Emulsifying Properties of Proteins: Evaluation of a Turbidimetric Technique

Kevin N. Pearce and John E. Kinsella*

The capacity of protein to stabilize emulsions is related to the interfacial area that can be coated by the protein. According to the Mie theory for light scattering, a simple relationship exists between turbidity and the interfacial area of an emulsion. In this study, turbidimetry was evaluated as a method for measuring emulsifying properties of proteins. Emulsions were made by homogenizing known amounts of proteins and peanut oil. These emulsions were serially diluted to give absorbances of 0.01–0.6 at 500 nm. Several factors, i.e., degree of homogenization, type of homogenizer, protein concentration, volume of dispersion, oil volume fraction; pH and type of oil affected emulsion formation. The relative emulsifying activity of various food proteins (casein, β-lactoglobulin, whey protein, yeast protein, ovalbumin, and succinylated yeast proteins) was determined. Using a simple formula based on the turbidity, volume fraction of dispersed phase and weight of protein, an emulsifying activity index, related to the interfacial area of the emulsion, was calculated. Succinylated yeast proteins showed high emulsifying activities. This appeared to be related to the solubility of proteins and their resistance to surface denaturation.

The intensive research concerning the development of novel and functional proteins has dramatized the need for standard methods for quantifying functional properties of proteins (Briskey, 1970; Kinsella, 1976). Emulsifying properties are important in many food applications of ingredient proteins and these commonly discussed in terms of emulsifying capacity (EC), emulsifying stability (ES), and emulsifying activity (EA). Various methods have been used to measure these indices.

The EC denotes the maximum amount of oil that is emulsified under specified conditions by a standard amount of protein. The first EC measurements by Swift et al. (1961) have been improved upon. Tsai et al. (1970) developed a microblender which allows emulsions to be formed in essentially air-free conditions thereby avoiding foaming. Webb et al. (1970) used the change in electrical resistance while Haq et al. (1973) monitored the alternating current impedance of the emulsion to provide an objective index of the emulsion collapse. Crenwelge et al. (1974) used the sudden fall in the electrical current drawn by the blender to indicate the fall in viscosity due to phase inversion. Marshall et al. (1975) and Wang and Kinsella (1976) found that the addition of an oil-soluble dye facilitated the visual recognition of the phase inversion that occurs when EC of the protein is exceeded. Several variables affect the determination of EC. The blender speed is one important variable, e.g., higher blending speeds give smaller apparent EC (Swift et al., 1961; Carpenter and Saffle, 1964; Inklaar and Fortuin, 1969; Crenwelge et al., 1974). Ivey et al. (1970) confirmed this but found that the surface area of the dispersed phase at phase inversion was independent of blender speed for emulsions stabilized by beef proteins.

Protein concentration, its solubility, pH of the medium, sucrose, and sodium chloride affect the emulsifying capacity of leaf proteins (Wang and Kinsella, 1976). Acton and Saffle (1972) found that oil volume fractions at emulsion collapse fell in a fairly narrow range of values for meat protein stabilized emulsions and the actual value depended largely on the method of emulsification. Other factors such as equipment design, shape of container, rate of oil addition, kind of oil, and nature of protein have also been investigated (Christian and Saffle, 1967; Saffle, 1968;

Kinsella, 1976). Thus EC is not solely a property of the protein under test but rather is a property of the emulsion system, the equipment and method used to produce the emulsion (Tornberg and Hermansson, 1977). Furthermore, it is not clear how EC is related to the amount of emulsifier required to produce a satisfactory emulsion when the amount of oil is less than that required for phase inversion. In cases where very viscous emulsions are formed, mixing of oil into the emulsion may be inefficient or incomplete and the observed EC value erroneous.

Emulsion Stability. Emulsions are not stable in a thermodynamic sense. Operationally a stable emulsion is one which is very slow to undergo the various processes which result in the separation of oil and water phases. These processes include creaming, flocculation, coalescence, and oiling-off (Becher, 1965). These processes may occur singly or in combination (Mulder and Walstra, 1974). Any definition of ES must recognize that different emulsions may break down by different processes and that the relative importance of these processes may depend upon temperature, gravitational field strength, and the concentration of oil in the emulsion. ES is commonly measured in terms of the amount of oil and/or cream separating from an emulsion during a certain period of time at a stated temperature and gravitational field (Carpenter and Saffle, 1964; Acton and Saffle, 1970; Smith and Dairiki, 1975; True et al., 1975). The time required for a specified degree of breakdown to occur is also used as a measure of stability (Hegarty et al., 1963; Trautman, 1964; Pearson et al., 1965; Helmer and Saffle, 1963). The amount of fat extractable from dairy cream by ether extraction has been used as an index of stability (Foley et al., 1971).

Several workers centrifuged heated emulsions and expressed the ES in terms of the height of the cream layer as a percentage of the initial height of the emulsion (Yasumatsu et al., 1972; Vananuvat and Kinsella, 1975; Wang and Kinsella, 1976; Wu and Sexson, 1976). The gravitational field (1300g) and time (5 min) cause all but the smallest oil globules to cream. Differences in ES measured in this manner reflect differences in the degree of packing in the cream layer and in the amount of oil separating. If the emulsion is unstable and complete oil separation occurs there will be no cream layer and ES = 0%. If the emulsion is stable and the oil globules undergo no changes during the test, then the ES value will depend solely on the degree of packing. Some stratification of oil globules according to size may occur because of the differences in the Stokes velocities and creaming rates. Close

Department of Food Science, Cornell University, Ithaca, New York 14853 (J.E.K.) and the New Zealand Dairy Research Institute, Palmerston North, New Zealand (K.N.P.).

Turbidimetric Evaluation

J. Agric. Food Chem., Vol. 26, No. 3, 1978 **717**

packed uniform spheres have a maximum volume of 74% and would give ES = 67.6% if the oil volume fraction is 0.5. Less homogeneous spheres and spheres which flatten at the point of contact can pack more densely. Strong repulsive forces between globules (expected in the most stable emulsions) do not increase the effective size of oil globules sufficiently to have a significant effect on the close packed volume as such forces are short-ranged compared with typical globule diameters. Thus the minimum ES value for a stable emulsion is nearly equal to the oil volume fraction expressed as a percentage.

A viscous continuous phase, small globule size and repulsive forces between globules will tend to hinder close packing of globules and could result in an ES value greater than 67.6% even though some oil separation and some coalescence occurred. Furthermore, the processes occurring during the centrifugation of the emulsion may not be characteristic of those occurring in a stored or heated emulsion. Thus, ES measured by the method of Yasumatsu et al. (1972) may not be a valid indication of emulsion stability.

Several other methods have been used to measure emulsion stability. Turbidimetric measurements have been used to study the stability of suspensions of fine wax particles (Bolton and Marshall, 1949). Light transmission by diluted ice cream emulsions (Keeney and Josephson, 1958; Govin and Leeder, 1971), temperature gradients in microwave heated emulsions (Petrowski, 1974), the diffuse light reflectance of pharmaceutical emulsions (Akers and Lach, 1976), and pulsed NMR measurements (Trumbetas et al., 1976) are other techniques which have been used to measure emulsion stability.

Because of the problems and shortcomings of many of the traditional approaches and the need for more reliable methods for the quantification of emulsifying properties by techniques requiring small quantities of protein, this study evaluated the potential of turbidimetry for measuring emulsification. In this paper we demonstrate some of the experimental factors affecting the determination of the emulsifying properties of proteins using the technique. A comparison of the relative emulsifying capacity of several proteins is presented.

EXPERIMENTAL SECTION

Turbidimetry. The ability of a protein to aid the formation of an emulsion is related to its ability to adsorb to and stabilize the oil–water interface. The capacity of a protein to stabilize an emulsion might be expected to be related to the interfacial area that can be coated by the available protein (Ivey et al., 1970). Similarly, the stability of an emulsion should be related to the constancy of the interfacial area. The Mie theory for light scattering by dispersed spherical particles (Kerker, 1969) indicates that there is a simple relationship between the turbidity and the interfacial area of an emulsion provided that certain conditions are met. Turbidity measurements are easily made using the common laboratory spectrophotometer and have been applied to the study of milk fat globules (Walstra, 1965) and coarse colloidal dispersions (Bagchi and Vold, 1975; Maron et al., 1963).

Theory. For a spectrophotometer in which none of the light scattered by the turbid sample reaches the photodetector and for a sample which does not adsorb light the turbidity of the sample is given by

$$T = \frac{2.303A}{l}$$

where A is the observed absorbance and l is the pathlength of the cuvette.

For a dilute dispersion of spherical particles which are large compared with the wavelength of the light the Mie theory for light scattering (Kerker, 1969) gives the following relationships:

Interfacial area = $2T$

$$\bar{R} = 3\phi/2T$$

$$N = 2T^3/(9\Pi\phi^2)$$

where ϕ is the volume fraction of the dispersed phase, $\bar{R}$ is the volume/area mean radius of the dispersed particles, N is the equivalent number density of particles (the hypothetical number density of particles, all of radius $\bar{R}$, which would have the same T as that observed for the polydisperse system.)

The above relationships are independent of the wavelength of the light and can be applied to a polydisperse system.

METHODS

Preparation of Emulsions. A measured amount of pure peanut oil and aqueous protein solution (generally 10 and 30 mL, respectively) were shaken together and homogenized by a hand-operated laboratory piston-type homogenizer (VWR Scientific, Rochester, N.Y.). Alternatively, the oil and protein dispersion were homogenized together for varying periods in a blender (Janke and Kunkel Model A10)(Chemical Rubber Co., Cleveland, Ohio) or Waring blender (Model 5011 fitted with Eberback Model No. 8580 semi-micro-container) (VWR Scientific, Rochester, N.Y.). Temperature was maintained at 20 °C. Duplicate preparations were made for each study.

Measurement of Turbidity. Aliquots (1 mL) of the emulsion were diluted serially with water and SDS (sodium dodecyl sulfate) solution to give final dilutions in the range 1/1000 to 1/5000 and a SDS concentration of 0.1%. The absorbance of the diluted emulsion was then determined in a 1-cm pathlength cuvette at a wavelength of 500 nm in a Spectronic 700 (Bausch and Lomb, Rochester, N.Y.) unless otherwise stated. A Hitachi-Perkin Elmer Model 356 spectrophotometer (Perkin-Elmer, Norwalk, Conn.) was used in the preliminary experiments. Identical cuvettes were used for all samples and were rinsed with a jet of distilled water between determinations. Absorbance of duplicate aliquots of each emulsion was measured in each case.

Determination of Adsorbed Protein. The emulsion was centrifuged at 25 000g for 15 min and aliquots of the aqueous layer and of the original protein solution were taken for protein determination by the Lowry method (Lowry et al., 1951). The adsorbed protein was determined by difference.

Oil Content of Emulsions. Aliquots (1 mL) of the emulsion and of the protein solution were weighed and were dried to constant weight (1 h) at 120 °C. The oil volume fraction (ϕ) of the emulsion was calculated from density and dry weight data, by the equation

$$\phi = \frac{C - A - E(B - C)}{C - A + (B - C)\{(1 + E)D_o/D_s - E\}}$$

where A denotes mass of beaker; B mass of beaker plus emulsion; C mass of beaker plus dry matter; D_0 density of oil; D_s density of protein solution, and E concentration of solutes (mass per unit mass of solvent).

Microscopic Examination. Both the undiluted and diluted emulsions were examined under the optical (light) microscope. A small quantity of the undiluted emulsion was placed directly on a glass microscope slide, was covered with a cover slip, and was examined at 100×, 400×, or

1000× magnification. Diluted emulsions were examined in a cuvette at 100× magnification. The cuvette was completely filled, stoppered, laid on the microscope stage, optical window up, and examined.

Emulsion Stability. The emulsion under test was held at constant temperature while being gently stirred. Periodically aliquots of the emulsion were taken for dilution and turbidity measurement as described above.

Materials. Several protein preparations were used in this study. Dialyzed and lyophilized preparations of egg albumin, β-lactoglobulin lysozyme (egg white), hemoglobin, and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, Mo.). Whey protein powder A ("Solac", New Zealand Milk Products, Inc., Rosemont, Ill.), prepared by ultrafiltration and partial demineralization by ion exchange, contained 67% protein. Whey protein powder B ("Foretein 29", Foremost Foods, San Francisco, Calif.) was a delactosed, partially demineralized whey. Soy protein isolates A (Profam 90) and B (promine F) were purchased from Grain Processing Corp. (Muscatini, Iowa) and Central Soya Co. (Chicago, Ill.), respectively. Sodium caseinate was obtained locally. Yeast protein was extracted from yeast by method of Vananuvat and Kinsella (1975). Succinylated yeast protein was prepared by the method described by Franzen and Kinsella (1976). Peanut oil (Planters) devoid of added emulsifiers or stabilizers was used. Sodium dodecylsulfate was purchased from Eastman Chemicals (Rochester, N.Y.). Other chemicals were reagent grade and distilled deionized water was used throughout.

RESULTS

Initially some of the experimental variables affecting turbidimetric measurements of protein stabilized emulsions were studied in order to standardize experimental conditions.

Microscopic Examination. All the emulsions studied contained polydisperse spherical oil globules. The smallest globules had diameters of less than 0.2 μm. In some emulsions clumping or flocculation of globules was apparent. Dilution of the emulsion with 0.1% SDS solution was effective in disrupting the flocs and gave a fully disperse system. Coalescence of flocculated particles and wetting of the glass slide with oil were observed under the microscope in undiluted emulsions of low stability.

Turbidity Measurements. The dilution of emulsions with 0.1% SDS solution produced turbid dispersions which were stable for several hours although in some cases periodic gentle shaking or stirring was necessary to minimize creaming. When emulsions were diluted with water alone, without SDS, less turbid solutions were frequently produced and microscopic examination revealed that these contained small flocs. The absence of SDS generally resulted in a marked deposition of oil onto the surfaces of containers and cuvettes.

In any particular emulsion sample the turbidity was proportional to the concentration of the diluted emulsion for absorbances up to about 0.4. At higher emulsion concentrations and higher absorbances, the relationship became nonlinear, presumably because of multiple scattering of light by the dispersed oil globules especially when oil globules were small. However, in the present study, errors did not exceed 7% for absorbances of less than 0.8.

Wavelength Dependence of Turbidity. Plots of absorbance vs. wavelength were obtained for an emulsion after it had been passed through the hand homogenizer one, four, and fifteen times (Figure 1). The data indicate that the greater the degree of homogenization the greater the absorbance and the greater the wavelength dependence
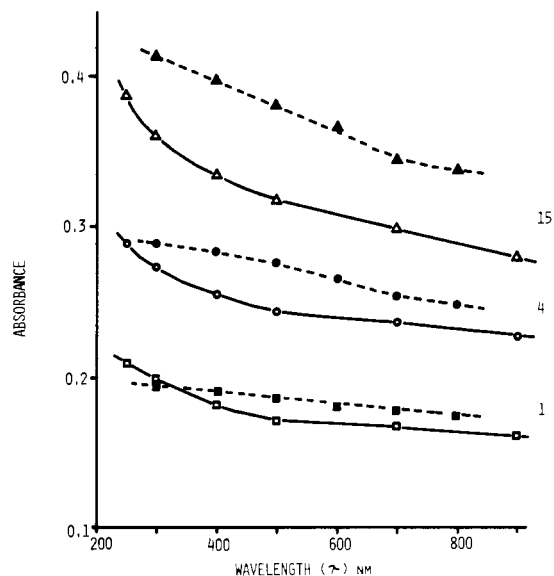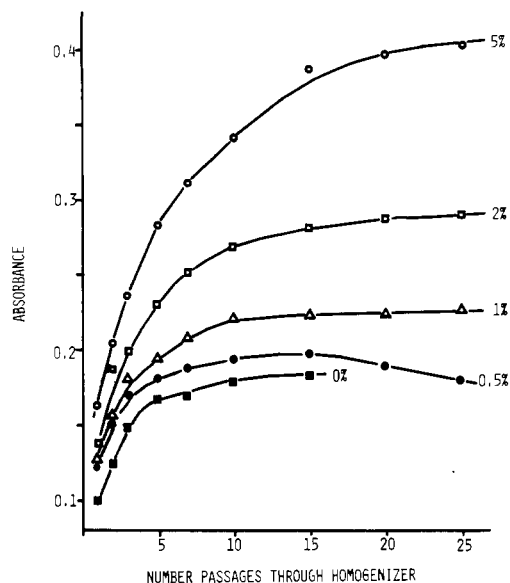

Figure 1. The absorbance of a protein-stabilized emulsion as a function of wavelength. The emulsion was stabilized by 2% whey protein solution, pH 8.0, ϕ = 0.25, prepared using a hand-operated homogenizer and diluted 1/2500 for absorbance measurements in a 1-cm pathlength cuvette. Numbers 1, 4, 15 denote the number of passages through the homogenizer; broken lines and solid lines denote measurements in Perkin-Elmer 356 and Spectronic 700 spectrophotometer, respectively.

of the absorbance. Microscopic examination of the emulsion revealed that the oil globules became smaller upon repeated homogenization. After a single passage through the homogenizer globules were highly polydisperse but they tended to become less polydisperse upon further homogenization. This was because larger globules were progressively broken down more readily than smaller globules.

In the Spectronic 700 spectrophotometer the cuvette is positioned about 6 cm from the photodetector window so that the detector angle of acceptance is relatively large (6°). Thus the light which is scattered through small angles by oil globules still falls on the detector and is measured as though it were transmitted light. Consequently, the absorbance values read from the spectrophotometer tend to be low. When the cuvette is placed in the Perkin-Elmer Model 356 spectrophotometer the cuvette to photodetector distance is 33 cm and the angle of acceptance (with a 4 × 13 mm masking slit fitted in front of the detector) is about 1° so that only transmitted light is detected. Thus the apparent absorbance is greater than that measured using the Spectronic 700 (Figure 1). In the present study a wavelength of 500 nm was used.

Degree of Homogenization and Effect of Protein Concentration. The effects of protein concentration and of the repeated passage of an emulsion through the hand-operated homogenizer are shown in Figure 2. The initial homogenization had the greatest effect on the absorbance but even after 25 passages through the homogenizer the absorbance was still increasing in those emulsions containing the higher levels of protein. In the less concentrated protein solutions the absorbance tended to become constant, or, in the case of 0.5% protein, even began to show a decrease when subjected to repeated homogenization. Homogenization by 15 passages was most acceptable under these conditions.

When an emulsion was diluted with an equal volume of water and then subjected to further homogenization, the absorbance tended to decrease. The limiting absorbance was similar to that for an emulsion prepared with half the

Turbidimetric Evaluation

J. Agric. Food Chem., Vol. 26, No. 3, 1978 **719**

Figure 2. Absorbance of protein stabilized emulsions as a function of the degree of homogenization and protein concentration. Emulsion stabilized by whey protein at concentrations of 0.5, 1, 2, and 5%, respectively, pH 8.0, ϕ = 0.25, 20 °C, and prepared using a hand-operated homogenizer. Diluted 1/2500 for absorbance measurements in a Spectronic 700 spectrophotometer at 500 nm.
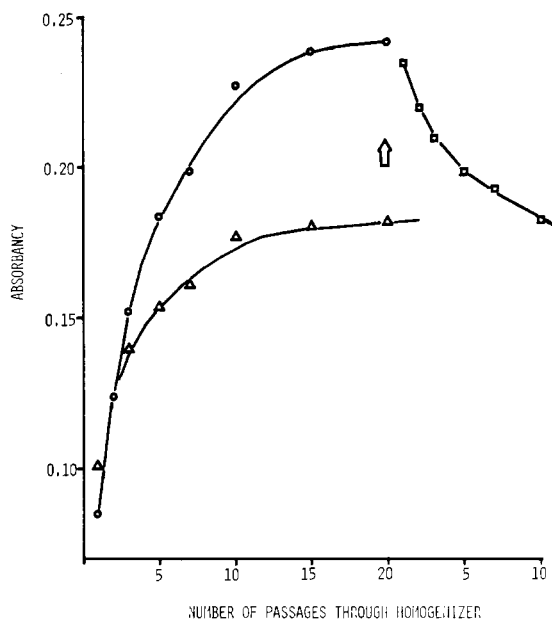
Figure 3. Effect of the dilution of an emulsion on the process of homogenization and absorbancy at 500 nm. Legend: (O) stabilized by 2% whey protein solution, pH 8.0, ϕ = 0.40, diluted 1/5000; (□) above emulsion after dilution with an equal volume of water, diluted 1/2500; (△) stabilized by 1% whey protein solution, pH 8.0, ϕ = 0.20, diluted 1/2500.

protein concentration and half the amount of oil (Figure 3).

Measurement of the amount of protein adsorbed by the oil phase (or otherwise rendered insoluble) indicated that in these experiments most of the added whey protein remained in solution and apparently was available for further emulsion formation. Thus, the limiting turbidity was not constrained by depletion of protein from solution. However, in other studies in which ovalbumin stabilized emulsions were made there was a marked depletion of protein from solution upon repeated homogenization and a corresponding decrease in the absorbance of the diluted
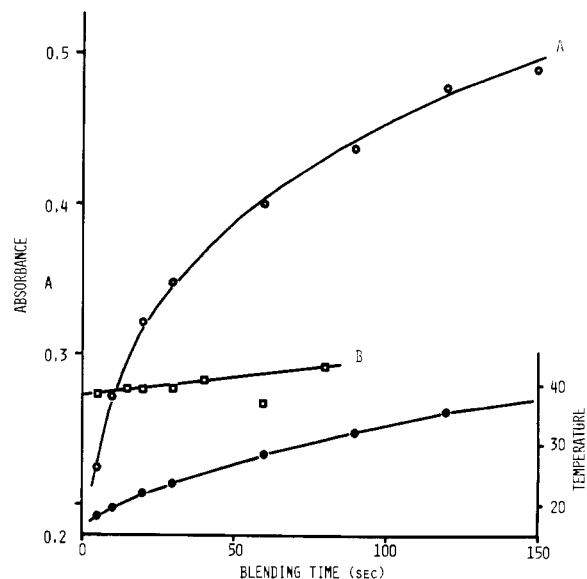
Figure 4. Absorbance at 500 nm of a protein stabilized emulsion as a function of blending time. Emulsions were stabilized by 2% whey protein solution, pH 8, ϕ = 0.4: (O) emulsion prepared in a Waring blender; diluted 1/1000 (A); (□) emulsion prepared in a Janke and Kunkel blender, diluted 1/2500 (B); (●) temperature in Waring blender.

emulsion. The ovalbumin was easily denatured during emulsification.

Effect of Liquid, Air, and Blender on Emulsion Formation. The total volume of liquid placed in either the Waring or Janke-Kunkel blender had a marked effect on the turbidity of the resulting emulsion. The greater the volume of liquid the lower was the observed turbidity. Consequently, care was needed to keep emulsion volumes constant (40 mL) during the different experiments. The formation of a vortex and the mixing of air into the emulsion in the Waring blender resulted in reduced turbidity compared with that obtained when air was eliminated from the blender jar. The incorporation of air into an emulsion typically reduced the turbidity to less than half.

The two blenders used in the present work had significantly different characteristics, as revealed when the effect of blending time on the absorbance was compared (Figure 4). The Janke-Kunkel blender produced nearly constant turbidity after only 5 s blending. The Waring blender produced a gradual rise in absorbance and a concomitant rise in emulsion temperature from 18.5 to 44 °C over the 150 s of blending. The Janke-Kunkel blender was used in preference to the Waring blender in most of the following experiments because of the rapid attainment of steady state turbidity without significant temperature change, the capacity for temperature control by water cooling, and the ability to consistently mix a smaller volume of sample, e.g., 25 mL.

A protein concentration effect was also apparent when emulsions were prepared in the blender (Figure 5). When no protein was present emulsions were unstable and the turbidity varied from experiment to experiment. In a few cases water-in-oil emulsions were formed. The presence of as little as 0.1% protein caused a marked increase in the stability of the emulsion and improved reproducibility. Larger amounts of protein caused a progressive increase in turbidity, a trend similar to that observed for emulsions prepared using the hand homogenizer (Figure 2).

Influence of Oil Volume Fraction. An increase in the amount of oil dispersed resulted in an approximately proportional increase in turbidity of emulsions produced
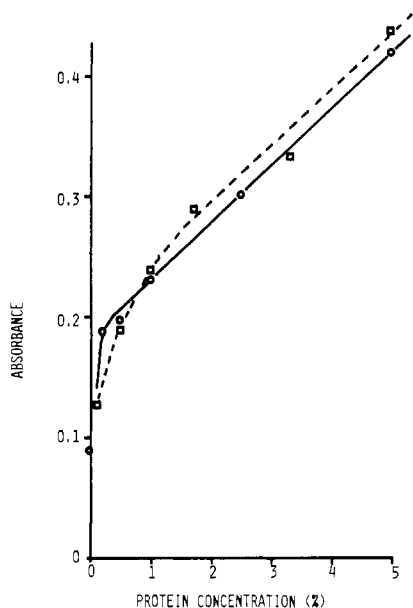
Figure 5. Absorbance of emulsions as a function of protein concentration. Emulsions stabilized by sodium caseinate solution, (□) pH 8, 0.1 M NaCl, ϕ = 0.25 diluted 1/2500, and by whey protein solution, (O) pH 8, ϕ = 0.25, diluted 1/2500. Temperature 20 °C, absorbance at 500 nm.
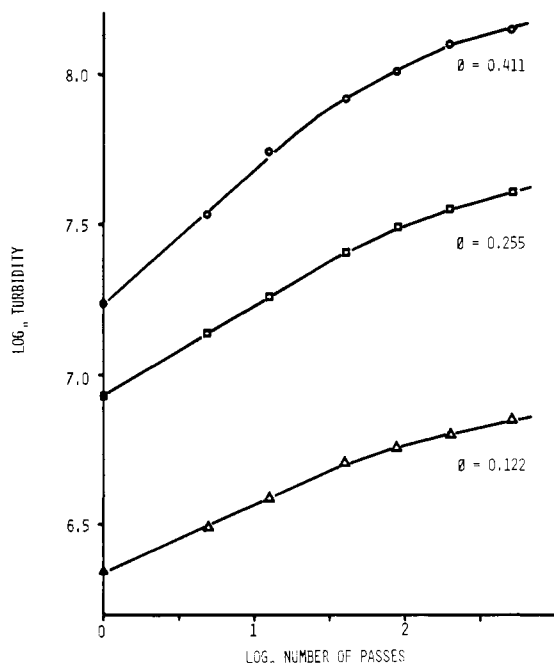
Figure 6. Turbidity of protein stabilized emulsions as a function of the degree of homogenization (number of passages through the homogenizer) and of the oil volume (ϕ) fraction. Emulsions stabilized by 2% whey protein solution, pH 8.0.

by either blender or homogenizer. The data for the hand homogenizer was plotted logarithmically (Figure 6). The constant initial slope was characteristic and suggested an empirical relationship between turbidity (T) and the number of passages (N) through the homogenizer that could be expressed by the equation: $\log T = A + B \log N$, where A and B are constants.

Influence of pH and Ionic Strength. Emulsions were prepared from solutions of whey protein having a range of pH values. The turbidity increased with pH for pH values above 5.5 but was constant below this value in emulsions formed with the blender. When detergent was omitted from the emulsion dilutions a minimum appeared

Table I.   Emulsion-Forming Properties of Various Oils[a]

| | Absorbance | |
Oil	JK[b] Blender	Waring Blender
Peanut	0.312	0.152
Safflower	0.301	0.146
Soybean	0.297	0.148
Corn	0.273	0.160
Paraffin	0.250	0.089

[a] Emulsions made with whey protein 2%, pH 8; ϕ = 0.25 and dilution of 1/2500.   Absorbance measured at 500 nm at 20 °C.   [b] JK = Janke-Kunkel blender.

in the absorbance curve at pH 5 presumably because of flocculation or minimum protein solubility. This pH corresponds closely to the isoelectric point of β-lactoglobulin, the major protein component of the whey powder.

Changes in ionic strength in the range 0–1 M (0–5.8% NaCl) had little effect on the turbidity of emulsions stabilized by whey protein or casein. However, in the absence of added salt at pH 6.5 casein stabilized emulsions showed more rapid flocculation than those emulsions containing NaCl concentrations above 0.1 M.

Influence of Oil Type. Various vegetable oils and paraffin oil were compared for their effects on emulsion turbidity, using the two types of blender (Table I). After 1 min of blending, the Waring blender produced lower turbidities than the Janke-Kunkel blender in all cases. However, the relative order of the oils differed for the two blenders. Whereas peanut oil gave the highest turbidity in the Janke-Kunkel blender, corn oil produced the highest turbidity in the Waring blender. Paraffin oil gave the lowest turbidity in both cases but differed less from the other oils when in the Janke-Kunkel blender. Safflower and soybean oils were very similar in both blenders. These data indicate a complex interaction between oil type and the type of equipment used to produce an emulsion.

Comparison of Various Proteins. Proteins differ in their ability to promote the formation of an emulsion. Using the information obtained in these preliminary studies, the relative emulsifying capacity of various proteins was measured using turbidimetry and applying the formula and rationale described in the section on theory. The equivalent number density of oil globules (N) as a function of the degree of homogenization for a variety of materials is shown in Figure 7.   Initially N increased almost linearly with repeated passages through the homogenizer. Egg albumin was unusual in showing a decrease in N after only three passages through the homogenizer. This protein is known to be readily denatured at surfaces (Cheesman and Davies, 1953) and a separate experiment showed there was a marked depletion of protein from solution during the formation of an emulsion from egg albumin. A similar effect may also occur for meat proteins (Swift et al., 1961) and for these and similar proteins there may be an optimum degree of homogenization for maximum dispersion. A wide disparity between proteins in their capacity to form emulsions was observed.   The β-lactoglobulin was the most efficient emulsifying protein being markedly superior to whey protein which contained mostly β-lactoglobulin and α-lactalbumin. The latter may have impaired the emulsifying capacity of the β-lactoglobulin by virtue of its ease of denaturation, though other components, i.e., salts or lactose may have deleteriously affected emulsion formation.

An emulsifying activity index (EAI) was defined: EAI = $2T/\phi C$, where C is the weight of protein per unit volume

Turbidimetric Evaluation

J. Agric. Food Chem., Vol. 26, No. 3, 1978 **721**
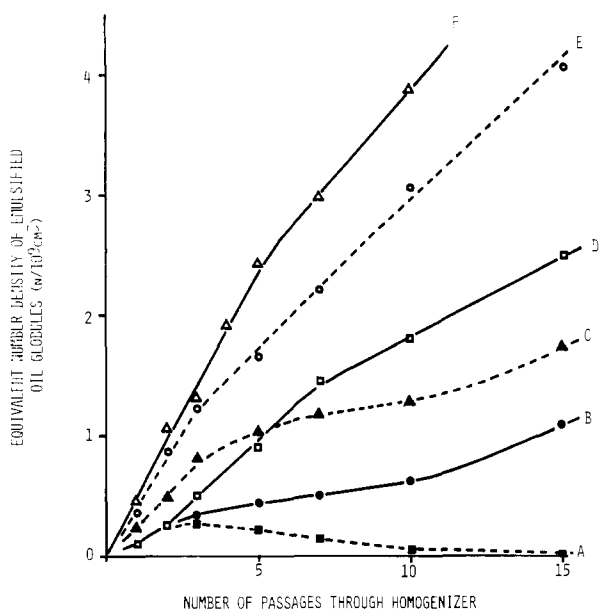
Figure 7. Comparison of various proteinaceous emulsifying agents. The equivalent number density of oil globules in the emulsion, N, was plotted against the number of passages through the hand-operated homogenizer. A variety of materials at a concentration of 0.5% and pH 6.5 were tested. Legend: A–F denote egg albumin, whey protein, bovine serum albumin, succinylated yeast protein, β-lactoglobulin, and sodium dodecylsulfate, respectively.

Table II. Emulsifying Activity Index Values for Various Proteins[a]

|	Emulsifying activity index, $(m^2\ g^{-1})$	
Protein	pH 6.5	pH 8.0
Succinylated (88%) yeast protein	322	341
Succinylated (62%) yeast protein	262	332
Sodium dodecylsulfate 0.1 detergent	251	212
Bovine serum albumin		197
Sodium caseinate	149	166
β-Lactoglobulin		153
Whey protein powder A	119	142
Succinylated (24%) yeast protein	110	204
Whey protein powder B	102	101
Soy protein isolate A	41	92
Hemoglobin		75
Soy protein isolate B	26	66
Yeast protein	8	59
Lysozyme		50
Egg albumin		49

[a] Protein concentration 0.5% in phosphate buffer, pH 6.5, $I = 0.1$.

of aqueous phase before the emulsion is formed. Turbidity (T) and volume fraction of dispersed phase (ϕ) were defined in theory section. The EAI has units of area of interface stabilized per unit weight of protein. The EAI is a function of oil volume fraction, protein concentration, and of the type of equipment used to produce the emulsion. Some EAI values for protein concentrations (0.5%) are given in Table II. The concentration of dissolved protein was not determined, but it would be expected to be less than 0.5%. The whey protein powders contained significant lactose while the soy protein isolates and some of the yeast proteins were not completely soluble under the conditions of these experiments. The highly soluble succinylated yeast protein demonstrated excellent emulsifying activity, especially when compared to the

Table III. Emulsifying Activity Index Values for Different Proteins from Yeast[a]

Protein sample	Succinylation (percent)	Emulsifying activity index, $m^2\ g^{-1}$
A	0	20
A*	0	58
B	24.4	70
C	12.8	90
D	44.5	221
D*	28.7	220
E	62.3	280
F*	54.4	328
G	88.2	356

[a] Proteins were dispersed at 0.4% concentration in phosphate buffer (pH 6.50, $I = 0.1$). Protein samples with asterisk were heated to 80 °C during isolation. Succinylation (percent) denotes the number of lysine groups succinylated in the yeast proteins, temperature 20 °C.
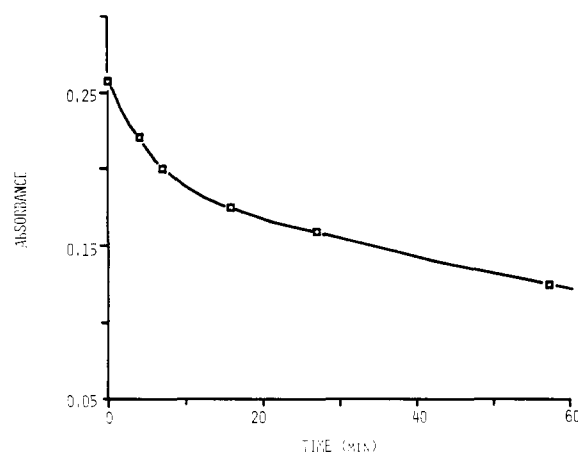
Figure 8. The heat stability of an emulsion stabilized by 0.1% SDS detergent, pH 8.0, 0.1 M NaCl, $\phi = 0.25$. Heated in a boiling water bath, diluted 1/2500 (ESI 3.3 h).

unmodified yeast protein or the easily denatured ovalbumin.

Modified yeast proteins are compared in Table III. Heating (80 °C) during extraction of the protein from disrupted yeast cells increased the EAI as did increasing the extent of the succinylation. The unmodified protein was not completely soluble under the conditions of the experiment and gave low values for the EAI. In contrast, the more highly modified proteins were completely soluble and gave high values for the EAI. It would seem that a wide range of EAI values can be achieved by suitably modifying yeast protein.

Emulsion Stability. The stability of a SDS stabilized emulsion heated in a boiling water bath is shown in Figure 8. The decrease in absorbance with time is approximately first order in absorbance. An Emulsion Stability Index (ESI) was defined: $ESI = T,\Delta t/\Delta T$ where ΔT is the change in turbidity, T, occurring during the time interval Δt. The ESI value for the emulsion of Figure 8 is 3.3 h. In this case there is a simple relationship between ESI value and the rate constant for the emulsion breakdown. In general, the relationship may be more complex, and the ESI value may depend on the time interval chosen for the measurements.

DISCUSSION

In order for the limiting form of the Mie theory to be applicable to the scattering of light by an emulsion it is necessary that all the conditions outlined in the theory

section of this paper be fulfilled. Microscopic examination of the emulsions showed the existence of only spherical globules in these preparations. However, many of the globules were not large compared with the wavelength of the scattered light. Although the total amount of oil present in the form of these small globules was generally small, the wavelength dependence of the absorbance (Figure 1) indicated significant error. The apparent mean globule radius derived from the absorbance readings fell near the minimum size for validity of the limiting form of the Mie theory. More complex equations are available for the treatment of small particle distributions but the applications of these requires some knowledge of the form of the size distribution, knowledge of the refractive indices, and measurements of turbidity over a range of wavelengths (Walstra, 1965). In the case of very fine emulsions, such as might be produced by high-pressure homogenization, the use of these more complex equations is essential.

The finite angle of acceptance of the photodetector is also a significant source of error, particularly in the case of coarse emulsions. Accessories are available to reduce acceptance angles and corrections can be calculated for small acceptance angles and small particles (Walstra, 1965). No attempt was made in the present work to correct for either the presence of very small particles or for the finite photodetector acceptance angles. Consequently, turbidities and derived quantities in this paper may contain significant errors and are only suitable for comparison with other similarly derived values.

Turbidity measurements are very simple to make although certain precautions are necessary. Rapid creaming, heavy flocculation, or the presence of foam may cause problems in obtaining representative samples of the emulsion for dilution. Rapid creaming of coarse emulsion globules during serial dilution or in the cuvette during actual turbidity measurements must be avoided. If due care is taken, reproducible data are readily obtained for any particular sample of a stable emulsion. The chief problems are concerned with the production of emulsions for study. Emulsion volume, the type of emulsifying apparatus, the speed and duration of its operation, and the type and amount of oil all affect the degree of dispersion of the oil and the value obtained for the EAI. Thus the EAI is not a property of the test material alone but is a property of the system as a whole. Careful standardization of equipment, oil, and procedures might allow reproducible measurements to be made in different laboratories. However, the values for the EAI so obtained apply only to the carefully defined system and they may not be applicable to food processing using large commercial emulsifying apparatus and a different oil from that specified in the test. In practice, this problem is not likely to be too serious because if a protein preparation has a high EAI under standard conditions then it is likely to be a good emulsifying agent under other similar conditions.

The degree of dispersion of oil in water seemed to reach a limiting value after prolonged blending (Figure 4) or repeated passages through a homogenizer (Figure 2). If the limiting emulsion is diluted (Figure 3) or transferred to a different machine and subjected to further blending or homogenization, there may be a change in particle size and again a limiting value is obtained. This suggests that the limiting state corresponds to a condition of dynamic equilibrium in which the processes leading to the breakup of globules to give small globules is balanced by processes involving recombination of globules. This latter process tends to produce coarse unstable emulsions while the former process produces stable emulsions. The conditions of high shear which exist in a blender or homogenizer increase the rate of collisions between oil globules and between oil globules and machine surfaces or air bubbles. Thus the EAI might be expected to be related to emulsion stability in those emulsions where the rate of breakdown is determined by collision processes.

In most of the systems studied in the present work depletion of protein from the aqueous phase during the formation of an emulsion was minimal. However, protein concentration did have a direct effect on the degree of dispersion (Figures 2 and 5). These results suggest that emulsifying capacity values for a protein are likely to be related to the stability of a concentrated emulsion under conditions of high shear rather than to the amount of oil that can be stabilized in dispersions by a limited amount of protein.

In stable emulsions the interfacial area does not change with time. Consequently, such emulsions have a constant turbidity. Coalescence and oiling-off cause an irreversible reduction in the interfacial area and the rates of these processes can be monitored using turbidimetry (Figure 8). Creaming removes oil globules from the bulk of the emulsion but does not in itself cause a reduction in the interfacial area of the whole emulsion. Flocculation can be reversed by dilution of emulsions with SDS solution and even if deflocculation could be avoided, the light scattering characteristics of flocs are poorly understood. Consequently, turbidimetry is unlikely to be useful in the study of either creaming or flocculation. Alternative direct methods are available for the study of creaming (Acton and Saffle, 1970; Yasumatsu et al., 1972).

Two indexes (EAI and ESI) were proposed in this paper for the characterization of emulsifying agents, especially proteins. These indexes are easily measured and, from theoretical and practical considerations, seem more likely to be related to practical performance of products than the EC, EA, and ES tests which are commonly used. Although further work is required to test this conjecture, the new indexes promise to be useful for testing the effects of chemical or enzymic modification of proteins and for testing proteins from novel sources as potential emulsifying agents.

LITERATURE CITED

Acton, J. C., Saffle, R. L., *J. Food Sci.* **35**, 852 (1970).
Acton, J. C., Saffle, R. L., *J. Food Sci.* **37**, 904 (1972).
Akers, M. J., Lach, J. L., *J. Pharm. Sci.* **65**, 216 (1976).
Bagchi, P., Vold, R. D., *J. Colloid Interface Sci.* **53**, 194 (1975).
Becher, P., "Emulsions: Theory and Practice", Reinhold, New York, N.Y., 1965.
Bolton, M. E., Marshall, A. W., *Soap Sanit. Chem.* **25**, 129 (1949).
Briskey, E. J., in "Evaluation of Novel Protein Products", Bender, A. E., et al., Ed., Pergamon Press, New York, N.Y., 1970.
Carpenter, J. A., Saffle, R. L., *J. Food Sci* **29**, 774 (1964).
Cheesman, D. F., Davies, J. T., *Adv. Protein Chem.* **9**, 439 (195).
Christian, J. A., Saffle, R. L., *Food Technol.* **21**, 1024 (1967).
Crenwelge, D., Dill, C. W., Tybor, P. T., Landman, W. A., *J. Food Sci.* **39**, 175 (1974).
Foley, J., Brady, J., Reynolds, P. J., *J. Soc. Dairy Technol.* **24**, 54 (1971).
Franzen, K., Kinsella, J. E., *J. Agric. Food Chem.* **24**, 788 (1976).
Govin, R., Leeder, J. G., *J. Food Sci.* **36**, 718 (1971).
Haq, A., Webb, N. B., Whitfield, J. K., Howell, A. J., Barbour, B. C., *J. Food Sci.* **38**, 1224 (1973).
Hegarty, G. R., Bratzler, L. J., Pearson, A. M., *J. Food Sci.* **28**, 663 (1963).
Helmer, R. L., Saffle, R. L., *Food Technol.* **17**, 1195 (1963).
Inklaar, P. A., Fortuin, J., *Food Technol.* **23**, 103 (1969).
Ivey, F. J., Webb, N. B., Jones, V. A., *Food Technol.* **24**, 1279 (1970).
Keeney, P. G., Josephson, D. V., *Ice Cream Trade J.* **54**(5), 32 (1958).

Sweet Analogue of Phyllodulcin

J. Agric. Food Chem., Vol. 26, No. 3, 1978 **723**

Kerker, M., "The Scattering of Light and Other Electromagnetic Radiation", Academic Press, London, 1969.

Kinsella, J. E., *Crit. Rev. Food Sci. Nutr.* **7**, 219 (1976).

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, P. J., *J. Biol. Chem.* **193**, 265 (1951).

Maron, S. H., Pierce, P. E., Ulevitch, I. N., *J. Colloid Sci.* **18**, 470 (1963).

Marshall, W. H., Dutson, T. R., Carpenter, Z. L., Smith, G. C., *J. Food Sci.* **40**, 896 (1975).

Mulder, H., Walstra, P., "The Milk Fat Globule", Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England, 1974.

Pearson, A. M., Spooner, M. E., Hegerty, G. R., Bratzler, L. J., *Food Technol.* **19**, 1841 (1965).

Petrowski, G. E., *J. Am. Oil Chem. Soc.* **51**, 110 (1974).

Saffle, R. L., *Adv. Food Res.* **16**, 105 (1968).

Smith, L. M., Dairiki, T., *J. Dairy Sci.* **58**, 1249 (1975).

Swift, C. E., Lockett, C., Fryer, A. J., *Food Technol.* **15**, 468 (1961).

Tornberg, E., Hermansson, A. M., *J. Food Sci.* **42**, 468 (1977).

Trautman, J. C., *Food Technol.* **18**, 1065 (1964).

True, L. C., Goodnight, K. C., Hartman, G. H., *J. Dairy Sci.* **58**, 800 (1975).

Trumbetas, J., Fioriti, J. A., Sims, R. J., *J. Am. Oil Chem. Soc.* **53**, 722 (1976).

Tsai, R. Y. T., Cassens, R. G., Briskey, E. J., *J. Food Sci.* **35**, 299 (1970).

Vananuvat, P., Kinsella, J. E., *J. Agric. Food Chem.* **23**, 613 (1975).

Walstra, P., *Neth. Milk Diary J.* **19**, 93 (1965).

Wang, J. C., Kinsella, J. E., *J. Food Sci.* **41**, 286 (1976).

Webb, N. B., Ivey, F. J., Jones, V. A., Monroe, J. R., *J. Food Sci.* **35**, 501 (1970).

Wu, Y. V., Sexson, K. R., *J. Food Sci.* **41**, 512 (1976).

Yasumatsu, K., Sawada, K., Moritaka, S., Misaki, M., Toda, J., Wada, T., IShii, K., *Agric. Biol. Chem.* **36**, 719 (1972).

An Intensely Sweet Analogue of Phyllodulcin: 2-(3-Hydroxy-4-methoxyphenyl)-1,3-benzodioxan
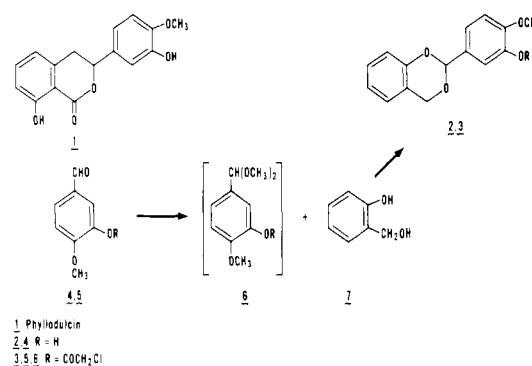
William E. Dick, Jr.* and John E. Hodge

2-(3-Hydroxy-4-methoxyphenyl)-1,3-benzodioxan, an easily prepared acetal of *o*–hydroxybenzyl alcohol (salicyl alcohol) and 3-hydroxy-4-methoxybenzaldehyde (isovanillin), mimics the structure and taste of phyllodulcin, a rare, intensely sweet isocoumarin derivative. Preliminary tests indicate a 2 mg % solution of the new acetal to be approximately isosweet with a 6% sucrose solution, but it is slowly hydrolyzed in water with loss of sweetness.

The present controversy over the healthfulness of dietary sweeteners underscores the need for a harmless, noncariogenic, intensely sweet, additive for dietary foods and pharmaceuticals. Some naturally sweet products of botanical origin, or derivatives prepared from such extracted products, are known to have saccharin-like sweetness (Hodge and Inglett, 1974), but candidate compounds often have structures too complex for facile synthesis and are not isolated economically from agricultural sources. These limitations make it unlikely that a sweetener such as phyllodulcin (1, Arakawa and Nakazaki, 1959; Asahina and Asano, 1931), an isocoumarin derivative isolated from *Hydrangea thunbergii Sieb.* and consumed as a tea-like decoction in Japan will be of commercial value, even though it is reported to be either 400 (Yamato et al., 1977) or 600–800 times sweeter than sucrose (Suzuki et al., 1977). A more likely source of suitable sweeteners will be easily prepared, harmless, synthetic compounds which contain the specific structural features required for expression of sweetness in the natural model. One such compound is 2, the acetal of 3-hydroxy-4-methoxybenzaldehyde (4) and *o*-hydroxybenzyl alcohol (7), which mimics the taste of phyllodulcin and contains some of its structural features.

RESULTS AND DISCUSSION

Synthesis of the sweet acetal, 2-(3-hydroxy-4-methoxyphenyl)-1,3-benzodioxan (2) is straightforward, offering

Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604.

Scheme I

overall yields of 60–65% (Scheme I). Selection of the chloroacetyl group to block the phenolic hydroxyl of 4 was a matter of convenience. Conversion of 3 to 2 is best carried out under mild conditions to avoid solvolytic losses of 2.

Structures of 2 and 3 were confirmed by ¹H and ¹³C nuclear magnetic resonance (NMR) and by high-resolution mass spectroscopy.

Solubility of 2 in water at 25 °C is low, slowing preparation of aqueous solutions of 2 unless the compound is predissolved in a solvent such as ethanol and then added to water. For an ethanolic stock solution containing 10 mg/mL, addition of 3 mL of stock solution to 100 mL of deionized water produces a saturated solution of 2.

Preliminary taste tests indicate that 2 is approximately 3000 times sweeter than sucrose with full expression of sweetness requiring 2–3 s, as does its analogue 1. However,