\ \mu m$) containing about 70% protein (Table 3) which, although

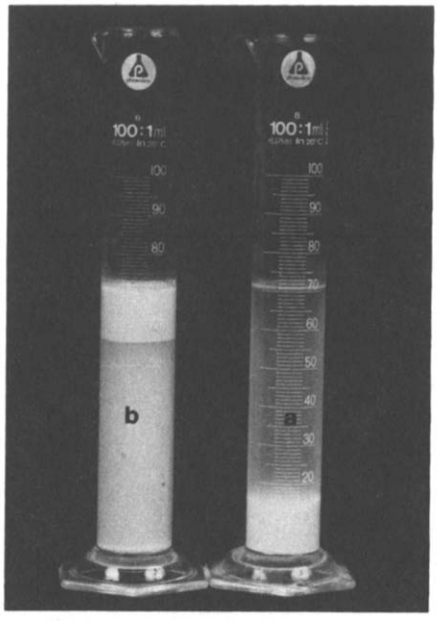

Fig. 8. Precipitation and creaming of hydrated tehina. (a) Tehina hydrated with 1·5 volumes of water, diluted 7-fold with water. (b) Tehina hydrated with 1·5 volume of 1M NaCl diluted 7-fold with 1M NaCl.

insoluble in water, readily absorb water. The partially hydrated solids, when dispersed in oil, flocculate to form large aggregates manifested by an increased sedimentation rate when dispersed in hexane (Fig. 6) and in lower packing efficiency of the solids. This also explains the increase in viscosity following addition of even small amounts of water to the anhydrous paste. At water contents of about 14% (water/meal = 0·4 in tehina with 59% of oil), the flocculated solids fill the whole volume of the material which does not flow but turns into a semi-solid. The whole mass is still homogeneous and easily disperses in hexane. This suggests that oil still forms a continuous phase. At a water content of 17%, (water/meal = 0·50) the whole mass becomes hydrophilic and does not disperse in hexane. At this point additional water added to the partially hydrated tehina forms a layer on the surface of the hydrated particles, which explains the increase in specific conductivity of the material (Fig. 7). At this stage of hydration most of the oil is still retained by the semi-solid mass. As more water is added, it accumulates at the interface between the flocculated solids and the oil which, at a water content of 24% (water/meal = 0·77), separates from the solids. At a water content of 30%, there is enough free water to form an oil/water emulsion with water as the continuous phase in which the hydrated solids can be easily dispersed. Upon further addition of water, the viscosity sharply decreases and the electrical conductivity increases. The exact water contents at which the physical changes occur during the hydration process depend upon the oil/solids ratio and on how finely the material is ground.

Hydration of tehina was studied with two solvent systems: water in which the main sesame protein, the α-globulin fraction, is insoluble, and 1 M NaCl in which this fraction is soluble. In both cases the physical changes occurring during the hydration process are similar. In hydrated tehina a stable dispersion of oil droplets is formed. Even when hydration is carried out with water in which most of the protein is insoluble, the aqueous phase contains substantial amounts of water-soluble low molecular weight protein which facilitates emulsification. The emulsification capacity of this protein fraction was not investigated. Observations on diluted tehina (Fig. 8) suggest that adhesion of the insoluble solids to the dispersed droplets provides an important mechanism by which the emulsion in tehina hydrated with water is stabilized. The stabilizing effect of the high content of solids in emulsified tehina explains its higher EA as compared to the corresponding BSA-stabilized emulsion and the relative small differences in EA in samples with large differences in protein solubilized in the aqueous phase (Table 7).

The solubility of the protein (Figs 2 and 3) was similar to that reported by Rivas *et al.* (1981) and Prakash (1986), indicating that the processing of sesame seeds to produce tehina did not alter the solubility properties of the meal proteins. It is not clear how far water penetrated inside the seeds when they were soaked in water prior to dehulling. The high content of Na^+ in the meal suggests that brine penetrated the seed interior during flotation on the brine, so that heat was applied on hydrated seeds during the drying process. The similarity in the amino acid composition of the purified salt-soluble proteins, extracted from tehina, to that reported for sesame α-globulins by Prakash and Nandi (1978), and the similarity in subunit pattern to those reported by Okubo *et al.* (1979*b*) and Yuno *et al.* (1986), suggest that no major breakdown took place on the subunit level during processing of sesame to tehina. The aggregating subunits in salt-soluble proteins from tehina are similar to the acidic and basic subunits in sesame (Yuno *et al.*, 1986). The main change observed was in the degree of aggregation of these subunits. Units of molecular weight of 170–180 000 (most probably 3 pairs of disulfide-linked acidic + basic subunits) are formed, instead of units of 360 000 as reported by Mohit and Kinsella (6 pairs of subunits, Kinsella & Mohit, 1985).

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