Surfactants Used in Food Industry: A Review

Iva Kralova & Johan Sjöblom

Taylor & Francis
Taylor & Francis Group

Surfactants Used in Food Industry: A Review

Iva Kralova and Johan Sjöblom

Ugelstad Laboratory, Department of Chemical Engineering, Norwegian University of Science and Technology (NTNU), Trondheim, Norway

The understanding of the formation, structures, and properties of emulsions is essential to the creation and stabilization of structures in food. The increasing use of surfactants, the identification of compounds with low toxicity and good surface activity properties is of great interest. The relevance of the major end points specified in the Organisation for Economic Co-operation and Development (OECD) guidelines for the hazard assessment of food chemicals is critically analyzed and main parameters are acute toxicity, subacute repeated studies, allergy, reproductive toxicity, long-term studies, and mutagenicity tests. We focus this article on surfactant association structures and food colloids. There is almost infinite number of combinations are organized and arranged in very complex internal microstructures with various types of assemblies such as dispersions, emulsions, foams, gels, etc. Low-mass surfactants are very mobile at the interface and they are particularly efficient reducing the interfacial tension. As a result, they rapidly coat the freshly created oil-water interface during emulsification. In this category, we mainly mentioned monoglycerides, lecithins, glycolipids, fatty alcohols and fatty acids. High-mass surfactants cover protein and polysacharide groups. The protein molecule may interpenetrate in the lipid phase to various degrees. The specific binding is predominantly electrostatic: The headgroups of the surfactants bind to groups of opposite charge on the protein. The saturation binding for anionic surfactants is pH-independent and seems to be controlled by the cooperative hydrophobic interactions. Polysaccharides and smallmolecule surfactants are two of the predominant groups of amphiphilic materials that have been explored for the stabilization of emulsions. One of the most important aspects of polymer-surfactant systems is their ability to control stability and rheology over a wide range of composition. Biocompatible, biodegradable, and/or nontoxic emulsion-based formulations have great potential for applications in the food. The combination of particular characteristics such as emulsifying, anti-adhesive and antimicrobial activities presented by biosurfactants suggests potential application as multipurpose ingredients or additives.

Keywords AGP (polyglycosides), biosurfactants, emulsifiers, emulsions, food application, food colloids, food industry, food processing, food products, food regulations, food safety, glycolipides, hydrocolloids, monoglycerides, proteins, stabilizers, surfactants, toxicity

INTRODUCTION

Surfactants have been used in the food industry for many centuries. Naturally occurring surfactants such a lecithin from egg yolk and various proteins from milk are used for the preparation of many food products such as

mayonnaise, salad creams, dressings, deserts, etc. Later, polar lipids as monoglycerides were introduced as emulsifiers for food products.[1] More recently, synthetic surfactants such sorbitan esters and their ethoxylates and sucrose esters have been used in food emulsions. Hence, the understanding of the formation, structures, and properties of emulsions is essential to the creation and stabilization of structures in food. In addition to the products just mentioned, whole milk and cream are emulsions, as butter, margarine, spreads, mayonnaises and dressings, coffee creamers, cream liqueurs, some fruit drinks and whippable toppings.[2]

Many foods are colloidal systems, containing particles and drops of various kinds. They particles may remain as individual units, but in most cases aggregation takes place to form three-dimensional structures, referred to as "gels." These aggregation structures may be formed particles or by

association of polymers and/or surfactants and are in the most general concept determined by the relative magnitudes of attractive (van der Waals forces) and repulsive forces. The latter can be electrostatic from charged interfaces or steric from adsorbed polymers or from vesicles in the continuous phase, depending on the compositions of the food formulations. The adsorbed surfactants are the cause of the repulsive forces. Ionic surfactants act mainly through eletrostatic repulsion; surfactants with a polymeric polar group give steric repulsion, which may be reinforced by electrostatic effects if the polar group is also charged. Interaction between surfactant and polymer molecule plays important major role in all system and will be briefly reviewed in this article. Interaction between proteins and hydrocolloids is very important especially vital; it leads to understanding interfacial properties and bulk rheology of the system.[3]

There are three main types of emulsion that are important in foods. In oil-in-water (o/w) emulsions, droplets of oil are suspended in an aqueous continuous phase. These are the most versatile of the emulsion types, which exist in many forms (mayonnaises, cream liqueur, creamers, whippable toppings, ice creams mixes) and their properties are controlled both the surfactants used and the components present in water phase. Other type is water-in-oil (w/o) emulsion, which are typified by butter, margarines, and fat-based spreads in general. The stability of these emulsions depends more on the properties of fat or oil and also surfactant used in water phase. The third of the emulsion types is water-in-oil-in-water (w/o/w),[4–6] which is, in effect, an o/w emulsion whose droplets themselves contain water droplets (i.e., are w/o emulsion). For better description, we can divide o/w food emulsions into three groups.[7] The first class (coffee creamers, cream liqueurs) only requires be stable toward creaming and coalescence during their shelf-life. The second class of emulsions[8] contains those which can be used as ingredients that participate in forming the structures of more complex products, that is, other components of food (proteins, polysaccharides) form a matrix in which fat globules are trapped or with which they are interact (yogurts, processed cheese). In the third class of emulsion, the droplets are required to create new structures during processing, such as in ice cream or whipped products, where the emulsion is destabilized and further interacts as a means of creating structure in the products.

TOXICITY OF SURFACTANTS

Because of the increasing use of surfactants, the identification of compounds with low toxicity and good surface activity properties is of great interest. Development of Quantitative Structure-Activity Relationship (QSAR) models provides a possible tool for this search. QSARs relate physicochemical data, such as molecular weight and solubility, to biological responses. The biological data describe properties such as toxicity, pharmacological effects and carcinogenicity. Immunity may be used as bioindicator for environmental toxic substances: Low level concentration of Pb, Cd, nitrosamines, benzopyrene, nicotine, and saturated fatty acids in food lead after application over months to a reduction of cellular and humoral immune response. After challenge by different infectious agents mortality was higher in the experimental group of mice. Until now, investigations with low level doses of toxic agents in food running over a longer time had not been performed[9] The six-step strategy for the construction of a valid QSAR model is based on statistical experimental design and multivariate modelling of the relationships between chemical descriptors and biological responses.[10] The steps are: formulation of classes; characterization of the selected class; selection of compounds to test; biological testing; model development; model validation and prediction of untested compounds.

Chemicals can generally be divided into four major classes according to their mode of toxic action. Class I compounds are relatively unreactive chemicals with a non-specific mode of action, also known as narcosis. Narcosis corresponds to the minimal level of toxicity and is also referred to as baseline toxicity. It is often assumed that neither a specific chemical nor a unique receptor is involved in narcosis. Narcosis due to environmental pollutants in aquatic organisms is, according to van Wezel,[11] defined as a nonspecific reversible disturbance of the functioning of the membrane, caused by accumulation of the pollutants in hydrophobic phases within the organism. More polar chemicals are defined as class II, reactive chemicals as class III and chemicals with a specific mode of action as class IV compounds. Compounds of classes II-IV cause mortality at much lower concentrations than corresponding baseline toxicity compounds.[12] However, this is a rough classification and to refine it, subclasses are required.

The relevance of the major end points specified in the OECD guidelines for the hazard assessment of food chemicals is critically analyzed in the following paragraphs.[13]

Acute Toxicity

A potential food component rules itself out if it is acutely toxic to a considerable extent. Therefore, determination of LD50 (acute dose that is lethal to half of the exposed animals) should not be required as a major end point for a food component. Only range-finding studies (e.g., a one-week multiple dose feeding study in rats) would be necessary to ensure that the ingredient proposed for use in food has a low acute toxicity.

Subacute/Subchronic Repeated Dose Studies

These are important for examining the safety of food components. The substance is added to the feed or drinking

water to imitate exposure to humans. Aspects to be checked are, for example, vitamin and mineral content and their bioavailability to avoid nutrient deficiencies, which could strongly influence the results of the toxicity studies and, thus, lead to erroneous conclusions.

Allergy

Testing for allergic sensitization is highly relevant. However, the commonly used animal models only detect substances that are active on the skin and/or after inhalation. Substances which are highly active in such tests are unsuitable as food components. Some food additives may cause intolerance reactions in certain individuals with symptoms similar to genuine allergic reactions. Therefore, there is a need for studying these end points in the testing of food ingredients. Currently, however, there is no animal model or in vitro test system available that unequivocally reveals intolerance.

Reproductive Toxicity

Reproduction toxicity tests of food components are necessary. They should include male and female fertility and reproduction, multi-generation, and teratogenicity tests.

Long-Term Studies

For food components, long-term studies may not always be necessary. In the guidelines of the Scientific Committee for Food of the European Union, a decision point approach is recommended. Similarly, chronic toxicity and carcinogenicity tests may be unnecessary for peptides, proteins, carbohydrates, and fats which by chemical analytical and metabolism studies can be shown to consist of well-known sequences of amino acids, mono- and disaccharides, and fatty acids.

Mutagenicity Tests

The testing of mutagenicity as an end point is a subject of discussion concerning its relevance to food components. The present state may be summarized as follows. The significance of mutagenicity per se as an end point for food components is not clear and no regulatory agency seems willing to use positive results in mutagenicity tests alone as grounds for nonadmittance of a food component. In addition, the faith in mutagenicity tests as prescreens for carcinogenicity is declining. A positive response does not need to be proof of carcinogenicity.

STANDARDS AND FACTORS FOR FOOD SAFETY

Toxicological standard setting is a process carried out by legally qualified national authorities to protect the public health or the quality of the environment. A toxicological standard for a substance can be defined as a limit value for its content in food, (drinking) water, soil, or air. These toxicological standards are not only based on toxicological knowledge, but are also the result of a thorough risk–benefit analysis. In the process of standard setting, toxicological guide values or health-based recommendations are weighed against technical feasibility and check possibilities, and socio-economical and political interests. For food additives, it was decided a long time ago by the Joint Food and Agriculture Organization of the United Nations (FAO)/Word Health Organization (WHO) Expert Committee on Food Additives (JECFA) that an acceptable daily intake (ADI) should be established that would provide ''an adequate margin of safety to reduce to a minimum any hazard to health in all groups of consumers.'' Crucial in this approach is the establishment of a threshold dose above which any functional or structural disturbance shows itself as a pathological effect of which the intensity increases with increasing exposure (due to both dose and duration). In evaluating the toxicological potential of substances (present in food), it is essential to distinguish between genotoxic substances, for which it is assumed that no thresholds exist, and nongenotoxic substances, which can be evaluated according to the threshold approach.[14]

The threshold dose for the most critical effect in a test is the highest exposure level without adverse, that is, toxicologically relevant, effects. It is called the no-observed-adverse-effect level (NOAEL). For the determination of the NOAEL, a series of doses is used. In order to establish the dose–effect relationship, the dose levels are chosen in such a way that the highest dose causes an adverse effect that is not observed after the lowest dose. Ideally, in a long-term toxicity study, the highest dose should evoke symptoms of toxicity without causing excessive mortality, and the lowest dose should not interfere with development, normal growth, and longevity. In between, doses should be selected sufficiently high to induce minimal toxic effects. The risk assessment is carried out by determining the NOAEL, which is the highest dose in the most sensitive animal species which causes no toxic effects. The NOAEL is then divided by a safety factor to set an ADI level. The ADI is an estimate of the amount of a food additive, expressed on a body weight basis that can be ingested daily over a lifetime without appreciable health risk. Substances that accumulate in the body are not suitable for use as additive. ADIs are only allocated to those additives that are substantially cleared from the body within 24 hours. Safety factors are used to set an ADI that provides an adequate safety margin for the consumer by assuming that man is 10 times more sensitive than the test animal. A further factor of 10 is included which assumes that the variation in sensitivity within the human population is within a 10-fold range. The no-effect level, determined in an appropriate animal study, is traditionally divided by a

safety factor of 100 (i.e., 10×10) to set the ADI. A food additive is considered safe for its intended use if the human intake figure is less than or equivalent to ADI. ADI is usually derived from the results of lifetime studies in animals and therefore relates to lifetime use in man. This provides a sufficient safety margin so that no particular concern is felt if man is exposed to levels higher than the ADI in the short term, provided that the average intake over longer periods does not exceed it.

RESPONSIBLE UNITS FOR STANDARDS

Within the framework of public health legislation, national regulatory authorities are responsible for standard setting with regard to food safety. The authorities can carry out the process of standard setting as a separate national affair, or adopt standards set by international bodies such as the WHO and the European Union. The WHO is an international advisory body with the overall aim of protecting human health. As far as toxicological risk assessment is concerned, it is not a legislative body. It backs national authorities in setting standards for the protection of human health. The International Program on Chemical Safety (IPCS) plays a guiding role in the international procedure of evaluating risks from chemicals and setting tolerances for residues of chemicals in food. Through the IPCS, the WHO participates in two joint committees of the WHO and the FAO. The JECFA and the Joint Meeting on Pesticide Residues (JMPR) serve as scientific advisory bodies of the Codex Alimentarius Commission, a Joint FAO/WHO commission that sets standards for chemicals in food. The JECFA, food contaminants and residues of veterinary drugs. JECFA first convened in 1956 with the mandate to formulate general principles governing the use of food additives and recommend, as far as practicable, suitable uniform methods for the physical, chemical, biochemical, pharmacological, toxicological, and biological examination of food additives and of any degradation products formed during the processing.

For food additives, ADIs or provisional ADIs (when the available information does not warrant a final conclusion) are calculated. This parameter indicates the safe daily dietary intake of a substance. The actual daily dietary intake should not exceed the ADI. Therefore, information on dietary intake is necessary. This can be obtained from market-basket or total diet studies. In the case of major food components and some novel foods (modified starches, polyols, modified celluloses), it is often not necessary to calculate an ADI since the effects observed in toxicity experiments concern the nutritional value. In such cases, no numerical value for the ADI is given (ADI not specified). These products are believed to be acceptable.

The development of new environmental friendly products requires such the products which are biodegradable by bacteria in nature. By enzymatic reaction, a surfactant molecule is ultimately converted into carbon dioxide, water and oxides of the other elements. If the surfactant does not undergo natural biodegradation then it is stable and persists in the environment. For surfactants the rate of biodegradation varies from 1–2 hours for fatty acids, 1–2 days for linear alkyl benzene sulphonates and several months for branched alkyl benzene sulphonates. The rate of biodegradation depends on the surfactant concentration, pH, and temperature. Two criteria are important when testing for biodegradation: (1) primary degradation that results in loss of surface activity; (2) ultimate biodegradation, that is, conversion into carbon dioxide, which can be measured using closed bottle tests. The rate of biodegradation also depends on the surfactant structure. For example, the surfactant must be water soluble. Lipophilic amphiphiles such a fluorocarbon surfactant may accumulate in the lipid compartments of the organism and break down very slowly. A third important factor in biodegradation is the presence of cleavage bonds in the alkyl chain, which depend on branching. Extensive branching of the alkyl chain tends to reduce the rate of biodegradation. This is probably due to steric hindrance preventing close approach of the surfactant molecule into the active site of the enzyme.

SURFACTANT ASSOCIATION STRUCTURES

A large number of surfactants traditionally used in foods are not water soluble and their action on emulsions is more complex than is covered by the common treatment of emulsion stability.[15] At first those of the compounds, which are below the Krafft point at room temperature do not limit their action to adsorbed mono-layers at the interface, but form additional phases to the two aqueous and oil liquids. In addtion the monoglyderides from natural sources have Krafft points well in excess of room temperature and their action as stabilizers of emulsions decisively depend on the temperature dependent phase behavior.

Even those of these surfactants, which have a Krafft point beneath room temperature, are classified as water insoluble as a contrast to ionic surfactants like soaps, which are classified as water soluble, because they form transparent aqueous solutions with concentrations at the level of 30–40%. In fact the solubility in water is much less than indicated; the divergent behavior is in reality based on differences in their self-association in water. The amphiphilic association in water is generally discussed as micellization.[16] As a result of this process, aqueous solutions with surfactant concentrations of up to 40% are transparent low viscosity liquids, which would indicate significant solubility in water. However, in order to understand emulsion stability it is essential to realize that the surfactant molecules are not at all soluble to this extent.
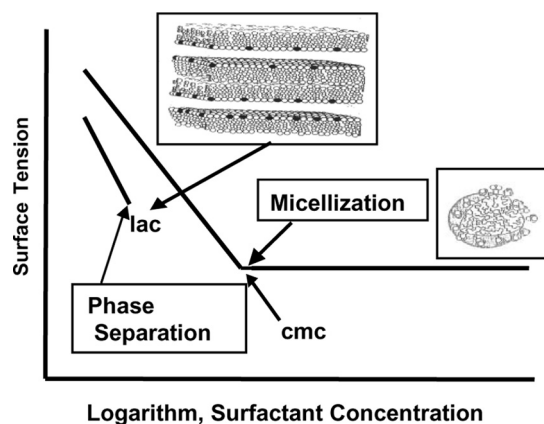
FIG. 1.   Surface tension of aqueous solutions of kinds of surfactants.

FIG. 2.   The possible arrangements for an emulsion with a liquid crystal as the third phase.

The second group of surfactants, the "insoluble" ones, differs from the first group only by the structure of the association. This difference may at first seem purely academic in nature, but is in reality crucial to comprehend food emulsion stability. Hence its consequences are analyzed in some detail. The code to comprehension of the emulsion stabilization is in the phenomena at the critical surfactant concentration, when the self-association in water is initiated (Figure 1). The features in the figure reveal the difference between the two categories of surfactants. For both of them association starts at a critical concentration, but the difference lies with what happens after the association has been initiated. For the water soluble surfactant the association is limited to spherical aggregates, micelles, (lower structure), which form a thermodynamically stable dispersion in water; for example, the system remains a one phase transparent liquid. For the water insoluble surfactant the association structure is a lamellar liquid crystal (top structure, Figure 1) and, since the structure as such does not have size limitation like the micelle, the association continues infinitely and a separate phase appears. At a first glance this distinction may seem mostly of limited scientific interest, but the consequences for emulsion properties are vast and a more detailed analysis was considered justified.

For the water soluble surfactants the adsorption of the surfactant to the interface increases with concentration in the aqueous solution until the critical micellization concentration is reached, at which the surfactant has formed a mono-layer at the interface. From that point the additional surfactant forms micelles.

The second category of surfactant behaves in a completely different manner. It forms a separate phase and the adsorption to the oil/water interface is now not a question of individual molecules; the adsorption is mainly monitored by the three interfacial free energies with four possible organizations of the dispersed structures (Figure 2).
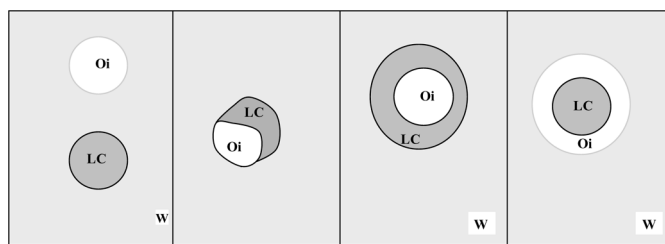
The arrangements (refer to Figure 2) do not depend entirely on the thermodynamic factors. In the most left part of the figure the repulsion between the liquid crystal (soft) particles and the oil drops are sufficient to prevent aggregation (provide colloidal stability) and they exist as separate entities. If flocculation occurs, three arrangements are possible depending on the magnitude of the interfacial free energies, γ(O/W), γ (O/LC) and γ (LC/W). The interfacial energies between the liquid crystal and the liquids is several magnitudes lower than between the two liquids and the arrangement second from the right in Figure 2 is the one expected. This is also what has been found experimentally.[17]

The emulsion now has now increased the number of phases from two to three, and the presence of the third phase has three vital consequences. It radically changes the volume ratios in the emulsion, it gives rise to another structure during emulsification and the temperature variation during and after the emulsification has decisive effect on the properties.

The first two effects are illustrated by a simple example with an emulsion stabilized by a mono-glyceride or similar compound with at least one unsaturated chain or a nonionic surfactant of short polar chain. The general features of a typical emulsion phase diagram are presented in Figure 3. The conditions in such an emulsion have recently been analyzed in detail;[18] in the present contribution the results will be briefly recounted. A realistic emulsion system is assumed, in which the two-phase region reaches to 15% surfactant in the oil phase (real systems vary from 0% to 40%) with negligible water solubilization and that the liquid crystal in equilibrium with the two liquids contains 50% water, 10% oil and 40% surfactant. The emulsion with no surfactant consists of 55% water and 45% oil. The drastic effect of the surfactant association is illustrated by the comparison of the emulsions (Figure 4). In a truly reversible emulsion with no association the only change with increased surfactant fraction would be from O/W to W/O at a surfactant fraction of 0.10 and would remain in this arrangement for additional surfactant added. Under the same condition of reversibility and, hence, the largest phase as the continuous one, the emulsion would change from O/W to LC/O/W at 7.35% surfactant, to O/LC/W
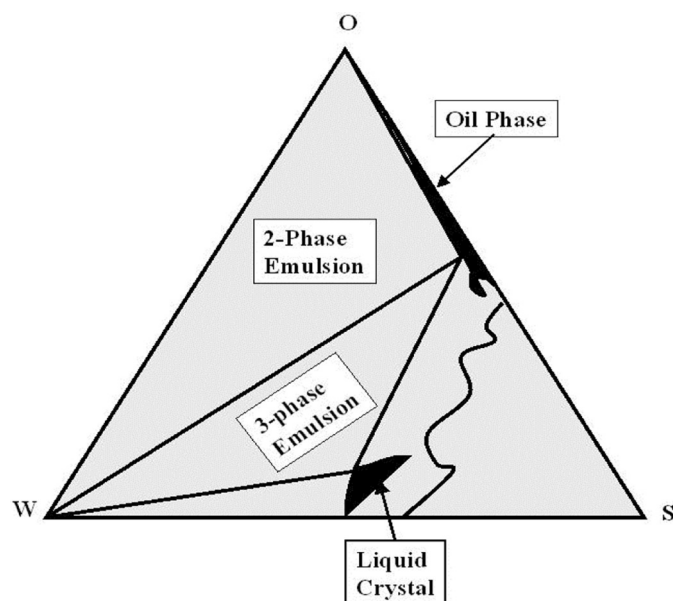
FIG. 3.   The general features of a phase diagram of an emulsion stabilized by lecithin or similar surfactant.

FIG. 5.   The weight fraction of liquid crystal, its vesicles of the dimension in the text, and the remaining aqueous phase.

at 9%, to W/O/LC at 18% and finally to O/LC at 33%. These are rather radical changes and the properties of these emulsions would be a highly interesting fundamental research project. However, the weight variations in Figure 4 are moderate compared to the changes taking place, because the intense emulsification used for food emulsions changes the liquid crystal to vesicles. The vesicles formed are assumed to be of realistic size with a core radius of 0.1 µm and a layer thickness of 0.0045 µm, a molecular.

The weight of the remaining aqueous phase (Figure 5) was calculated as its total weight in the emulsion minus the liquid for the core of the vesicles and for the minimum aqueous phase to disperse the oil drops and the vesicles. The latter was set at a conservative 25% of their weight. The fact that the formation of vesicles results in serious demands on the aqueous phase is obvious from the results.
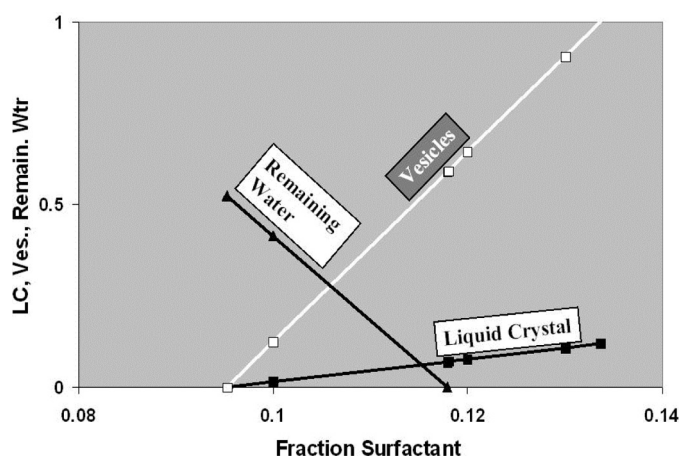
The entire aqueous phase of more than 50% was used for an increase of the surfactant fraction of 2%! The example illustrates the conclusive effect of the association structures of the common food surfactants, whose molecular structure includes at least one unsaturated hydrocarbon chain. For the more common food emulsifiers with saturated chains an additional (and comparably important) determinant is the temperature dependence of the water/emulsifier system. It is the key factor in their performance in a series of products like margarine etc.[19,20] Figure 6 shows partial phase diagram of distilled mono-glycerides.

Applying these conditions to an emulsion, dispersion is found of the lamellar liquid crystal and oil drops in water (Figure 7), analogously to the conditions in Figure 3. Cooling under conditions close to equilibrium would mean that the surfactant crystallizes into β crystals and the layered stabilizing structure at the interface (or the vesicles) would be lost. Fast cooling, contrariwise, retains the layered structure of the lamellar liquid crystal (Figure 7, left), but with reduced transversal mobility of the surfactants in the
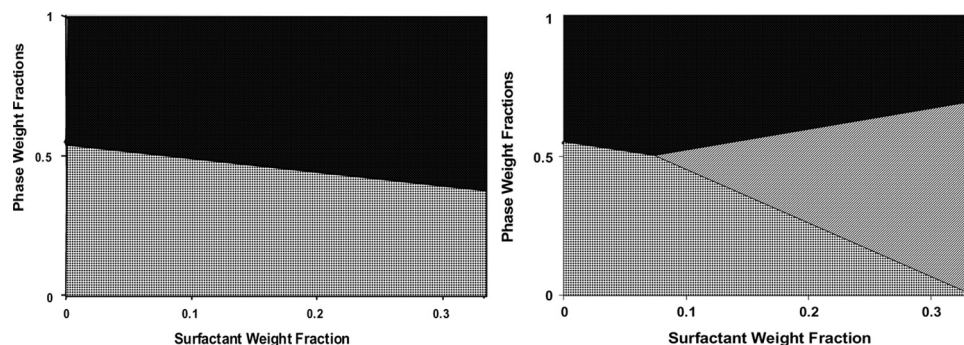

FIG. 4.   (Right) The fractions of the aqueous phase, the oil phase and the liquid crystal versus weight fraction of the surfactant. (The left diagram is added as a comparison to a case for which no phase changes would occur.)
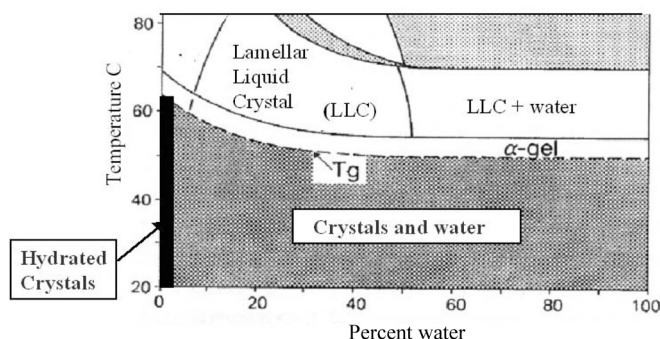
FIG. 6. Partial phase diagram of distilled mono-glycerides, adapted after Krogh.[21]

layers. The L_β phase has become a "gelly" L_β phase with excellent stabilizing action on the emulsion. However, the L_β phase is not thermodynamically stable leading to long-term instability is a difficulty. The problem is alleviated by the addition of compounds, which retard the recrystallization.

There are a large number of liquid crystalline structures according to Larsson[19] and Krogh,[21] but their effects on food emulsions are less direct than that of the lamellar variety.

EMULSIONS AND FOOD COLLOIDS

The most complex colloids and emulsions are those of food and food products, which are difficult to stabilize, because a large number of microstructures of combinations of proteins, carbohydrates, fats and lipids are present. This almost infinite number of combinations are organized and arranged in very complex internal microstructures with various types of assemblies such as dispersions, emulsions,
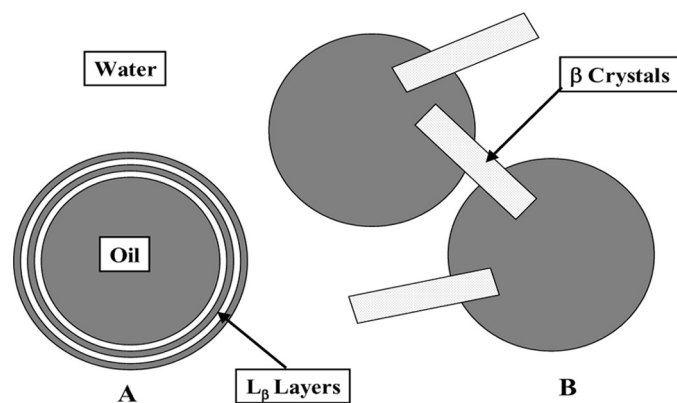

FIG. 7. The L_β layers around emulsion drops (left) are excellent stabilizers, but the slow conversion to the thermodynamically stable state as β crystals (right) results in a loss of stabilization.

foams, gels, etc. In addition, Mother Nature has provided us with many small molecular weight molecules that are known, in general, as food additives (vitamins, antioxidants, acidulants, enzymes, flavors, etc.). The additives have many functional properties and play significant role in food quality and long-term stability.[22]

Amphiphilic molecules, which are part of these additives, play a major role in determining the microstructure of the product and in affecting its structural and textural stability. These molecules are known as dispersing agents, emulsifiers, foamers, stabilizers, etc. (proteins, polysacharides, lipoproteins, glycolipids, polar lipids, or other functional macromolecules.[23] For many technologists, the ability to control the properties and stability of a product, as well as the rheology, texture, foam, crystallization phenomena, is a key factor in the development of better products or bringing new amphiphiles to be used as emulsifiers and stabilizers.[24]

The emulsifier reduces the interfacial tension between the two phases and to some extent reduces the amount of work required to overcome the surface energy in order to disperse the liquids one into the other and also serves to stabilize the final dispersion by preventing flocculation, coalescence, "rupture" and separation into two immiscible phases.[25] Even the simplest and most common emulsifiers have a complex action as illustrated in the preceeding section.

The emulsifying capability of an agent may be classified according to the hydrophile-lipophile balance (HLB) in its molecules. This is defined as the ratio of the weight percentage of hydrophilic groups to the weight percentage of hydrophobic groups in the molecule. HLB values for commercial emulsifying agents range from 1 to 20. Agents with low values, 3–6, promote the formation of w/o emulsions (glycerol esters, propylene glycol fatty acid esters, polyglycerol esters, sorbitol fatty acid esters). On the other hand, those with high values, 8–16, favor to formation of o/w types (proteins, phospholipids, potassium and sodium salts, hydrocolloids, alginates, polyoxyethylene fatty acid esters, CMC, guar gum, etc.).[26] Table 1 shows typical food colloids and their stabilization. These colloids consist of complex blends of monomeric and polymeric materials, hydrocolloids and proteins. The competitive adsorption of the different emulsifiers is based on principle that, with time, the more surface active surfactant replaces the less active material[27] and different amphiphilic association structures develop.

In food industry we can focus on two categories low-molecular mass surfactants (leader groups are fatty alcohols, glycolipids and fatty acids) and macromolecular (high-molecular-mass) emulsifiers extracted from naturally occurring materials, polysaccharides and proteins. The increasing environmental concern about chemical surfactants triggers attention to microbial-derived surface-active

TABLE 1
Typical food colloids and its stabilization (O = oil, A = air, W = aqueous phase)

Food	Type	Method of preparation	Surfactant (stabilizer)	Mechanism of stabilization
Milk	O/W	Natural product	complex of phospholipids, proteins, enzymes, vitamins	Protein membrane
Cream	A+O/W	Centrifugation	Emulsifiers (Mono-, diglycerides)	Protein membrane and particle stabilization of air
Cream liqueurs	O/W	Centrifugation	Emulsifier (Sodium Caseinate)	Protein membrane
Ice cream	A+O/W	Homogenization	Emulsifiers (Mono-, diglycerides), Stabilizers (alginates, carrageenan, gums, gelatine)	Protein membrane and ice network
Butter and margarine	W/O	Churning and in votator	Emulsifier, Stabilizer (mono-, triglycerides, lecithin)	Fat crystal network
Sauces	O/W	High-speed mixing and homogenization	Emulsifier (lecithin) Stabilizer (gum)	Protein and polysaccharide
Mayonnaise	O/W	High-speed mixing	Emulsifier (lecithin)	Polysaccharide
Fabricated meat product	O/W	Low-speed mixing and chopping	Emulsifiers (soluble proteins)	Gelled protein matrix
Bakery products	A+O/W	Mixing	Emulsifiers (Proteins)	Starch and protein network

compounds (biosurfactants) essentially due to their low toxicity and biodegradable nature. Darling and Birkett[28] reported that recent progress in the area of proteins as food emulsifiers is related to a better understanding of the three stages in the stabilization of interfaces by proteins, adsorption, denaturation and coagulation. Each state requires activation energy, which once overcome, results in a successive lowering of interfacial energy. The effect of small molecule surfactants in protein-stabilized dispersions is to decrease the equilibrium surface concentration of protein. The ability of small-molecule surfactants to displace macromolecules from interfaces is related to their higher adsorption energy compared to individual segments of the macromolecule.

LOW-MASS SURFACTANTS

Low molecular weight (LMW) surfactants are very mobile at the interface and they are particularly efficient reducing the interfacial tension. As a result, they rapidly coat the freshly created oil-water interface during emulsification.[29]

MONOGLYCERIDES

Fats and oils are an excellent source for many amphiphilic molecules since they are inexpensive, easy to extract and easy to handle.[30] One of the most common emulsifiers for water-in-oil emulsions is monoglyceride of fatty acids. Hydrogenated oils or natural fats, when transesterified with glycerol, will yield mixtures of mono and diesters of fatty acids. These categories of products are considered

generally recognized as safe (GRAS) and can be used in many food products, cosmetics, and pharmaceuticals without any limitations. The products are hydrophobic in nature, dissolve easily in oils, but swell in water to form lyotropic liquid crystalline structures. Tremendous efforts have been made by many chemists, as well as enzymologists to, in situ, treat fats by fatty acids to form the monoglycerides. These attempts include the use of advanced (coated, activated, encapsulated, etc.) alkaline or acidic reagents and biotechnology methods or enzymes (lipases from different sources treated in various manners) to form alpha-monoglycerides in high yields, high specificity (only alpha-mono-glycerides without any beta-mono modification) and low cost, without the need of an expensive and difficult molecular distillation process.[21]

Lipid fractions (oleoresins), in which the monoglycerides content is somewhat higher (up to 2–3 wt%), have been explored and used for some applications. Many of the oleoresins extracted from fruits, flowers, spices, leaves, etc., consist of various triglycerides, nonsaponifiable fats (waxes) and monoglyceride derivatives. These fractions are sometimes "self-emulsifiable" and can form 'in situ' water-in-oil emulsions. Monoglycerides of saturated fatty acids associated to form lamellar liquid crystalline phases at low concentrations as outlined in the preceding section. These condensed layers form at the oil-water interface at and above the critical temperature T_C, which is the temperature used for emulsification (Figure 8). The new trends in this area are to extract the oleoresins, to remove from it the active matter (lycopene color in tomato, antioxidants
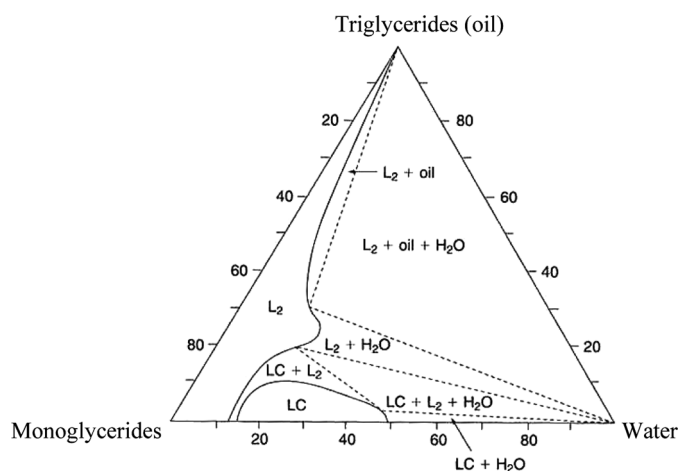
FIG. 8. Ternary phase diagram of soybean oil-sunflower oil monogly-ceride and water.

FIG. 9. The structure of typical products based on lecithins.

such as carnosic acid in rosemary and lutein in Marigold, etc.), to enrich the residual oil phase, by further extraction, with monoglycerides, and to use these fractions as "natural occur-ring self-emulsifying oils" for w/o emulsions.

LECITHINS AND LYSOLECITHINS

Lecithins are essentially hydrophobic molecules, but they find their use in aqueous solutions. Attempts have been made to describe the phase diagrams of lecithins in water, but due to the complexity of their structure and internal composition it was always a difficult task. In gen-eral, one can claim that although lecithins form lamellar liquid crystalline structures in water, it will be difficult to use them as emulsifiers for stabilization of either water-in-oil or oil-in-water emulsions.

Crude oils of all origins always contain small quantities of phospholipids, in addition to triglycerides which are their main components. These are mainly derivatives of phosphonyl-3-glycerol, whose alcohol functions in position 1 and 2 are esterified by two different or identical fatty acids, whereas the phosphoric residue can be esterified either by an amino alcohol or a polyol. These phospholi-pids (PL) are found mainly (around 60%) in cell membranes of living organisms.[31] They have strong amphiphilic characteristics, which explains the sensitivity of the bilayer formed towards parameters such as hydra-tion, salinity, pH, temperature, presence of cholesterol, etc. The intermolecular forces of the amphiphilic molecule, such as the forces of electrostatic origin (quite weak), the Van der Waals interactions, interactions between charged and induced electric dipole, induced dipoles/induced dipole interactions, as well as lipophilic interactions up to hydro-phobic interactions, have been illustrated and quantified.
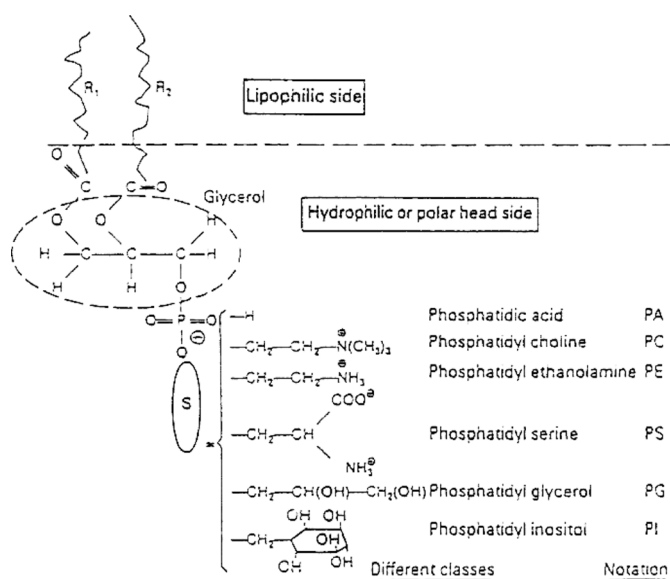
Lecithins consisting of phospholipids and sphingolipds (sphingosine and phytosphingosine) (certain phospholipds that do not have the glycerol ester structure, but have the phosphoric ester of a long carbon chain hydroxy amino base) have been extracted for generations from products such as soya, wheat, oat, eggs, etc. and are, in crude mixture, a very inexpensive materials. There are many manufacturers who treat the crude oily lecithin in various manners and extract products with various degrees of pur-ity and specificity (Figure 9). Products such as plastic lecithin, deoiled lecithin, phosphatidyl choline-enriched lecithin, phosphatidyl choline (PC), phosphatidyl serine (PS), phosphatidyl ethanolamine (PE), phosphatidyl inosi-tol (PI), and phosphatidic acid (PA) of mixture of fatty acids or of any particular fatty acid have been separated and are available on the market.[23]

GLYCOLIPIDS AND SAPONINS

Phospholipids are always accompanied by nonpho-sphorous molecules with amphiphilic characteristics. They are mainly diglyceride ethers with a mono- or disacchar-ides. Ceramides and phytoceramides (amides of fatty acids with sphingosine or phytosphingosine) may be linked with galactose (animals) or with glucose (plants). The com-pounds thus formed are cerebrosides.[31] Recently, investi-gators from different labs have been making attempts to extract molecules such as digalactosyl-diglycerides (DGDG) from cereals (oats, wheat, and soya) and to study the surface properties of these molecules. Many scientists believe that eventually, the advanced separation methods will decrease extraction cost and the DCDG and

trigalactosyl-diglyc-erides (TGDG) will be the future water-in-oil and oil-in-water natural emulsifiers.

The unsaponifable part of a given fat comprises all of its constituents which have very low solubility in water and are mostly soluble in fat solvents. Those products include diverse hydrocarbons, terpenic compounds, fatty alcohols and fat-soluble vitamins. Recent studies have given results which were interpreted as the formation of stable "surface complexes" at various oil-in-water interfaces by saponins combined with other monomeric emulsifiers and thus stabilize the emulsions in an improved manner.[32] These amphiphiles belong to a new category of food products, the nutraceuticals.

FATTY ALCOHOLS

The synthesis of fatty alcohol ethoxylate (AEO) and alkylpolyglucosides (APGs) involves polymerization processes. In the synthesis of AEOs fatty alcohol is reacted with ethylene oxide using a base catalyst and water elimination. APGs are synthesized by direct glucosidation between an excess of alcohol and glucose using an acid catalyst and water elimination.[33] The final surfactant product is of technical grade that is, a complex mixture of surfactants with quite abroad range of molecular weights. To further complicate matters the hydrophobic part of the surfactant product can originate from a mixture of fatty alcohols.[34] The linkage between the glucose units is usually referred to as a glucoside linkage or glucoside bond. In nature, the formation and breakdown of these glucoside bonds are enzymatically controlled by glucosidases.[35] This property makes these surfactants potentially more easily biodegradable. Fatty alcohols are amphiphilic molecules consisting of a hydrophilic headgroup and a hydrophobic tail. The headgroup may be ionic, zwitterionic or polar nonionic and the tail may consist of one or two hydrocarbon chains.[36]

FATTY ACIDS

These compounds are generally classified as saturated, monounsaturated or polyunsaturated, and properties of fats depend on the fatty acids composing them. Within an unsaturated fatty acid molecule, one of two configuration forms can occur around one double bond. The *cis* form has the two parts of the carbon chain bent towards each other, and the *trans* form has the two parts almost linear, similar to saturated fatty acids.[37] In general, fats containing a majority of saturated fatty acids are solid at room temperature, and those containing mostly unsaturated fatty acids are usually liquid at room temperature and are called oils. Some common saturated fatty acids in food include palmitic, stearic and mysristic acids. One common monounsaturated fatty acids is oleic acid, and one most common polyunsaturated fatty acid in food is linoleic acid

(Table 2). Fatty acids are frequently represented by a notation such as C18:2 that indicate that the fatty acid consists of an 18-carbon chain and 2 double bonds. There are several C18:2 linoleic acid variants (known like "conjugated linoleic acid" (CLA)) such as 9,11-CLA and 10,12-CLA which correspond to 9,11-octadecadienoic acid and 10,12-octadecadienoic acid. The principal dietary isomer of CLA is cis-9,trans-11 CLA, also known as rumenic acid. CLA is found naturally in meats, eggs, cheese, milk and yogurt. Most common dietary sources of the trans fat in a typical American diet comes from commercially baked and fried foods that are made with vegetable shortening, some margarine (especially hard margarines) or oils containing partially hydrogenated oils and fats. French fries, donuts, pastries, muffins, croissants, cookies, crackers, chips and other snack foods are high in *trans* fatty acids.

Fatty acid sugar esters are nonionic surfactants with high emulsifying, stabilizing and detergency effect, which are widespread uses as W/O emulsifiers in food products.[38] Selection of proper emulsifier is very important in the manufacture of food additives; the emulsifier must possess suitable functional properties to confer stability against droplet coalescence during the shelf-life and they have to be nontoxic.[39] Sugar fatty acid esters used in ice cream, soup and mayonnaise, are marked as E 473. Fructose esters can be used as antibacterial agents that suppress the cell growth of Streptococcus mutans, causative organism of dental caries. Among the different carbohydrate esters, fructose laurate showed the highest growth inhibitory effect.[40] Therefore, lipase- catalyzed synthesis of carbohydrate esters has potential for developing antibacterial agents applicable to food additives. Sugar fatty acid esters are produced from renewable and inexpensive substances, are completely biodegradable under aerobic and anaerobic conditions, nontoxic, nonskin irritants, odorless, and tasteless.[41,42] Chemical synthesis of sugar fatty acid esters is generally performed as a high temperature esterification in the presence of an alkaline catalyst, which is accompanied by high energy consumption, browning of products and low selectivity toward the various hydroxyl groups in sugars.[43] Some of the chemically synthesized fatty acid sugar esters are also toxic and/or not readily biodegradable.[42,44] Lipase-catalyzed synthesis of fatty acid fructose esters was performed in a mainly solid-phase system consisting of insoluble fructose, fatty acid and product in a small amount of organic solvent-adjuvant (2-methyl 2-butanol, t-butanol, acetone and ethyl methylketon), which is maintaining a catalytic phase for the action of the lipase. Sabeder et al.[45] proved that the highest conversion of 82% after 72 hours of reaction was achieved in ethyl methylketon, which is biocompatible for the production of food additives. The highest conversion for the synthesis of fructose palmitate in 2-methyl

TABLE 2
Common fatty acids in food market

Chemical names and descriptions of some common fatty acids

Common name	Carbon atoms	Double bonds	Scientific name	Sources
Butyric acid	4	0	butanoic acid	butterfat
Caproic acid	6	0	hexanoic acid	butterfat
Caprylic acid	8	0	octanoic acid	coconut oil
Capric acid	10	0	decanoic acid	coconut oil
Lauric acid	12	0	dodecanoic acid	coconut oil
Myristic acid	14	0	tetradecanoic acid	palm kernel oil
Palmitic acid	16	0	hexadecanoic acid	palm oil
Palmitoleic acid	16	1	9-hexadecenoic acid	animal fats
Stearic acid	18	0	octadecanoic acid	animal fats
Oleic acid	18	1	9-octadecenoic acid	olive oil
Ricinoleic acid	18	1	12-hydroxy-9-octadecenoic acid	castor oil
Vaccenic acid	18	1	11-octadecenoic acid	butterfat
Linoleic acid	18	2	9,12-octadecadienoic acid	Grape seed oil
Alpha-linolenic acid (ALA)	18	3	9,12,15-octadecatrienoic acid	flaxseed (linseed) oil
Gamma-linolenic acid (GLA)	18	3	6,9,12-octadecatrienoic acid	borage oil
Arachidic acid	20	0	eicosanoic acid	peanut oil, fish oil
Gadoleic acid	20	1	9-eicosenoic acid	fish oil
Arachidonic acid (AA)	20	4	5,8,11,14-eicosatetraenoic acid	liver fats
EPA	20	5	5,8,11,14,17-eicosapentaenoic acid	fish oil
Behenic acid	22	0	docosanoic acid	rapeseed oil
Erucic acid	22	1	13-docosenoic acid	rapeseed oil
DHA	22	6	4,7,10,13,16,19-docosahexaenoic acid	fish oil
Lignoceric acid	24	0	tetracosanoic acid	small amounts in most fats

2-butanol (78%), was achieved under optimized reaction conditions: 10% (w/w of substrates) of Novozym 435, 12.1% (w/w) of molecular sieves, 60°C and stirring rate of 600 rpm.

Saturated Fatty Acids

Based on international dietary recommendations, generic benchmarks were developed to evaluate foods and beverages on their content of trans fatty acids, saturated fatty acids, sodium and sugars. In principle, the developed generic benchmarks can be applied globally for any food and beverage product. The whole Unilever global foods and beverages portfolio has been evaluated and actions have been taken to improve the nutritional quality. The advantages of this method over other initiatives to assess the nutritional quality of foods are that it is based on the latest nutritional scientific insights and its global applicability.[46,47] It has been suggested that milk fat, due to its content of saturated fatty acids, may have a thrombogenic effect.

A method using gas chromatography/electron ionization-mass spectrometry (GC/EI-MS) in the selected ion monitoring (SIM) mode was developed for the analysis of fatty acids as methyl esters (FAMEs) in order to determine their percentage contribution to the fatty acid profile in food.[48]

Unsaturated Fatty Acids

Monounsaturated fatty acids have only one unsaturated (double) bond. Monounsaturated oils are liquid at room temperature but start to solidify at refrigerator temperatures. For example, salad dressing containing olive oil turns cloudy when refrigerated but is clear at room temperature. Monounsaturated fatty acids can help decrease LDL cholesterol when substituted for saturated fats in the diet. Monounsaturated fatty acids are found in canola, olive, and peanut oils, avocados and nuts. Other group of unsaturated fatty acids is polyunsaturated fatty acids having more than one unsaturated (double) bond. Polyunsaturated fatty acids can help lower LDL cholesterol when

substituted for saturated fats in the diet. They are found in safflower, sesame, sunflower, corn and soybean oils, fatty fish (salmon, mackerel, smelt, herring and trout), and some nuts (walnuts) and seeds.[49] Most naturally occurring dietary unsaturated fatty acids in vegetable oils or polyunsaturated fatty acids of fish oils are of the *cis* configuration.[50] Some of the unsaturated fatty acids ingested by ruminants are partially hydrogenated by bacteria in the rumen. In consequence, milk, fat, dairy products, and beef and mutton fat also contain *cis* and *trans* fatty acid isomers, although the proportions are somewhat different. Structured lipids (SL) containing n-3 highly unsaturated fatty acids (n-3 HUFA) have been produced with immobilized sn-1,3 specific and nonspecific lipases as biocatalysts. HUFA such as eicosapentaenoic (EPA, 20:5 n-3), docosahexaenoic (DHA, 22:6 n-3), linolenic (18:3 n-3), and g-linolenic (GLA, 18:3 n-6) acids are important in foods, nutrition, and pharmaceutical applications. For the most part, the position of the HUFA in the glycerol moiety is the key to their functionality in foods and absorption when consumed.[51] These designer lipids may replace conventional fats and oils in certain specialty applications because of their structure-health (nutraceutical or medical lipids) and structure-function (functional lipids) attributes. In most cases insertion of the desired HUFA at the sn-2 position will provide max. nutritional benefits.

Trans Fatty Acids

Trans fatty acids (trans fats) are a specific type of fat formed when liquid oils are made into solid fats like shortening and hard margarine.[52] This is a process known as "partial hydrogenation." Trans fats are also found naturally in small amounts in certain foods (e.g., dairy products, beef, and lamb). Also, small amounts of trans fats are formed during the refining of liquid vegetable oils (e.g., canola and soybean oil). Trans fats raise total and LDL cholesterol and lower HDL cholesterol. They are found in foods made with or fried in partially hydrogenated oils. Hydrogenation heightens the melting point of fats, which makes it possible to convert fats in liquid form to semi-solids and solids that are useful in many dietary products, increasing shelf life and the flavor stability of unsaturated fatty acids. Through hydrogenation, oils such as soybean, safflower, and cottonseed oil, which are rich in unsaturated fatty acids, are converted to margarines and vegetable shortening.[53] Thus, trans fatty acids are produced artificially and commercially today. They are present in variable amounts in a wide range of foods, including most foods made with partially hydrogenated oils such as baked goods and fried foods, and some margarine products.

The production and use, and in particular, the separation, synthesis and recovery of polar lipid-rich fractions containing eicosapentaenoic acid (EPA), docosahexaenoic

acid (DHA), docosapentaenoic acid (DPA(n-3) or DPA(n-6)), arachidonic acid (ARA), and eicosatetraenoic acid (C20:4 n-3) from microorganisms, genetically modified seeds and marine organisms (including fish and squid) and their use in human food, animal feed, pharmaceutical and cosmetic applications is described.[54]

The companies have produced a family of novel carriers enabling water solubilization of highly lipophilic molecules. The compound carriers were synthesized by conjugating polyethylene glycol to alpha-tocopherol, tocotrienols, beta-sitosterol or cholesterol via an alkanedioyl linker.[55] These PEG- conjugates were amphiphilic and formed comicelles with a wide range of molecules including vitamins, carotenoids, ubiquinones, poly-unsaturated fatty acids and polyene macrolide antibiotics. The resulting formulations were water-soluble, nontoxic and had excellent stability. Long-chain polyunsaturated fatty acids (LCPUFA) are primarily referred to eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) in the omega-3 series and arachidonic acid (AA) in the omega-6 series.[56] The beneficial health effects of omega-3 fatty acids are related to their protection against cardiovascular disease, autoimmune disorders, diabetes, inflammation, arthritis and arrhythmia. These omega acids belongs to group called "essential fatty acids."

These fatty acids use the Greek alphabet (α, β, γ, ..., ω) to identify the location of the double bonds. The "omega" is the last carbon of the chain because omega is the last letter of the Greek alphabet. Linoleic acid is an omega-6 fatty acid because it has a double bond six carbons away from the "omega" carbon. Linoleic acid plays an important role in lowering cholesterol levels. Alpha-linolenic acid is an omega-3 fatty acid because it has a double bond three carbons away from the "omega" carbon. For arachidonic acid, we subtract 14 from 20 to obtain 6; therefore, it is an omega-6 fatty acid. This type of terminology is sometimes applied to oleic acid which is an omega-9 fatty acid. Figure 10 shows these structures and double bond location.

DHA (docosahexaenoic acid) and AA (arachidonic acid) are both crucial to the optimal development of the brain and eyes. The importance of DHA and AA in infant nutrition is well established, and both substances are routinely added to infant formulas. Excessive amounts of omega-6 polyunsaturated fatty acids and a very high omega-6/omega-3 ratio have been linked with
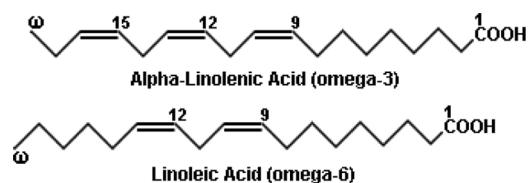

FIG. 10. Types of essential fatty acids.

pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases. The ratio of omega-6 to omega-3 in modern diets is approximately 15:1, whereas ratios of 2:1 to 4:1 have been associated with reduced mortality from cardiovascular disease, suppressed inflammation in patients with rheumatoid arthritis, and decreased risk of breast cancer. Food sources of the two main dietary polyunsaturated fatty acids (linoleic acid and alpha-linolenic acid). Linoleic acid (Omega 6 family) is extracted from vegetables, fruits, nuts, grains, seeds. As good sources are accounted oils made from: safflower, sunflower, corn, soya, evening primrose, pumpkin, wheatgerm. alpha-linolenic acid (omega 3 family) is acid which main source is fish. But there are other sources of this acid as flaxseed oil (contains twice as much as is found in fish oil), mustard seeds, pumpkin seeds, soya bean, walnut oil, green leafy vegetables, grains, spirulina.

In clinical studies, *trans* fatty acids found in hydrogenated fats and partially hydrogenated cooking oils tend to raise total blood cholesterol and LDL ("bad") cholesterol levels and lower HDL ("good") cholesterol levels when used instead of *cis* fatty acids or natural oils. These changes tend to increase the risk of heart disease and stroke. Strong epidemiological evidence also indicates *trans* fatty acid intake is associated with an increased risk of coronary heart disease. The Food and Drug Administration (FDA) passed a regulation requiring *trans* fat to be listed on the nutrition label. The American Heart Association's Nutrition Committee strongly advises that healthy Americans over age 2 limit their intake of saturated fat and *trans* fat to no more than 10% of total calories. Saturated fat intake should be less than 7% if you have coronary heart disease, high LDL cholesterol or diabetes. Individuals should adjust total fat intake to meet their caloric needs. Finally, it is recommended eat a healthy diet low in saturated fat, *trans* fat, and cholesterol, and low in sodium. Good choices include lean meats, fish, skinless poultry, low-fat or nonfat dairy products, fruits, vegetables and whole-grain products.

HIGH-MASS SURFACTANTS

Proteins as Surfactants

The protein may adopt a folded or unfolded conformation at the oil/water interface. In addition, the protein molecule may interpenetrate in the lipid phase to various degrees.[57] It is a linear chain of amino acids that assumes a three-dimensional shape dictated by the primary sequence of the amino acids in the chain. The side chains of the amino acids play an important role in directing the way in which the protein folds in solution. The hydrophobic (nonpolar) side chains avoid interaction with water, while the hydrophilic (polar) side chains inside and the hydrophilic side chains outside.[58] The final shape of protein (helix, planar or "random coil") is a product of many interactions, which form a delicate balance.

Protein structure is described in terms of four levels.[59] The primary structure is the amino acid sequence and the location of any disulfide bridges. The secondary structure refers to regular local structure of linear segments of 10 polypeptide chains, for example, the α-helix and the β-sheet. These regular structures are cooperative in nature. In globular proteins, the α-helix consists of 10–15 residues that are held together by hydrogen bonds formed between backbone carbonyl oxygens and backbone amide hydrogens four residues ahead in the amino acid sequence. β-sheets consist of regularly folded β-strands (3–10 residues in globular proteins), which are not stable by themselves but are stabilized by hydrogen bonding in the backbone. The secondary structure elements fold into structural units, called domains, which comprise the tertiary structure. The folding of the secondary structure elements into the tertiary structure is cooperative.[60] The tertiary structure is maintained by four types of interaction between side chain groups of amino acid residues: (1) hydrogen bonding, (2) ionic interactions between oppositely charged groups (salt bridges), (3) hydrophobic interactions, and (4) disulfide cross-linkages; these covalent links are much stronger than the noncovalent interactions (1–3). Disulfide bridges are said to increase the stability of the native state by reducing the number of unfolded conformations: The greater the number of unfolded conformations of a protein, the higher the entropic cost of folding that protein into its single native state.[61] Quarternary structure refers to the spatial arrangement of the subunits.

Many proteins, such as caseins, whey proteins (lactoglobulins, lysozymes, ovalbumins) bovine and human serum albumins, gelatins, etc. are known for many decades as emulsifiers and enormous amount of research, has been carried out to clarify the microstructures of emulsions, like milk, ice cream, and dairy products. Protein stabilized emulsions can provide both types of stabilization, although full coverage of the interface is required. If the surface is poorly covered, destabilization by bridging flocculation can occur. With protein films, the presence of interfacial shear viscosity will tend to retard film thinning. Therefore, attention was paid to the viscoelastic properties of adsorbed protein films at the oil-water interface. Many food systems are composed of a combination of monomeric and macromolecular emulsifiers. Efforts have been made to evaluate the contribution of each emulsifier at the interface. Typical example of native proteins is lysozyme, an enzyme that dissolves certain bacteria by cleaving the polysaccharide component of their cell walls. Lysozyme has a helical content of 30%, and the content of β-sheet is 10%. The tertiary structure consists of two domains,

TABLE 3
Amino-acid side-group in proteins used for chemical modification[63]

Group	Modification
Amino (Lys)	Acylation, alkylation
Carbonyl (Asp, Glu)	Esterification, amide formation
Disulphide (cystine)	Reduction
Sulphydryl (Cys)	
Thioether (Met)	Alkylation; oxidation
Imidazole (His)	
Indole (Trp)	
Phenolic (Phe)	Acylation
Guanidino (Arg)	Condensation by dicarbonyls

TABLE 4
Changes in functional properties of some chemically phosphorylated proteins

Protein	Number of phosph. Groups (mol/mol protein)	Changes in functional properties
BLG	14	Increased creaming stability, viscosity
Casein	7	Increased viscosity, water absorption, decreased emulsifying capacity
Lysozyme	6	Increased water absorption
Soy protein isolate	Not available	Increased solubility, emulsifying capacity, foaming properties
Yeast protein	4	Increased heat stability, emulsifying activity, foam stability

separated by a cleft that comprises the active site.[62] Lysozyme is an unusually stable protein: its thermal stability is high and characterized by a transition temperature of $77°C$ at neutral pH and dilute salt, and it is known to form dimers in alkaline solution.

Since many of the proteins are too hydrophobic, or too hydrophilic, it was essential to slightly modify them chemically or enzymatically in order to render them more surface active. Table 3 summarizes some of the possible chemical reactions. The most widely studied derivatization is acylation of the e-amino-group of lysine. The acylation affects surface, thus, it leads to improved emulsion stability. In a similar manner succinylation was carried out on serum albumin, on yeast and soy proteins and excellent results were observed. Heat-coagulated whey protein was succinylated (on the lysyl residues) to obtain enhanced water and fat adsorption together with emulsification properties, etc.[64] Such modified proteins, especially those with an iso-electric point in the pH range of 9 to 10, are potentially useful for formulating new food products, since there is the possibility of forming electrostatically stable complexes between anionic native proteins and protein derivatives with a net positive charge. The phosphorylation of yeast proteins leads to enhanced emulsifying and foaming properties by increasing the net negative charge on the protein and by formation of thicker and better covered layers on the oil droplets (Table 4). Some of these chemical modifications led to products that are significantly better than the native proteins and we can see them in the market, being offered by many companies (modified soy proteins, egg proteins, whey proteins). Lactoglobulins, lysozymes, ovalbumins, bovine and human serum albumins are common examples of native emulsifiers used in dairy products like milk, ice cream, and so on.

Enzymatic reactions have been carried out on certain native proteins in order to improve their surface activity. Most reactions are aimed at introducing hydrophobic substituents. Proteins that form micelles, namely caseins, are major protein fraction in bovine milk (about 80% of the total milk protein). Several components may be identified, namely α_S-casein, β-casein, and χ-casein.[65] Large spherical casein micelles are formed by association of these types in presence of free phosphate and calcium ions. Molecules are held together by electrostatic and hydrophobic interactions. The α_S- and β-caseins are surrounded by the flexible hydrophilic χ-casein, which forms the surface layer of micelle by electrostatic repulsion. The micelle diameter varies between 50 and 300 nm. Koczo et al.[66] suggested a new stability mechanism for food emulsions resulting from the layering of sodium caseinate submicelles in thin liquid films. They found that films thinned stepwise by stratification. The heights of the film step-transitions were in the same range as the effective size of the casein submicelles (about 20 nm). This showed that microlayering of submicelles took place in the stratifying film and a layer left the film via step-transitions. This new mechanism of microlayering in emulsion films could play an important role in the stability of food emulsions. Xu et al.[67] tested the effects of surfactants, protein, and fat substitutes on the fat particle structure and stability of food emulsions. They claimed two conclusions from the experimental results: (1) Oil-soluble or water-soluble surfactant-stabilized food emulsions are very unstable under shear stress; a very poor fat particle structure is developed after the shear; (2) protein plays a very important role in the stability of food emulsions by the formation of an adsorption layer on the surface of fat particles and microlayering of protein submicelles around fat particles. It is found that by varying the

protein concentration and ratio between protein and surfactants, the fat particle packing structure and stability of food emulsions can be controlled.

Proteins and polar lipids coexist in biological systems, mainly unassociated with each other, but also as composite structures with specific actions. They have a very important physical property in common, an inherent amphiphilic nature, which provides the driving force for formation of association structure of lipids as well as stabilizing some food colloids. Proteins have a strong affinity both to polar lipids and to aromatic surfactants. The interactions with nonionic surfactants are very limited. Proteins also strongly interact with water-insoluble polar lipids (electrostatically dependent interaction). Guo et al.[68] have proposed different models to describe the saturated protein-SDS complex: (1) the "rod-like particle model," which was proposed on the basis of viscosimetric measurements, describes the complex as a rigid rod with a cross-sectional radius of about 18 Å and a length proportional to the protein molecular weight; (2) the "flexible helix model," which is a theoretical model that describes the complex as a flexible cylindrical micelle formed by the SDS molecules, on the surface of which hydrophilic segments of the protein are bound; and (3) the "necklace model," which is based on results from the free-boundary electrophoresis technique and proposes an unfolded protein with SDS micelle-like clusters bound to it. This model is based on CD measurements that indicate the structure of the protein of protein-SDS complexes is constituted of the α-helix and the random coil. Lundahl and co-workers[69] concluded on the basis of a small-angle netron scattering (SANS) study that protein decorated, spherical micelles are formed, rather than a cylindrical structure as proposed earlier. Other SANS studies,[70] as well as viscometry[71] is also compatible with the necklace model. Turro et al.[72] in a study combining fluorescence, electron spin resonance (ESR) and NMR, concluded that the unfolded protein wraps around the micelles.

Protein–Surfactant Interaction

The specific binding is predominantly electrostatic: the headgroups of the surfactants bind to groups of opposite charge on the protein. A change in pH will cause a change in the net-charge of the protein and consequently in the binding. In general, if the pH is lowered, the anionic binding isotherm is shifted to a lower surfactant concentration, the cationic binding isotherm to a higher concentration.[73] Interfacial composition and competitive adsorptions have been discussed by many researchers and is illustrated in Figure 11. However, for specific binding to occur, the hydrocarbon chain length seems to be important: in a study of the binding of sodium n-alkyl sulfates to lysozyme (at pH 3.2) it was found that both sodium decyl and dodecyl sulfates show specific binding,
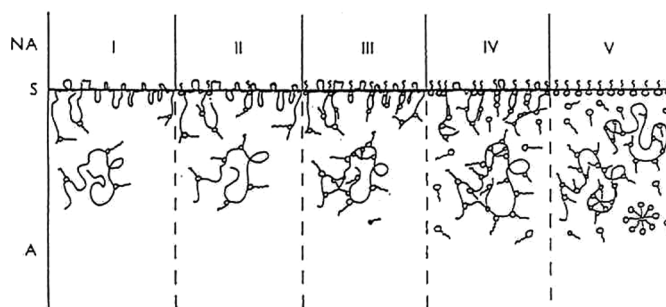

FIG. 11. Protein-surfactant binding and competitive adsorption as a function of increasing surfactant concentration.

but not sodium octyl sulfate, which seems to interact cooperatively only with lysozyme.

Similar results were found for the binding of n-alkyl trimethylammonium bromides to BSA: decyltrimethyl ammonium bromide does not exhibit a sharp transition from the specific binding to the cooperative binding region, in contrast to the surfactants with longer n-alkyl chains. BSA showed the specific binding region for sodium dodecyl, decyl and alsofor octyl sulfates. It should be mentioned that BSA might be unusual in this respect, since it is capable of binding low numbers (3–10) of anionic amphiphiles with very high affinity. The number of specific binding sites was found to increase with hydrocarbon chain length for the interaction between BSA and homologous series of n-alkyl sulfates and sulfonates. Furthermore,[74] the onset of specific binding occurs at a lower surfactant concentration with increasing chain length. Cooperative binding means that the binding affinity increases as more surfactant is bound.

Piazza et al.[75] provides phenomenological observations of changing interactions between neighboring polymer molecules in protein/polysaccharides/lipids foaming systems that were isolated from the roasted ground coffee. This study proved that the relative strength of the two-dimensional physical network at the air-water interface is mainly dependent on the interaction between protein-like macromolecules and lipids. In the case of real food system, like in the case of the espresso coffee beverage, due to the complexity of the system, the foaming mechanisms can be described by appropriate mathematical descriptions of the surface viscoelastic phenomena, containing the terms of transport of surfactant biopolymers to the interface and describing the coagulation of particles taking place here. The structure and the rheological properties of the interface can affect many aspects of the physical properties of foam systems.[76,77] This is the principle reason why interfacial characteristics, such as tensiometry and interfacial rheology, are receiving a growing interest when dealing with foam systems.[78,79] If the flow properties, texture and even sensory properties

of a foam or emulsion could be controlled by simply manipulating the interfacial rheological properties of the solution, this would provide manufacturers with a powerful processing tool.

The saturation binding for anionic surfactants is pH-independent and seems to be controlled by the cooperative hydrophobic interactions. Precipitation of the protein-anionic surfactant complex at pH values below the isoelectric point at low surfactant concentration is common and has been reported for the lysozyme-SDS system (at pH 3.2),[80] as well as for the BSA-SDS system (at pH 4.3). Below its isoelectric point a protein carries a net positive charge.

POLYSACHARIDES (HYDROCOLLOIDS)

Polysaccharides and smallmolecule surfactants are two of the predominant groups of amphiphilic materials that have been explored for the stabilization of emulsions.[81] The polysaccharides that have been commonly employed are alginate, carrageenan, chitosan, pectin, rhamsan, xanthan, dextran, carboxymethylcellulose, hydroxypropyl methylcellulose, and scleroglucan, while the small molecule surfactants most commonly used are sodium dodecyl sulfate (SDS), mono- and diglycerides, sorbitan esters, and phospholipids.[82,83] To be a good emulsifier, then, a macromolecular species should have the capacity to adsorb rapidly at the nascent oil-water interface created during emulsification and so protect the newly formed fine droplets against recoalescence. On the other hand, the role of a good stabilizer is to keep droplets apart in the emulsion once it has been formed during long-term storage. Polysaccharides make good stabilizing agents because of their hydrophilicity, high molecular weight and oil-water interfaces.[84] The gelation behavior which leads to the formation most studied hydrocolloid as an emulsifier is caused by a gum of macromolecular barriers in the aqueous medium. Polysaccharides are rigid, water-soluble, and consist of at least three different fractions colloids. Therefore, they are not considered as classical emulsifiers. The most studied hydrocolloid as an emulsifier is gum Arabic that is widely used in citrus drinks.[85] The active fraction in gum Arabic strongly supports the concept that was postulated by Dickinson with regard to the activity of the hydrocolloids.[86] Guar and fenugreek gums[87] have been isolated and purified to the extend that most of the proteineous matter was removed and the adsorption isotherms indicated some adsorption. Emulsions prepared with purified guar or fenugreek gums are quite stable even in the very dilute form and strong birefringency was detected on the oil-water interface indicating formation of thick oriented layer. In some cases competitive adsorption takes place and eventually the protein will displace the gum from the surface. The hydrocolloid will serve as a stabilizer, or as a protective colloid. However, there are many studies from which it can be clearly derived that under proper heat and humidity conditions the gums can interact with the via the Maillard reaction in situ, to form a new compound composed of hydrophilic and hydrophobic moieties that will be able to better adsorb on the oil-water interfaces.[88]

Polysacharide–Surfactant Interaction

Due to starch polysacharides central role in many food-related applications, most of the research on starch surfactant interactions has involved the use of food-grade emulsifiers such a long chain (C_{14}-C_{18}) monoglycerides and esters of sucrose. One of the most important aspects of polymer-surfactant systems is their ability to control stability and rheology over a wide range of composition.[89] Surfactant molecules that bind to a polymer chain generally do so in clusters that closely resemble the micelles formed in the absence of polymer. If the polymer is less polar or contains hydrophobic regions or sites, there is an intimate contact between the micelles and polymer chain. In such situation, contact between one surfactant micelle and two polymer segments will be favorable. The cross-linking of two or more polymer chains can lead to network formation and dramatic rheological effects.[90] For nonionic surfactants, there is a little to gain in forming micelles in the presence of a polymer and, hence, the interaction between nonionic surfactants and polymers is relatively weak. Grant et al.[91] examine sorbitan esters and chitosan as a small molecule surfactant-biopolymer combination for preparation of stable emulsions. Chitosan is mixed with sorbitan esters to form a surfactant-biopolymer complex that produces stable emulsion and cream formulations. Combinations of chitosan and three sorbitan esters were evaluated in order to determine the influence of the chemical structure of the sorbitan ester on the physicochemical and rheological properties of the emulsion. The length and degree of saturation of the surfactant hydrocarbon chains and chemical architecture have a significant impact on the development of chitosan-surfactant complexes. The chitosan-sorbitan monooleate cream may be used for the development of stable emulsions for applications in the food delivery industries.

Sorbitan esters, commonly referred to as Span or Tween, are nonionic surfactants that are formed from mixtures of partial esters of sorbitol and anhydrides in addition to fatty acids. The wide range of sorbitan esters that are commonly employed as surfactants in various industries differ in terms of their chemical (i.e., composition, structure), physical (i.e., hydrophile-lipophile balance (HLB), color, and state at room temperature), and functional properties (i.e., critical micelle concentration (CMC), density, viscosity).[92,93] Chitosan is a natural polysaccharide that can also be obtained synthetically by the deacetylation of chitin to produce poly- (1,4-â-D-glucopyranose)

molecules. The properties of chitosan such as molecular weight and degree of deacetylation may be varied to suit formulation design.[94] Chitosan is GRAS listed for use in food preparations.[95] The biocompatibility and toxicity of the chitosan-surfactant cream is currently being evaluated in vitro and in vivo.

BIOSURFACTANTS

The term biosurfactant has been used very loosely and refers to any usable and isolated compound obtained from microorganisms that has some influence on interfaces. Rhamnolipids from *Pseudomonas aeruginosa*, surfactin from *Bacillus subtilis*, emulsan from *Acinetobacter calcoaceticus* and sophorolipids from *Candida bombicola* are some examples of microbial-derived surfactants. Originally, biosurfactants attracted attention as hydrocarbons dissolution agents, but the interest in these molecules have been increasing considerably in the past five decades as alternative to chemical surfactants (carboxylates, sulphonates and sulphate acid esters) specially in food, pharmaceutical and oil industry.[96] Microbial surfactants are categorized by their chemical composition and microbial origin. Rosenberg and Ron[97] suggested that biosurfactants can be divided into low-molecular-mass molecules, which efficiently lower surface and interfacial tension, and high-molecular-mass polymers, which are more effective as emulsion stabilizing agents. The major classes of low-mass surfactants include glycolipids, lipopeptides, and phospholipids, whereas high mass includes polymeric and particulate surfactants. Most biosurfactants are either anionic or neutral and the hydrophobic moiety is based on long-chain fatty acids or fatty acids derivatives whereas the hydrophilic portion can be a carbohydrate, aminoacid, phosphate or cyclic peptide. Table 5 shows the major biosurfactant classes and the microorganisms involved.

Biosurfactants have special advantages over their chemically manufactured counterparts because of their lower toxicity,[99] biodegradable nature,[100] effectiveness at extreme temperatures, pH, salinity, and the biosynthesis. Certain hydrocarbon-degrading bacteria and yeast produce appreciable amounts of phospholipids and fatty acids when grown on n-alkanes. These surfactants are very interesting from a scientific point of view and some of them exhibit very unique properties such as formation of microemulsion. But, since the production cost is still very high in comparison to plant (soya) phospholipids it is hard to see when these products will become commercial. Table 6 lists only the important biotechnology products used in the food industry. Some of them are purified end-products of fermentation, others are chemically modified. Most work on biosurfactants applications has been focusing on bioremediation of pollutants;[102] however, these microbial compounds exhibit a variety of useful properties for the

TABLE 5
Major types of microbial surfactants[97,98]

Surfactant class	Microorganism
Glycolipids	
Rhamnolipids	*Pseudomonas aeruginosa*
Trehalose lipids	*Rhodococcus erithropolis, Arthobacter sp.*
Sophorolipids	*Candida bombicola, Candida apicola*
Mannosylerythritol lipids	*Candida antartica*
Lipopeptides	
Surfactin/iturin/fengycin	*Bacillus subtilis*
Viscosin	*Pseudomonas fluorescens*
Lichenysin	*Bacillus licheniformis*
Serrawettin	*Serratia marcescens*
Phospholipids	
	Acinetobacter sp., Corynebacterium lepus
Fatty acids/neutral lipids	
Corynomicolic acids	*Corynebacterium insidibasseosum*
Polymeric surfactants	
Emulsan	*Acinetobacter calcoaceticus*
Alasan	*Acinetobacter radioresistens*
Liposan	*Candida lipolytica*
Lipomanan	*Candida tropicalis*
Particulate biosurfactants	
Vesicles	*Acinetobacter calcoaceticus*
Whole microbial cells	*Cyanobacteria*

food industry especially as emulsifiers, foaming, wetting, solubilizers,[96] antiadhesive, and antimicrobial agents.[103]

The main distinctive features of microbial surfactants that can be of interest for food processing are related to their surface activity (good surfactant can lower surface tension (ST) of water from 72 to 35 mN/m and the interfacial tension (IT) water/hexadecane from 40 to 1 mN/m);[102] tolerance to pH, temperature and ionic strength; biodegradability (easily degraded[100] and particularly suited for environmental applications such as bioremediation;[98] antimicrobial activity; emulsifying and demulsifying ability and low toxicity. When comparing the toxicity of six biosurfactants, four synthetic surfactants, and two commercial dispersants. Poremba et al.[104] found that most biosurfactants were degraded faster, except for a synthetic sucrose-stearate that showed structure homology to glycolipids and was degraded more rapidly than the biogenic glycolipids (rhamnolipids, trehalose lipids, sophorose lipids). These authors also reported that biosurfactants showed higher EC_{50} (effective concentration to decrease 50% of test population) values than synthetic dispersants.

TABLE 6
Biotechnology products associated with food production and preparation[101]

Product	Uses
Organic acids, their salts and derivatives	pH control agents, acidulants, preservatives, flavouring agents, flavor enhancers, adjuvants, color stabilizers gelling enhancers, melt modifiers, turbidity reducers, etc.
Mono/oligosacchardies	Sweeteners for diet and health food
Polysaccharides	Thickeners, water-binding agents, gellants, foaming agents, rheology modifiers, nutritive supplements.
Amino acids, peptides	Constituents of protein hydrolyzates for flavoring, anti-microbial agents (nisin, bacteriocins), monosodium glutamate as taste enhancer
Proteins	Single-cell proteins (SCP) as food and feed additives
Enzymes	Microbial rennets, meat tenderizers, flour modifying proteases, beer stabilizers/ clarifiers, amylases, glycoamylases and pullulanases for starch hydrolysis, glycose isomerase for fructose and high-fructose syrup production, pectin-degrading enzymes (fruit juice production), lipases as inter-esterification catalysts (e.g., in food surfactant production), glucose oxidase as oxygen scavenger, invertase for confectionery products
Lipids and derivatives	Speciality fats and oils, emulsifying and de-emulsifying agents, lubricants, die releasing aids, wetting agents, fat-blooming preventers, etc.
Other substances of interest in food production	B-group vitamins, L-ascorbic acid, special flavors (vanilla, fruit, mushroom, mint, onion, etc.), coloring agents, taste enhancers (5∞-nucleotides)

Apart from their obvious role as agents that decrease surface and interfacial tension, in bakery and ice cream formulations biosurfactants act by controlling consistency, retarding staling and solubilizing flavor oils; they are also utilized as fat stabilizer and antispattering agent during cooking of oil and fats.[105] An improvement of dough stability, texture, volume and conservation of bakery products was obtained by the addition of rhamnolipid surfactants.[106] The authors also suggested the use of rhamnolipids to improve properties of butter cream, croissants and frozen confectionery products. L-Rhamnose has a considerable potential as precursor for flavorings. It is already used industrially as precursor of high-quality flavor components like Furaneol (trademark of Firmenich SA, Geneva). L-Rhamnose is obtained by hydrolyzing rhamnolipid surfactants produced by P. aeruginosa.[107]

Their other potencial application in food industry is like antiadhesive agents which could be used against forming bacterial biofilms onto food surfaces. These biofilms are potencial sources of contamination which may lead to food spoilage and disease transmission.[108] Due to the fact that food processors have a zero tolerance levels for pathogens like Salmonella and also (in most countries) for Listeria monocytogenes, a single adherent cell may be as significant as a well-developed biofilm; thus controlling the adherence of microorganisms to food contact surfaces is an essential step in providing safe and quality products to consumers. The bioconditioning of surfaces through the use of microbial surfactants have been suggested as a new strategy to reduce adhesion. Pretreatment of silicone rubber with S. thermophilus surfactant inhibited by 85% the adhesion of Candida albicans,[109] whereas surfactants from Lactobacillus fermentum and Lactobacillus acidophilus adsorbed on glass, reduced by 77% the number of adhering uropathogenic cells of Enterococcus faecalis.[110] The use of biosurfactants released by Lactobacilli strains is very promising once these microorganisms are naturally present in human flora and have also a probiotic effect.[103] The use of biosurfactants, which disrupts biofilms and reduce adhesion, in combination with antibiotics could represent a novel antimicrobial strategy. Antibiotics are in general less effective against biofilms than planktonic cells; the disruption of biofilm by biosurfactant can facilitate the antibiotic access to the cells. An interesting work regarding the use of biosurfactants to inhibit the adhesion of the pathogen L. monocytogenes in two types of surfaces classically used in food industry has been conducted by the group of Meylheuc et al.[111] The preconditioning of stainless steel and polytetrafluoroethylene (PTFE) surfaces with a biosurfactant obtained from Pseudomonas fluorescens inhibits the adhesion of L. monocytogenes L028 strain. A significant reduction (>90%) was attained in microbial adhesion levels in stainless steel whereas no significant effect was observed in PTFE. Further work demonstrated that the prior adsorption of P. fluorescens surfactant in stainless steel also favored the bactericidal effect of disinfectants.[112] Considering the interesting properties demonstrated by biosurfactants

we can think on their future utilization as multipurpose ingredients, which exhibit emulsifier, antiadhesive, and antimicrobial activities simultaneously and thus, suitable for many food applications.

BIOSURFACTANT PRODUCTION FROM FOOD AND WASTE

Another interesting approach for food industries is to take advantage of their byproducts or residues as substrates for biosurfactant production. The main alternative sources for biosurfactant production comprise oily residues, milk and distillery wastes, and carbohydrate rich residues. Most oils and fats are used in the food industry, which generates great quantities of wastes and so, their disposal is a growing problem. *Candida antarctica* and *Candida apicola* synthesized surfactants (glycolipids) in a cultivation medium supplemented with oil refinery waste, either with soapstock (5–12% v/v) or post-refinery fatty acids (2–5% v/v). The efficiency of glycolipids synthesis was increased from 7.3 to 13.4 g/L and from 6.6 to 10.5 g/L in the medium supplemented with soapstock and post-refinery fatty acids.[113] Equally Nitschke et al.[114] evaluated edible oil soapstocks as alternative low-cost substrates for the production of rhamnolipids by *P. aeruginosa* LBI strain. Wastes obtained from soybean, cottonseed, babassu, palm, and corn oil refinery were also tested with result that vegetable oils and residues from vegetable oil refinery are among the most used low-cost substrates for rhamnolipids production.

Dubey et al.[115] reported biosurfactant production from synthetic medium and industrial wastes such as distillery and whey by sludge isolate *P. aeruginosa* BS2. The wastes were good substrates for growth and proliferation of bacteria and biosurfactant production in distillery and whey wastes reached maximal amounts of 0.9 and 0.92 g/L, respectively, after 96 hours of incubation. Rodrigues et al.[116] performed a screening for *Lactobacillus* strains able to produce surfactants. The acid lactic bacteria *Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus pentosus,* and *Lactobacillus coryniformis torquens* were selected as surfactant- producing organisms with *L. pentosus* and been considered the most promising strain and whey as a potential alternative substrate.

Nitschke et al.[109] reported new potential usage of Cassava wastewater as useful for surfactant biosynthesis. This is a carbohydrate-rich residue generated at large amounts during the processing of cassava flour. This residue proved to be an appropriate substrate for biosurfactant biosynthesis, providing not only bacterial growth and product accumulation but also useful properties for many industrial applications.

Molasses is a byproduct of the sugar industry that is low in price compared to other conventional sugar sources like sucrose or glucose and is rich in other nutrients such as minerals and vitamins.[118] Molasses and corn steep liquor were used as the primary carbon and nitrogen sources for production of rhamnolipid biosurfactants by *P. aeruginosa* GS3; the interfacial tension of culture medium against crude oil was reduced from 21 to 0.47 mN/m.[119]

CONCLUSIONS

Biocompatible, biodegradable, and/or nontoxic emulsion-based formulations have great potential for applications in the food.

Biosurfactants show several properties that could be useful in many fields of food industry; recently their antiadhesive activity has attracted attention as a new tool to inhibit and disrupt the biofilms formed in food contact surfaces. The combination of particular characteristics such as emulsifying, antiadhesive, and antimicrobial activities presented by biosurfactants suggests potential application as multipurpose ingredients or additives. Scant information regarding toxicity, combined with high production costs seems to be the major cause for the limited uses of biosurfactants in food area. However, the use of agroindustrial wastes can reduce the biosurfactants production costs as well as the waste treatment expenses, and also render a new alternative for food and food-related industries not only for valorizing their wastes but also to becoming microbial surfactant producers.

REFERENCES

[1] Xu, W., Nikolov, A., Wasan, D.T., Gonsalves, A., and Borwankar, R.P. (1998) *J. Food Sci.*, 63(2): 183–188.

[2] Larsson, K. and Friberg, E. (1990) In *Food Emulsions*, edited by S.E. Friberg, K. Larsson, and J. Sjoblom, 2nd edition; New York: Marcel Dekker.

[3] Tadros, T.F. (2005) *Applied Surfactants, Principles and Application*; Weinheim, Germany: Wiley-VCH.

[4] Tadros, T.F., Dederen, C., and Talman, M.C. (1995) *Cosmet. Toiletries*, 112: 75–86.

[5] Benichou, A., Aserin, A., and Garti, N. (2004) *Adv. Colloid Interface Sci.*, 108–109: 29–41.

[6] Morais, J.M., Santos, O.D.H., Nunes, J.R.L., Zanatta, C.F., and Rocha-Filho, P.A. (2008) *J. Dispersion Sci. Technol.*, 29: 63–69.

[7] Abe, M. and Scamehorn, J.F. (2005) *Mixed Surfactant Systems,* 2nd edition; New York: Marcel Dekker.

[8] Dickinson, E. and Euston, S.R. (1992) *Adv. Colloid Interface Sci.*, 42: 89–148.

[9] Stickl, H.A. (1991) *International Journal of Hygiene and Environmental Medicine*, 191(2–3): 232–240.

[10] Jonsson, J., Eriksson, L., Sjöström, M., and Wold, S. (1989) *Chemom. Intell. Lab. Syst.*, 5: 169–186.

[11] van Wezel, A.P. and Opperhuizen, A. (1995) *Toxicology*, 25: 255–279.

[12] Verhaar, H.J.M., van Leeuwen, C.J., and Hermens, J.L.M. (1992) *Chemosphere*, 25: 471–491.

[13] Feron, V.J. (1997) In *Food Safety and Toxicity*, edited by J. de Vries; New York: CRC Press.

[14] van Leeuwen, F.X.R. (1997) In *Food Safety and Toxicity*, edited by J. de Vries; New York: CRC Press.

[15] Binks, B.P. (1998) *Modern Aspects of Emulsion Science*; Cambridge, UK: Royal Society of Chemistry.

[16] Laughlin, R.G. (1994) *The Aqueous Phase Behavior of Surfactants*; San Diego, CA: Academic Press.

[17] Friberg, S.E. (1992) *Langmuir*, 8: 8–14.

[18] Friberg, S.E. (2006) *Colloids Surfaces A*, 282–283: 369–376.

[19] Larsson, K. (2004) In *Food Emulsions*, edited by S.E. Friberg, K. Larsson, and J. Sjöblom; New York: Marcel Dekker; pp. 93–106.

[20] Larsson, K., Quinn, P., Sato, K., and Tibergl, F. (2006) *Lipids: Structure, Physical Properties and Functionality*; Bridgewater, UK: The Oily Press.

[21] Krogh, N.J. and Sparto, F.V. (2004) In *Food Emulsions*; New York: Marcel Dekker.

[22] Robins, M.M. and Wilde, P.J. (2003) In *Encyclopedia of Food Science and Nutrition,* 2nd edition; London: Academic Press.

[23] Garti, N. (1999) *Colloids and Surfaces, A*, 152: 125–146.

[24] Sjöblom, J. (1996) *Emulsions and Emulsion Stability*; New York: Marcel Dekker.

[25] Brennan, J.G. (2006) In *Food Processing Handbook*; Weinheim, Germany: Wiley-VCH.

[26] Dickinson, E. and McClemens, D.J. (1996) *Advances in Food Colloids*; Glasgow: Blackie.

[27] Hasenhuettl, G.L. and Hartel, R.W. (1997) *Food Emulsifiers and Their Applications*; New York: Chapman and Hall.

[28] Darling, D.F. and Birkett, R.J. (1987) In *Food and Foams*, edited by E. Dickinson; London: Royal Society of Chemistry.

[29] Wilde, P.J. (1996) *Current Opinion in Colloid and Interface Science*, 5: 176–181.

[30] Hamilton, R.J. (1995) *Developments in Oils and Fats*; London: Blackie Academic and Professional.

[31] Karleskind, A. (1996) *Oils and Fats Manual*; Hampshire, UK: Intercept.

[32] Mitra, S. and Dungan, S.R. (2000) *Colloids and Surfaces B*, 17: 117–133.

[33] Holmberg, K. (2003) *Novel Surfactants*; New York: Marcel Dekker.

[34] van Os, N.M. (1998) *Nonionic Surfactants*; New York: Marcel Dekker.

[35] Stryer, L. (1981) *Biochemistry*; New York: W. H. Freeman and Company.

[36] Israelachvili, J.N. (1992) *Intermolecular and Surface Forces*; London: Academic Press.

[37] Institute of Food Science & Technology: Trans Fatty Acids, 1999: http://www.ifst.org/hottop9.htm

[38] Yan, Y., Bornscheuer, U.T., Cao, L., and Schmid, R.D. (1999) *Enzyme Microb. Technol.*, 25: 725–728.

[39] Partal, P., Guerrero, A., Berjano, M., and Gallegos, C. (1999) *J. Food Eng.*, 41: 33–41.

[40] Watanabe, T., Katayama, S., Matsubara, M., Honda, Y., and Kuwahara, M. (2000) *Current Microbiology*, 41: 210–213.

[41] Degn, P., Pedersen, L.H., Duus, J., and Zimmermann, W. (1999) *Biotechnol. Lett.*, 21: 275–280.

[42] Tarahomjoo, S. and Alemzadeh, I. (2003) *Enzyme Microb. Technol.*, 33: 33–37.

[43] Yan, Y., Bornscheuer, U.T., Stadler, G., Lutz-Wahl, S., Reuss, M., and Schmid, R.D. (2001) *J. Am. Oil Chem. Soc.*, 78(2): 147–152.

[44] Cao, L., Fischer, A., Bornscheuer, U.T., and Schmid, R.D. (1997) *Biocatal. Biotransform.*, 14: 269–283.

[45] Sabeder, S., Habulin, M., and Knez, Z. (2006) *J. Food Eng.*, 77: 880–886.

[46] Nijman, C.A.J., Zijp, I.M., Sierksma, A., Roodenburg, A.J.C., Leenen, R., van den Kerkhoff, C., Weststrate, J.A., and Meijer, G.W. (2007) *European Journal of Clinical Nutrition*, 61(4): 461–471.

[47] Tholstrup, T., Marckmann, P., Hermansen, J., Holmer, G., and Sandstrom, B. (1999) *Br. J. Nutr.*, 82(2): 105–113.

[48] Saskia, T. and Walter, V. (2005) *J. Agric. Food. Chem.*, 53(23).

[49] Morgado, N., Gallequillos, A., Sanhuueza, J., Garrido, A., and Nieto, S. (1998) *Lipids*, 33: 669–673.

[50] IFIC review (1998): http://ificinfo.health.org/review/ir-fat.htm

[51] Akoh, C.C. (2001) *ACS Symp. Ser.*, 788.

[52] Innis, S.M., Green, T.J., and Halsey, T.K. (1999) *J. Am. Coll. Nutr.*, 18.

[53] Federal register (1999), 64(221), 62749: http://www.fda.gov/OHRMS/DOCKETS/98fr/111799c.pdf

[54] Bernard, K., Wulf, B., and Ruben, A.J. (2002) *PCT Int. Appl.*

[55] Borowy-Borowski, H., Sodja, C., Docherty, J., Walker, P.R., and Sikorska, M. (2004) *Journal of Drug Targeting*, 12(7): 415–424.

[56] Finley, J.W. and Shahidi, F. (2007). *ACS Symposium Series*, 788.

[57] Nnanna, I.A. and Xia, J. (2001) *Protein-Based Surfactants*; New York: Marcel Dekker.

[58] Nonius, D. and Miller, R. (1999) *Proteins and Liquid Interfaces*; Amsterdam: Elsevier Science.

[59] Creighton, T.E. (1993) *Proteins Structures and Molecular Properties*; New York: W. H. Freeman and Company.

[60] Lehninger, A. (1982) *Principles of Biochemistry*; New York: Worth Publishers, Inc.

[61] Branden, C. and Tooze, J. (1999) *Introduction to Protein Structure*; New York: Garland Publishing Inc.

[62] Fukushima, K., Murata, Y., Nishikido, N., Sugihara, G., and Tanaka, M. (1981) *Bull. Chem. Soc. Jpn.*, 54: 3122–3127.

[63] Kinsella, J.E. and Shetty, K.J. (1979) *ACS Symp. Ser.*, 92.

[64] Ibel, K., May, R.P., Kirschner, K., Szadkowski, H., Mascher, E., and Lundahl, P. (1990) *Eur. J. Biochem.*, 190: 311–318.

[65] Dickinson, E., Golding, M., and Povey, M. (1997) *J. Colloid Interface Sci.*, 185: 515–529.

[66] Koczo, K., Wasan, A.D., Wasan, D.T., Borwankar, R.P., and Gonsalves, A. (1996) *J. Colloid Interface Sci.*, 178: 694–702.

[67] Xu, W., Nikolov, A., and Darsh, T.W. (2005) *J. Food Eng.*, 66: 107–116.

[68] Guo, H. and Chen, S.H. (1990) *Chem. Phys.*, 149: 129–139.

[69] Ibel, K., May, R.P., Kirschner, K., Szadkowski, H., Mascher, E., and Lundahl, P. (1990) *Eur. J. Biochem.*, 190: 311–318.

[70] Guo, X.H., Zhao, N.M., Chen, S.H., and Teixeira, J. (1990) *Biopolymers*, 29: 335–346.

[71] Shinagawa, S., Kameyama, K., and Takagi, T. (1993) *BBA*, 1161: 79–84.

[72] Turro, N.J., Lei, X.G., Ananthapadmanabhan, K.P., and Aronson, M. (1995) *Langmuir*, 11: 2525–2533.

[73] Sarmiento, F., Ruso, J.M., Prieto, G., and Mosquera, V. (1998) *Langmuir*, 14: 5725–5729.

[74] Jones, M.N. and Manley, P. (1984) *Surfactants in Solution*; London: Plenum.

[75] Piazza, L., Gigli, A., and Bulbarello, A. (2008) *J. Food Eng.*, 84: 420–429.

[76] Ispen, R. and Otte, J. (2004) *Nordic Rheology Society*, 21: 143–178.

[77] Langevin, D. (2000) *Adv. Colloid Interface Sci.*, 88: 209–222.

[78] Murray, B.S. (2002) *Curr. Opin. Colloid Interface Sci.*, 7: 426–431.

[79] Wilde, P. (2000) *Curr. Opin. Colloid Interface Sci.*, 5: 176–181.

[80] Wiliams, P.A., Philips, G.O., and Randall, R.C. (1990) In *Gums and Stabilizers for the Food Industry*, edited by G.O. Philips, P.A. Wiliams, and D.J. Wedlock; Cambridge, UK: IRL Press.

[81] Sjöblom, J. (1992) *Emulsion-A Fundamental and Practical Approach*; Boston: Kluwer Academic Publishers.

[82] Mollett, H. and Grubenmann, A. (2001) *Formulation Technology: Emulsions, Suspensions, Solid Forms*; Cambridge: Wiley-VCH.

[83] Bais, D., Trevisan, A., Lapasin, R., Partal, P., and Gallegos, C.J. (2005) *Colloid. Interface Sci.*, 290: 546–556.

[84] Laplante, S., Turgeon, S.L., and Paquin, P. (2005) *Carbohyd. Polymers*, 59: 425–434.

[85] Dickinson, E., Calazka, V.B., and Anderson, D.M.W. (1990) In *Gums and Stabilizers for the Food Industry*, edited by G.O. Philips, P.A. Wiliams, D.J. Wedlock; Cambridge, UK: IRL Press.

[86] Garti, N., Madar, Z., Aserin, A., and Sternheim, B. (1997) *Food Sci. Technic.*, 30: 305.

[87] Matsudomi, N., Inoue, Y., Nakashima, H., and Kato, A. (1995) *J. Food Sci.*, 60: 265.

[88] Dickenson, E. and Waltra, P. (1993) In *Food Colloids and Polymers*; Cambridge, UK: Royal Society of Chemistry.

[89] Goddard, E.D. and Ananthapad-Manqabhan, K.P. (1992) *Polymer-Surfactant Interaction*; Boca Raton, FL: CRC Press.

[90] Banat, I.M., Makkar, R.S., and Cameotra, S.S. (2000) *Appl. Microbiol. Biotechnol.*, 53: 495–508.

[91] Grant, J., Cho, J., and Allen, C. (2006) *Langmuir*, 22: 4327–4335.

[92] Korhonen, M., Hirvonen, J., Peltonen, L., Antikainen, O., Yrjanainen, L., and Yliruusi, J. (2004) *Int. J. Pharm.*, 269: 227–239.

[93] Peltonen, L., Hirvonen, J., and Yliruusi, J.J. (2001) *Colloid Interface Sci.*, 240: 272–276.

[94] Illum, L. (1998) *Pharm. Res.*, 15: 1326–1331.

[95] Ogawa, S., Decker, E.A., and McClements, D.J. (2003) *J. Agric. Food Chem.*, 51: 2806–2812.

[96] Banat, I.M., Makkar, R.S., and Cameotra, S.S. (2000) *Applied Microbiology and Biotechnology*, 53: 495–508.

[97] Rosenberg, E. and Ron, E.Z. (1999) *Appl. Microbiol. Biotechnol.*, 52: 154–162.

[98] Deleu, M. and Paquot, M. (2004) *Comptes Rendus Chimie*, 7: 641–646.

[99] Flasz, A., Rocha, C.A., Mosquera, B., and Sajo, C. (1998) *Medical Science Research*, 26(3): 181–185.

[100] Mohan, P.K., Nakhla, G., and Yanful, E.K. (2006) *Water Res.*, 40: 533–540.

[101] Desai, J.D. and Desai, A.J. (1993) In *Biosurfactants-Production, Properties, Application*; New York: Marcel Dekker.

[102] Mulligan, C.N. (2005) *Environmental Pollution*, 133: 183–198.

[103] Singh, P. and Cameotra, S.S. (2004) *Trends in Biotechnology*, 22(3): 142–146.

[104] Poremba, K., Gunkel, W., Lang, S., and Wagner, F. (1991) *Environ. Toxicol. Water Qual.*, 6(2): 157–163.

[105] Kosaric, N. (2001) *Food Technology and Biotechnology*, 39(4): 295–304.

[106] Van Haesendonck, I.P.H. and Vanzeveren, E.C.A. W.O. 2004/040984 2004.

[107] Linhardt, R.J., Bakhit, R., Daniels, L., Mayerl, F., and Pickenhagen, W. (1989) *Biotechnol. Bioeng.*, 33: 365–368.

[108] Hood, S.K. and Zottola, E.A. (1995) *Food Control*, 6(1): 9–18.

[109] Velraeds, M.M.C., van der Mei, H.C., Reid, G., and Busscher, H.J. (1996) *Appl. Environ. Microbiol.*, 62(6): 1958–1963.

[110] Irie, Y., O'Toole, G.A., and Yuk, M.H. (2005) *FEMS Microbiol. Lett.*, 250: 237–243.

[111] Meylheuc, T., van Oss, C.J., and Bellon-Fontaine, M.N. (2001) *J. Appl. Microbiol.*, 91: 822–832.

[112] Meylheuc, T., Renault, M., and Bellon-Fontaine, M.N. (2006) *Int. J. Food Microbiol.*, 109: 71–78.

[113] Bednarski, W., Adamczak, M., Tomasik, J., and Plaszczyk, M. (2004) *Bioresour. Technol.*, 95: 15–18.

[114] Nitschke, M., Costa, S.G.V.A.O., Haddad, R., Goncalves, L.A.G., Eberlin, M.N., and Contiero, J. (2005) *Biotechnol. Progr.*, 21(5): 1562–1566.

[115] Dubey, K. and Juwarkar, A. (2001) *World J. Microbiol. Biotechnol.*, 17: 61–69.

[116] Rodrigues, L., Moldes, A., Teixeira, J., and Oliveira, R. (2006) *Biochem. Eng. J.*, 28: 109–116.

[117] Nitschke, M. and Pastore, G.M. (2006) *Biosource Technology*, 97: 336–341.

[118] Makkar, R.S. and Cameotra, S.S. (2002) *Appl. Microbiol. Biotechnol.*, 58: 428–434.

[119] Patel, R.M. and Desai, A.J. (1997) *Lett. Appl. Microbiol.*, 25: 91–94.