EINE UNTERSUCHUNG DER EMULGIERENDEN EIGENSCHAFTEN VON DREI NAHRUNGSMITTELSPROTEINEN

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Sowohl die emulgierenden als auch die phasenflächigen Eigenschaften von drei Nahrungsmittelsproteinen, repräsentiert von einem Sojaproteinisolat, einem Molkenproteinkonzentrat (WPC) und einem Natriumkaseinat wurden untersucht um die Eigenschaften dieser Proteine feststellen zu können.

Bei der Untersuchung von der phasenflächigen Spannung wurde eine Apparatur, gebaut nach dem Tropfenvolumenprinzip, benutzt und die Kinetik der Proteinadsorption an der Grenzfläche wurde als unterscheidbare Verlaufsgeschwindigkeitsstufen analysiert.

Bei den Emulgierungsuntersuchungen wurde die Vorbereitung der 40- gewichtsprozentigen Sojabohnenöleemulsionen in einem isothermischen Kreislaufsystem gemacht. Der Kraft- und Energieverbrauch während der Emulgierung wurde gemessen und kontrolliert. Die Eigenschaften der hergestellten Emulsionen wurden mit der Verteilung von den Fettpartikelgrössen und der adsorbierten Proteinmenge per Einheit Phasengrenzfläche gekennzeichnet.

Die Untersuchungsergebnisse der Grenzfläche sowohl die der Emulgierung deuten darauf hin, dass die Grenzfläche hauptsächlich durch die Adsorption von Kaseinmonomeren aus der Masse bedeckt wird, während die Sojaproteine zur Grenzfläche als assoziierte Komplexe wandern, wo sie auseinanderfallen und die Grenzfläche bedecken. Die Molkenproteine geben gewöhnlich einen verhältnismässig dünnen Film um die Fettkügelchen herum, besonders wenn sie in 0.2 M NaCl dispensiert werden.

A STUDY OF THE EMULSIFYING BEHAVIOR OF THREE FOOD PROTEINS

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Both the emulsifying and interfacial properties of three food proteins represented by a soy protein isolate, a whey protein concentrate (WPC), and a sodium caseinate have been studied in order to evaluate the emulsifying characteristics of proteins.

In the interfacial tension studies an apparatus based on the drop volume principle has been used, and the kinetics of protein adsorption at the interface has been analysed in terms of distinguishible rate-determining steps.

In the emulsification studies, preparation of emulsions of 40% soybean oil by weight has been performed in an isothermal, recirculating system. Power and energy consumption during emulsification have been measured and controlled. The emulsions formed were characterized in terms of fat particle size distribution and amount of protein adsorbed per unit area of fat surface.

Both the interfacial and the emulsification studies suggest that the caseinates mainly cover the interface by adsorption of casein monomers from the bulk, whereas the soy proteins migrate to the interface by the associated complex, which disintegrates at the interface in order to cover it. The whey proteins give in general comparatively thin protein films around the fat globules, especially when dispersed in 0.2 M NaCl.

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UNE ETUDE DE LA CARACTERISTIQUE EMULSIFIANTES DE TROIS PROTEINES ALIMENTAIRES

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Les qualités émulsifiantes ainsi que les interfaciales de trois protéines alimentaires représentées d´un isolé de soy protéine, d´un concentré de petit-lait-protéine (WPC) et d´un sodium caséinate ont été étudiées pour évaluer les caractéristiques émulsifiantes de protéines.

En étudiant la tension interfaciale un appareil construit selon le principe du volume de goutte était employé et la cinétique de protéine adsorbée était analysée et précisée en termes distinguants les degrés de la vitesse de l´opération.

En étudiant l´émulsification la préparation des émulsions de l´huile de soy de 40% de poids était exécutée dans un système de circulation isothermique. La consommation de la force et de l´énergie a été mesurée et controlée. Les émulsions formées étaient caractérisées en termes de la distribution de la grandeur des particles de gras et de la quantité de protéine adsorbée par unité de la surface de gras.

Les études interfaciales ainsi que les études d´émulsification indiquent que les caseinates couvrent l´interface principallement par l´adsorption des monomères de caseine de la masse tandis que les protéines de soy se déplacent à l´interface comme des complexes associés qui disintègrent à la surface en la couvrant. Les petit-lait-protéines donnent ordinairement un film relativement mince autour des globules de gras, particulièrement quand elles sont dispersées en 0.2 M NaCl.

ИЗУЧЕНИЕ ЭМУЛЬГИРОВАНИЯ У ТРЕХ ПИЩЕВЫХ БЕЛКОВ

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РЕЗЮМЕ

Изучались свойства эмульгирования и поверхностного напряжения у трех пищевых белков /представляемых одним изолятом сойевого белка, одним концентратом сывороточного белка /WPC/ и одним казеинатом натрия / с целью определения качеств эмульгирования белков.

При изучении поверхностного напряжения употреблялось устройство, основанное на принципе капельного объема а кинетика адсорпции белков к поверхности анализировалась по степеням определения скорости.

При изучении эмульгирования образование эмульсии 40% / по весу / сойевого масла осуществлялось в изотермической замкнутой / рециркуляционной / системе. Потребление эффекта и энергии во время эмульгирования измерялось и регулировалось. Образовавшиеся эмульсии характеризовались по распределению жировых частиц и количества белка, которое адсорбируется на единицу поверхности жира.

Как изучение поверхностного напряжения так и изучение эмульгирования указывает на то, что казеинаты покрывают поверхность гл.образом путем адсорпции мономеров казеина снизу из основной фазы, в то время как сойевые белки мигрируют к поверхности в виде ассоциированного комплекса, который распределяется по поверхности для покрытия ее. Сывороточные белки образуют обычно сравнительно тонкие белковые пленки вокруг жировых шариков, особенно при их дисперсии в С.2 М NaCL.

A STUDY OF THE EMULSIFYING BEHAVIOR OF THREE FOOD PROTEINS

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INTRODUCTION

The addition of non-meat proteins, such as sodium caseinate and soy protein products, to sausages is already in common use. But the influence of these proteins on the physical properties of the sausage and how they act as emulsifiers and gel-forming substances, respectively, is still a subject of concern to meat research workers.

During later year it has been questioned if there really is any emulsification during chopping of the meat and the fat in a bowl chopper. The discussion emerges from the photomicrographs taken by van den Oord (1), which demonstrate that in a sausage mixture the fat is normally present as intact fatty tissue cells. Emulsification during chopping can only take place if some of the cells are broken and the liquid part of the fat is pressed out from the cell tissue. During the subsequent heating of the sausage the membranes surrounding the fat cells are further disrupted and the meat and added proteins coagulate into a gel matrix, where water, solid particles, free fat, emulsified fat and fat cells are entrapped. With a certain exchange of non-meat proteins problems associated with poor fat and water binding of the sausage can be reduced, but to what extent these properties are related to the emulsifying or the gelling capacity of the added protein is a difficult task to elucidate.

In this study the investigations have been restricted to the emulsifying properties of three food proteins in a model system. The emulsions formed have been made up of 40 wt. % soybean oil and 60 wt. % of protein dispersion of 2.5 wt. % protein content. Furthermore, the interfacial behavior of the proteins have been studied giving a possibility to evaluate the emulsifying behavior on a more general basis. Three protein products have been examined namely a soybean protein isolate, a sodium caseinate and a whey protein concentrate. These proteins have then been exchanged with meat protein in a sausage product, and it has been checked whether the evaluated emulsifying characteristics of the proteins wereof any significance for the properties of the sausage.

MATERIALS

Soy protein isolate. A soy protein isolate produced under mild conditions was kindly provided by Central Soya and it is described elsewhere (2). The protein (N x 6.25) content is 96.1% dry weight. Solubility determined according to Hermansson (3) in distilled water and in 0.2 M sodium chloride solution at pH 7, denoted as (0 - 7) and (0.2 - 7), is 97.3% and 74.1%, respectively. For the experiments made with the sausage product, Promine-D (Central Soya) a commercially available sodium soybean proteinate was used.

Caseinate. Spray blend caseinate (DMV, Holland), a commercially available sodium caseinate, was used. The protein (N x 6.43) content is 93.4% (dry weight). Solubility determined as above in (0 - 7) and (0.2 - 7) is 100% in both cases.

Whey protein concentrate (WPC). The production of the ultrafiltered and spraydried WPC is described elsewhere (2). The protein (N x 6.55) content is 69.8% (dry weight). Solubility in (0 - 7) and (0.2 - 7) is 94.1% and 94.9%, respectively.

INTERFACIAL BEHAVIOR

The interfacial tension decay of the three food proteins at the air-water interface at 25°C has been monitored with an apparatus based on the drop volume technique. A full description of the interfacial tension apparatus is given elsewhere (4). The interfacial tension depression of the proteins has been measured as a function of time and at different initial subphase concentrations (2). For description of the procedure used cf. ref. (5).

The concentration dependence of the interfacial tension of the three food proteins can be followed in Figure 1, where the surface pressure $\Pi = \gamma_0 - \gamma$ (γ_0 is the initial interfacial tension) attained after 40 minutes, $\Pi_{40\ minutes}$, is plotted against the initial subphase concentration. At high concentrations (10^{0} - 10^{-1} wt. % of the initial subphase concentration), the surface activity of all the proteins is high and almost equal, whereas at lower concentrations the differences in surface behavior of the proteins become evident. The caseinate (0.2 - 7) system is most effective as a surface active agent and is more or less independent of concentration in the concentration range of 10^{-1} - 10^{-3} wt. %. The opposite behavior is observed for the soy proteins, which gradually lose their surface activity with decreasing subphase concentration. WPC (0 - 7) and caseinate (0 - 7) have a rather similar concentration dependence in this range, and the curves are in between those of the caseinate (0.2 - 7) and the soy proteins. The addition of 0.2 M NaCl to the WPC dispersions raises the surface activity of the WPC not far beyond that of the caseinate (0.2 - 7). The increase in lowering of interfacial tension due to salt addition is also observed for the other two proteins.

The time dependence of the interfacial tension of the three food proteins at different concentrations has been analysed in terms of distinguishable rate-determining steps. The theory on which the kinetic analysis is based is given earlier (5), and for a detailed description of the interfacial behavior of the three food proteins the reader is referred to Tornberg (2). A summarized evaluation of the interfacial behavior of the three food proteins at different ionic strengths is given schematically in Figure 2.

The soy proteins diffuse slowly to the interface in comparison with the other two proteins, which has been interpreted as a higher particle weight of the migrating unit in the case of the soy proteins. This is in accordance with the soy proteins, consisting mainly of the 7S and 11S globulins, having a complex quaternary structure in bulk with a particle weight ranging from 180 - 363000 (6,7). According to Wolf (8) and Koshiyama (9) association and aggregation of the 7S and 11S globulins are favoured at 0.1 - 0.2 M NaCl, which has been illustrated in Figure 2. For the soy proteins diffusion is slower in distilled water than in 0.2 M NaCl solution.

There is a high spreading ability of the already adsorbed soy protein aggregates compared to the other proteins, which is most pronounced when soy protein is dispersed in 0.2 M NaCl solution.

Although the caseinate has a complex quaternary structure like the soy proteins, it has a very different surface behavior. The diffusion step of the molecules to the interface is almost as rapid as that of WPC at concentrations above 10^{-3} wt. %. This can be explained if the migration to the interface of the caseinates is performed mainly by the free casein molecules, which are in equilibrium with those in the submicelles. The results obtained from the work by Creamer and Berry (10) show that the casein micelle subunit (particle weight of approximately 250,000 (11)), which sodium caseinate is supposed to mainly consist of, are in equilibrium with their components caseins. The results obtained indicate that the very flexible, random coil-like casein molecules have simpler kinetics than the other proteins studied, involving diffusion to the interface and direct anchoring of the freely available hydrophobic segments having a small adsorption barrier to overcome. Due to higher electrostatic repulsion between adsorbed species at the interface for caseinate (0 - 7) compared to caseinate (0.2 - 7), the caseinate (0.2 - 7) molecules probably can more densly pack at the interface.

The whey proteins diffuse quickly to the interface, which is in accordance with the whey proteins consisting of mainly small molecules and molecular complexes. The WPC (0 - 7) diffuses somewhat slower than WPC (0.2 - 7) to the interface, a relationship which has also been observed for the other two proteins. WPC (0.2 - 7) covers the interface to a higher degree by diffusion from the bulk phase than WPC (0 - 7), which indicates that WPC (0 - 7) spreads or unfolds more easily at the interface.

EMULSIFYING BEHAVIOR

A quantity of 50 grams of emulsion was emulsified in a valve homogenizer incorporated into a recirculating system as previously described (12). The flow rate of the emulsion through the recirculating system was held constant at 250 ± 25 ml min^{-1}. The system was cooled during the emulsification procedure to keep the temperature of the emulsion at 25 ± 2 during processing. The emulsions formed were stored for 24 hr at 20°C and thereafter characterized in terms of fat particle size distribution (13) and amount of protein adsorbed per unit area of fat surface (mg/m^2) (13). The interfacial area of the fat particles expressed in m^2 fat/ml emulsion is derived from the particle size distribution according to calculations given elsewhere (13).

The amount of protein adsorbed in mg per m^2 fat surface area (protein load) and the percentage protein adsorbed from bulk are plotted against fat surface area in Figures 3 and 4, respectively, for all the protein stabilized emulsions. The number of passes has been held constant at 10 and the surface area increase has been obtained by augmenting the power input, i.e. the pressure drop in the valve homogenizer. The most striking feature to be observed in Figures 3 and 4 is the very different behavior shown by the caseinates as compared to the soy proteins. The emulsions stabilized by the latter have a very high protein load at small surface areas, whereafter it diminishes almost exponentially as the surface area expands. The caseinates, though, give low protein coverage for emulsions of small surface area, but the protein load grows as a function of the surface area to a maximum at about 1.5 m^2/ml emulsion, after which it decreases slightly. The different behavior shown by the two proteins corresponds in Figure 4 to a higher percentage adsorbed by the soy proteins at small surface areas and a lower percentage adsorbed at the larger surface areas as compared to the caseinates. It can also be deduced from Figure 4 that in order to cover the interface with protein at larger surface areas, the caseinates, especially in 0.2 M NaCl, predominantly supply protein from the bulk. The soy proteins, though, to a lesser extent supply protein from the bulk, especially at larger areas than 1.8 m^2/ml, where percentage adsorbed is more or less independent of fat surface area. This indicates that at these large surface areas the newly created interface is mostly covered by spreading or unfolding of already adsorbed soy protein molecules at the interface.

This emulsifying behavior of the two proteins seems to be in accordance with the interfacial behavior suggested from the interfacial tension measurements. The high protein coverage of the soy proteins at small surface areas is in accordance with the soy proteins migrating to the interface in an aggregated form, whereas the low percentage adsorbed for the caseinates at these surface areas support the assumption that casein monomers are adsorbed at the interface. Moreover, the interfacial tension results indicated that the soy proteins spread relatively easily at the interface, especially in 0.2 M NaCl solution, and this is mainly the way the soy proteins cover the enlarged interface during emulsification, as suggested from Figure 4. Percentage caseinate adsorbed is more or less directly proportional to the fat surface area, as seen in Figure 4, which suggests that the larger the fat surface area created the more casein monomers are extracted from the casein subunits. This behavior is especially pronounced, when caseinate is dispersed in 0.2 M NaCl solution.

The WPC (0.2 - 7) stabilized emulsions have the lowest protein load (≈ 2 mg/m^2) at fat surface areas between 1.0 and 2.0 m^2/ml, whereas at larger surface areas the soy protein (0 - 7) stabilized emulsions have as low values as those stabilized with WPC (0.2 - 7). It is interesting to note that an increase in ionic strength to 0.2 M NaCl does not increase the amount of protein adsorbed in the case of the whey proteins. In fact the opposite is observed in contrast to the behavior of the other two proteins.

The emulsifying behavior of the whey proteins is not as easily correlated with the interfacial behavior as that of the other proteins. The interfacial data showed that WPC (0 - 7) spreads relatively easy on the interface in relation to WPC (0.2 - 7), but in Figure 4 the supply of molecules from bulk by WPC (0 - 7) exceeds that of WPC (0.2 - 7). This might be explained if the more frequently occurring unfolding of the WPC (0 - 7) molecules gives rise to an enhanced amount of hydrophobic groups exposed to the bulk, which induces further adsorption from bulk and, hence, multilayer formation.

BEHAVIOR OF ADDED PROTEINS IN A SAUSAGE PRODUCT

Just to check whether the emulsifying properties of the proteins is of any significance in order to improve the fat and water binding properties of a sausage product, a few studies of these properties on a sausage system have been performed, where 40% of the meat protein has been exchanged to one of the studied proteins (14). The protein/water and protein/fat ratio was set constant to 0.2 and 0.3, respectively, and the water, fat and protein content of the sausage mix was 51.3%, 34.2% and 10.3%, respectively. The ingredients were mixed and chopped at low speed in a cutter for 6.5 min. The sausages were cooked at 80°C to a centre temperature of 70°C, and slices of the sausage product were then fried on a frying pan at a temperature of 175 ± 5°C.

Table 1. Weight, moisture and fat losses during frying of a sausage product, where 40% of the meat protein has been exchanged.

Exchanged protein	Weight loss (%)	Fat loss based on fat content (%)	Moisture loss based on water content (%)
No	41.2 ± 4.3	48.4 ± 3.8	61.5 ± 2.8
WPC	36.7 ± 0.8	30.8 ± 0.8	59.5 ± 0.5
Sodium caseinate	26.8 ± 3.4	23.4 ± 3.6	50.0 ± 2.3
Promine-D	34.8 ± 2.4	34.5 ± 2.4	58.5 ± 1.5

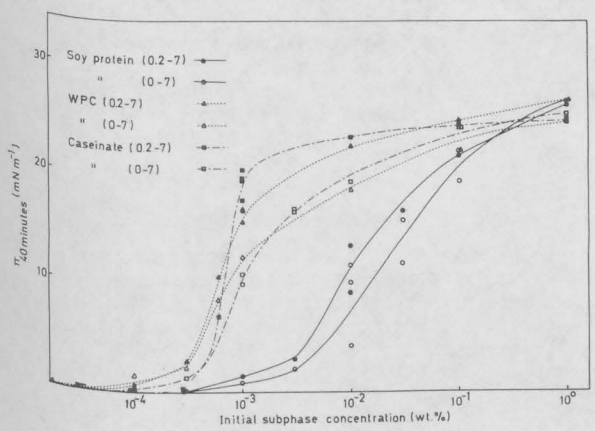

Figure 1. The surface pressure attained after 40 minutes, $\Pi_{40\ minutes}$, as a function of the initial subphase concentration for all the proteins studied at different ionic strengths (2).

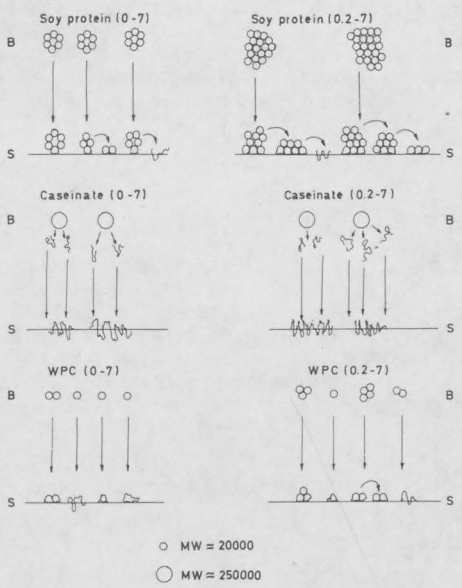

Figure 2. A highly schematic representation of the differing interfacial behavior of the three food proteins at different ionic strengths.

B : in the bulk phase

S : at the interface

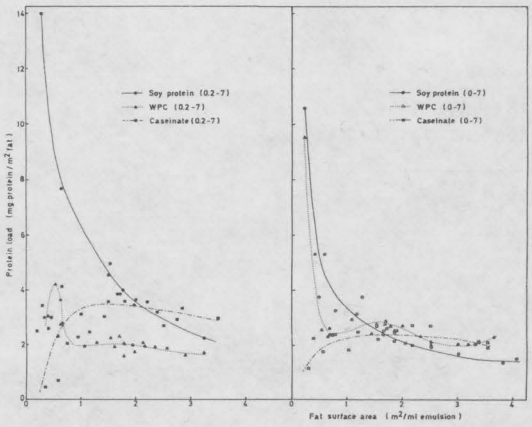

Figure 3. Protein load of the protein stabilized emulsions as a function of the fat surface area during variation of power supply in a valve homogenizer at a constant number of passes of 10 (13).

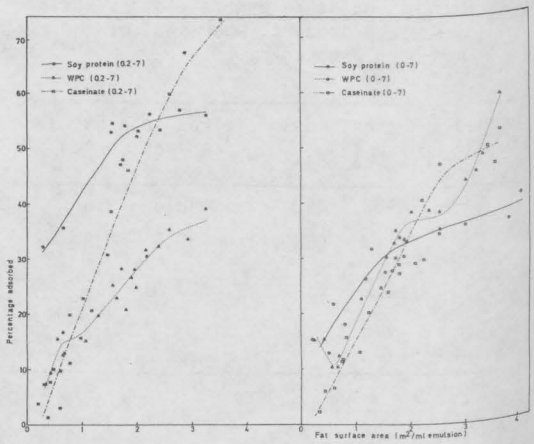

Figure 4. Percentage protein adsorbed from the bulk phase to the interface of the protein stabilized emulsions as a function of the fat surface area created during variation of power supply in a valve homogenizer using a constant number of passes of 10 (13).

The weight, moisture and fat losses (15) during frying were measured and are given in Table 1.

As can be seen in Table 1 the sausage product with no exchange of meat protein is not a good one, which might be due to the relatively high fat content and to the relatively ineffectively performed chopping in the cutter. It can further be seen in the table that the sample where sodium caseinate substitutes the meat protein, the best fried sausage is obtained with regard to both fat and moisture losses. Sodium caseinate does not gel at elevated temperatures and has a high surface activity in salt solutions according to the elucidation of both the interfacial and the emulsifying behavior, which implicate that good emulsifying properties of an exchange protein can be of importance in improving a badly prepared sausage product.

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