A REVIEW OF METHODOLOGY FOR EMULSIFICATION PROPERTIES OF PLANT PROTEINS[1]

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ABSTRACT

Emulsifying properties of proteins play an important role in a large number of food applications. The development of a standardized method to measure such properties would help food processors select the right protein for a given application. Because of the large diversity of food emulsions, it is difficult to develop one model system to measure emulsification properties which would be applicable in all cases. Therefore, it is proposed that three model systems be developed to simulate three general types of food emulsions, namely, comminuted meat sausages, low-fat milk-type emulsions, and high-fat mayonnaise-type emulsions. Composition, preparation, and methods of evaluation of the emulsion stability of such model systems are discussed.

More and more protein products are becoming available for food use, making the evaluation of their functional properties in standardized model systems an ever-increasing necessity.

Emulsifying properties of proteins are important in a large number of different food applications, and thus it is one of the more important functional properties which needs to be evaluated.

Practically all food emulsions are of the oil-in-water type, but they differ greatly in composition and properties. Because of this diversity, it is difficult (perhaps impossible) to develop a single model system which would be applicable to each food use.

Therefore, I would like to propose the development of three model systems to simulate three general types of food emulsions, *i.e.*: a) comminuted meat sausages, such as frankfurters and bologna, b) low-viscosity emulsions, such as milk, coffee whitener, and liquid whipped topping, and c) high-viscosity emulsions, such as mayonnaise or salad dressings.

The reason for this classification lies not only in differences in their compositions, but also in the differences in problems associated with these emulsions. In a low-viscosity, low-fat emulsion, creaming is the usual form of breakdown, and the contribution of protein to the whitening effect of the emulsion is important. In high-fat, high-viscosity emulsions, creaming is less important, and coalescence and inversion into water-in-oil emulsions are the most likely breakdown problems. The function of protein in meat emulsions is multifold; it helps to emulsify the fat, and contributes to water and fat absorption, consistency, and texture of the product. Thus, in meat emulsions a more complete evaluation is needed.

These three types of food emulsions differ considerably, and a protein most suited for one type of emulsion may not be the best for another type.

In developing these model systems for the evaluation of the emulsification properties of proteins, three major factors need to be assessed; composition of the emulsion, preparation of the emulsion, and evaluation of emulsion stability.

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COMMINUTED MEAT EMULSIONS

Most of the published methods for the measurement of the emulsification properties of proteins are actually attempts to measure the ability of these proteins to stabilize meat emulsions. Since the breakdown of a meat emulsion usually manifests itself in "greasing out," *i.e*, separation of fat upon heat processing, Swift *et al.* (1) developed a method to measure the maximum amount of fat emulsified by a given protein dispersion under standardized conditions. This was termed "emulsion capacity." In such a test, oil is added at a given rate to a constantly stirred protein dispersion under carefully specified conditions, until the emulsion inverts into a w/o emulsion, as indicated by a sudden drop in emulsion viscosity. Swift *et al.* (1) determined heat stability by preparing the emulsion in the same way, but they terminated oil addition when the emulsion attained maximum viscosity, *i.e.*, just before inversion, and subsequently heated the emulsion to 75°C for 30 min. There are numerous modifications of this method, consisting of changes in equipment, speed of stirring, rate of oil addition, means of detection of end point, protein concentration, etc. (2–12).

Some of these modifications improved the precision of the method but, at the same time, some of them destroyed even the limited resemblance of the test method to the actual conditions of meat emulsion preparation. The continuous addition of liquid oil to a very dilute protein dispersion, stirred by a propellor-type mixer until inversion occurs, has very little relation to the ability of a protein to stabilize a meat emulsion or even to the stability of any given liquid emulsion.

Most protein additives have low solubility at the pH of the meat, and consequently they give poor results in such tests (7). Since these protein additives proved to be useful in practical application, Inklaar and Fortuin (13) suggested that emulsion stability should be measured at neutral pH so that the test results correlate better with practical application.

It has been demonstrated that although enzyme-modified soy protein isolates showed higher "emulsion capacity" than unmodified soy proteins, they scored poorly in "emulsion stability" tests, and were unacceptable in meat emulsions (14). Smith *et al.* (15) demonstrated that fish protein concentrate and soy protein concentrate with low nitrogen solubility gave the lowest results in the "emulsion capacity" test, but contributed the most to meat emulsion stability in a high-fat frankfurter. They also stated that any empirical functional property determined in a model system using a high moisture:protein ratio is of little predictive value for estimating the actual contribution of protein additives to the stability of meat emulsions.

The more recent methods developed to evaluate protein additives for meat emulsions attempt to simulate actual sausage preparations more closely. Hayes (16) describes making an emulsion in a bowl cutter using 1 part protein additive, 5 parts water, and 5 parts fat, and then evaluating the formed emulsion by frying it at 325°F, stuffing it into a casing, and cooking it in water to an internal temperature of 165°F, and also by canning the emulsion and retorting at 250°F for 45 min. Observations of the strength and stability of the emulsion were made after each treatment. The advantages of this method are: a) the proportions of protein:water:fat more closely approximate the proportions present in meat emulsions, b) it uses the same equipment for emulsification as is used in sausage production, and c) it allows the emulsification of solid fat at low temperature

rather than oil. The disadvantage of such a method is that the evaluation of emulsion characteristics is a subjective procedure. However, objective methods could be developed to measure emulsion stability and consistency. The addition of sodium chloride and phosphate salts to this system would allow one to study the effect of these salts on emulsion formation and stability. Although this method can be used to screen protein additives for meat emulsions, the results must be viewed with caution, as it has been shown that the protein which gives a high score in such a test will not always work well in an actual meat emulsion.[2]

Since the ultimate test for any protein additive lies in actual preparation of meat emulsions, it makes sense to develop a standardized comminuted sausage formulation to evaluate protein additives. But meat is such a variable commodity that the results may not be reproducible. However, it is possible to develop an internal control, such as the use of a standard protein additive, and compare other proteins to this control. Such a standardized formulation should contain a limited amount of meat so that the protein additive is truly needed to stabilize the meat emulsion.

The cooked stability of the finished meat emulsion could be determined by the method of Meyer *et al.* (17), which measures both fat and moisture loss upon cooking; or by the methods of Townsend *et al.* (18) and Morrison *et al.* (19), which measure total weight loss on cooking; or by the method of Helmer and Saffle (20), which measures fat separation upon cooking.

The physical properties of the cooked comminuted sausages could be determined by a trained panel (15,19,21) or, preferably, by objective textural measurement using an Instron or similar instrument.

As mentioned above, the function of proteins in comminuted sausages is multifold, and thus the results of such an evaluation should not be called emulsification properties, but rather "meat emulsion stabilization" properties.

LOW-VISCOSITY FOOD EMULSIONS

Although such food emulsions may contain a number of different ingredients, and fat content may vary from 2 to 25%, a simplified model system containing 10% fat, 1% protein, and 89% water is satisfactory for studying such emulsions.

It is desirable to use piston-type homogenizers to prepare the emulsion, in order to simulate practical conditions as closely as possible. Mixer-type homogenizers may produce too much foaming, and particle-size distribution may not be similar to the normally homogenized samples. Inadequate homogenization will cause creaming in each case, thus differences may not be observable.

The simplest and most frequently used method for measuring emulsion stability consists of centrifuging the emulsion and measuring the percentage of the emulsified layer remaining (22). Such a method is rapid and easy to carry out; however, it has several disadvantages. Creaming is bound to occur in any dilute emulsion if the phases are not exactly equal in density. After such centrifugation, it will be difficult to observe differences in low-fat, low-viscosity emulsions. Also, as two layers are formed during creaming, an oil-rich emulsion on top and an oil-poor emulsion on the bottom, the "emulsified layer" remaining will stay at 100%.

[2]H. Czarnecki, Central Soya Co. Personal communication.

The method of Titus *et al.* (23) and a modification thereof (24) give a more accurate measurement of fat separation in such emulsions. In this method, the change in fat content in the lower portion of an undisturbed emulsion is measured after a given holding time or treatment. If either creaming or coalescence occurs, the fat content in the lower portion of the undisturbed emulsion will be decreased to various extents, and this change is measured. The disadvantage of such a method is that repetitive fat analysis is time consuming.

Keeney and Josephson (25) described the measurement of change in light transmission of diluted emulsions at 540 nm as a means of detecting de-emulsification. The percentage of light transmission is directly proportional to particle size and quantity, and hence an increased percentage of transmission indicates instability. Considerable dilution of the emulsion is required to measure light transmission; however, light reflectance can be measured without such dilution. It has been demonstrated that the change in light reflectance, as measured by the Agtron instrument, can be correlated to emulsion stability.[3] However, such measurement is also affected by the nature of the protein, and thus it is difficult to compare emulsions stabilized with different proteins.

Addition of an oil-soluble red dye to the oil, and measurement of the change in "redness" at a specific wavelength in the lower portion of an undisturbed emulsion, may serve as a rapid, quantitative determination of fat separation.

The above-described methods which measure creaming or coalescence are able to detect only gross differences in emulsion stability. On the other hand, measuring changes in particle-size distribution will reveal slight changes in emulsion stability, even before any visual phase separation occurs. Thus, comparing size distribution and its change with time would give the most accurate estimation of emulsion stability. Groves and Freshwater (26) reviewed the various methods for determining the particle-size distribution of emulsions. They concluded that a combination of Coulter counter and centrifugal photosedimentometer techniques is needed to provide a complete size-distribution measurement, since the Coulter counter is not accurate below 0.5-μ particle diameter, whereas the centrifugal photosedimentometer is limited to particles below about 10 μ in diameter. Matsumoto and Fukushima (27) studied several food emulsions using the centrifugal photosedimentometer technique, and demonstrated that a large number of oil globules are distributed in the submicron range. These have major influences on the properties of the emulsions. However, to evaluate emulsion stability, the use of a Coulter counter to detect change in size distribution may be sufficient in most cases.

HIGH-VISCOSITY, HIGH-FAT EMULSIONS

Since high-fat food emulsions such as mayonnaise and salad dressings usually contain vinegar, it may be desirable to include it in the model system. Such a model system may be made up using 65% salad oil, 10% vinegar, 2% protein, and 23% water. The simplest way to prepare an oil-in-water emulsion with this composition is to add the oil continuously to a stirred protein dispersion. However, this may not be practical for commercial operation, and thus the model emulsion should be prepared as is done in practice, that is, using a

[3]G. Puski, Central Soya Co. Unpublished data.

homogenizer with addition of oil in one step.

The methods described to evaluate the stability of low-fat emulsions can also be used here. In addition, the product should be checked for phase inversion and coalescence with time or treatment. Viscosity or consistency of such emulsions is also important, and should be evaluated.

CONCLUSION

The evaluation of each new food protein product in a diversity of processed food preparations is a costly and time-consuming matter. It has been recognized that there is a serious need for functional-property evaluation methods employing model systems which correlate well with actual food use. Numerous model systems have been proposed, but in many cases there is little correlation with performance in end use. It is easy to criticize previous methods proposed for the measurement of the emulsification properties of proteins, but it is much more difficult to develop better ones. One purpose of this presentation is to encourage wider consideration of the existing problem. Hopefully, with further effort, generally acceptable methods which correlate well with practical applications can be developed. Insofar as emulsifying properties are concerned, it is suggested that the model system method should simulate actual food processing conditions as closely as possible.

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