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Stabilization of Food Colloids: The Role of Electrostatic and Steric Forces

Camillo La Mesa and Gianfranco Risuleo

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Abstract

The role that some forces exert on food colloid stability is discussed. The focus is on the combination of different energy terms, determining particle-particle attraction or repulsion. The forces are relevant in dispersion stabilization and macroscopic phase separation. The observed features depend on the energies at work and colloid concentration. Examples deal with food manipulations giving cheese, yogurt, and mayonnaise. All products result from the overlapping of forces jointly leading to aggregation or phase separation in foods. The combination of attractive, van der Waals (vdW), and repulsive, double-layer (DL) forces results in the dominance of aggregation or dispersion modes, depending on the particle concentration, on the force amplitude, and on their decay length. DL and vdW forces are at the basis of Derjaguin-Landau-Verwey-Overbeek (DLVO) theory on colloid stability. That approach is modified when these forces, jointly operating in bio-based colloids, overlap with steric stabilization and depletion modes. Steric effects can be strongly dispersive even at high ionic strength, despite this is rather counterintuitive, when depletion ones favor the nucleation in a single phase.

Keywords: van der Waals forces, double-layer forces, Poisson-Boltzmann equation, steric stabilization, depletion, food-based colloids

1. Introduction

Optimization of food properties is of fundamental interest because of growing demand for its widespread availability [1]. Substantial efforts tend to optimize the steps required in advanced food chain. Scientists and technicians focus on all preparation stages, from the collection of raw matter to transformation in the required form. Freezing, cooking, drying, salting, and all procedures which are part of human knowledge since thousand years are considered [2–6].

Protein-rich preparations, such as anchovy paste [7], stockfish, dried venison, and cheese [8], are relevant examples. There is an urgent need to render old-fashioned preparations reliable and safe to a huge number of potential users. It is also necessary to ensure them good quality, together with homogeneous and "permanent" textures. Old-dated preparations give high-quality matter but operate in small scale, with drawbacks due to costs and durability. These preparations must be optimized to fulfill industry and safety requirements. Foods shall be stable for long times, still retaining their peculiar quality and taste. This is one of the reasons why modern preparations use stabilizers [9]. Storage must not require conditions hardly at hand in developing countries; think of the lack of low temperature and storage chains.

The focus is on semifluid matrices, such as creams and pastes, in other words, on items arbitrarily defined as *soft matter food*. Such products (ice creams, mayonnaise, pastes, sauces, etc.) are stabilized by addition of salts, lipids, proteins, and/or polysaccharides [10, 11]. Stabilizers are taste-neutral, fully biocompatible, and not expensive. They are obtained in large amounts, from the same sources as the products to be stabilized. In addition, food colloids must have peculiar rheological properties.

We do not consider explicitly the biological quality of preparations, which must fulfill the standards required from national/international panels. Many chemical, biochemical, and physicochemical properties characterize the features of stabilizers used in the food industry. Such properties always imply the stabilizer's capability to adsorb onto surfaces [12–14]. The latter is
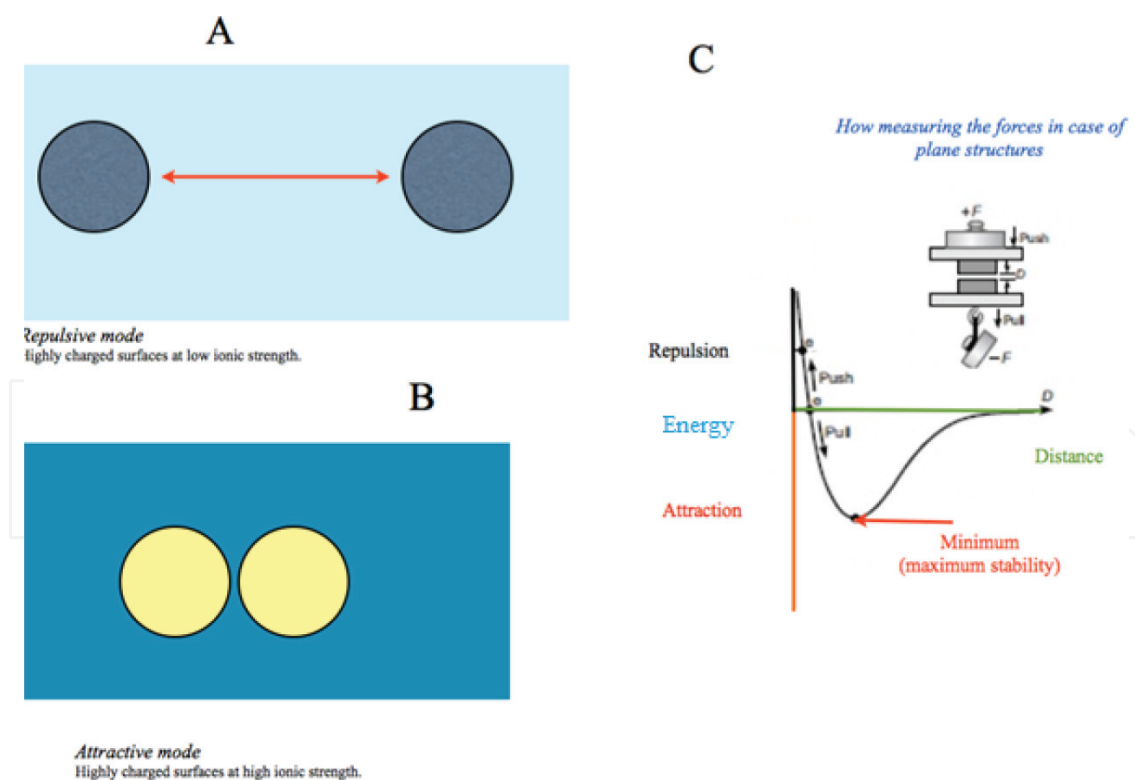

Figure 1. Electrolyte-modulated interactions among charged colloids. In (**A**), the case where repulsive electrostatic effects dominate is drawn; (**B**) refers to the reverse case (at high ionic strength). Light or dark blue colors indicate low and high ionic strength media, respectively. (**C**) The experimental determination of attractive/repulsive forces as a function of distance among two surfaces. The green horizontal line in that graph indicates equilibrium, i.e., $\Delta G = 0$.

the result of wrapping [15], steric [16, 17], osmotic [18], and electrostatic effects [19] and combinations thereof [20]. It is hard to ascertain whether the desired effect is due to the combination of more contributions. Think of the role played by proteins and polysaccharides as food stabilizers!

Electrostatic effects due to such stabilizers are relevant in most cases considered here. With this in mind, we report on the role that some forces exert in food stabilization. To proceed along this line, it is required to know the fundamental aspects of food biocolloids, interpreted according to the so-called Derjaguin-Landau-Verwey-Overbeek (DLVO) theory [21, 22]. In its original form, it is simple to handle and applies to all colloid mixtures, irrespective of their nature and physical state. DLVO theory combines attractive, van der Waals (vdW), and repulsive, double-layer (DL) forces. Refinements and modifications of the original theory are available [23–27]. The theory explains why food colloids remain dispersed, or coagulated, depending on the experimental conditions. This is because DL counteracts with vdW terms and their combination tunes the interaction modes. Similarly charged surfaces undergo long-range repulsions, and the energy barriers keeping them apart may be several K_BT units high [28]. However, if the electrolyte concentration in the medium increases, a secondary minimum in force *vs.* distance plots is observed (see **Figure 1**) [29]. The repulsive forces are minimized, and attractive ones dominate, i.e., coagulation occurs as σ approaches zero.

2. Some food preparation procedures

We report first on qualitative descriptions of food-making procedures. For some of them, the role that physical forces exert in the stabilization or phase separation is evident, in others much less. Enzymatic reaction steps are common. Energy barriers must be overcome to make the required processes effective; that is the reason for the need of heating during some preparation steps. Food preparation may occur in one or more stages.

Though they share the same raw product in common, significant difference occurs between cheese-making and yogurt-making procedures, briefly reported below. The former proceed by controlling milk fermentation, to get a product with specific organoleptic requirements in terms of appearance, flavor, taste, and texture. Such properties must be reproducible every time cheese is made. In fact, a particular cheese needs a specific preparation. In modern industrial cheese-making, the craft elements are retained to some extent, but there is more science than craft. In contrast, individual cheese-makers and craft-based factories operate on small scale and sell "handmade" products. In cases of the like, each batch may differ from another, as commonly occurs in the manipulation of natural products.

Some cheeses are deliberately left to ferment under the action of spores and bacteria; this leads to products of high added value in a niche market, such as *Roquefort*. In culturing the cheese-maker brings pasteurized milk in the vat to the thermal range promoting the growth of bacteria that feed on lactose. That sugar ferments into lactic acid. Bacteria may be wild, with non-pasteurized milk, added from a given culture, frozen or freeze-dried concentrates. Those producing only lactic acid are homofermentative; the ones producing CO_2, alcohol, aldehydes, ketones, etc. are heterofermentative.

Both homo- and heterofermentation produce cheeses with typical features in terms of taste, macroscopic textures, consistency, elasticity, presence of bubbles, and bubble size. When the cheese technicians judge that enough lactic acid has been developed, they add *rennet*, which precipitates casein. *Rennet* contains *chymosin* which converts *κ-casein* to *para-κ-caseinate*, the main component of cheese curd (see **Figure 2**). There is also a *glycomacropeptide*, almost always lost in the cheese *whey*. After adding the *rennet*, milk is left to form curds over a period of time. As curds are formed, milk fat is trapped in a casein matrix; *whey* must be released once cheese curds are fully developed. There are several ways to do that.

The presence of water and bacteria encourages further decomposition. Therefore, water or *whey* must be removed. When cheese curds are formed, a partial, sometimes significant, dehydration occurs. This gives rise to good quality products keeping their main features over time. In a stage termed *cheddaring* (from cheddar), curd acidity increases. When it has reached the required level, the curd is milled in pieces, and salt is added to arrest acid development. After some other stages, pressed cheese blocks are removed from the *molds* and waxed or stored for maturation. Vacuum packing removes O_2 and prevents fungal growth during maturation. This process is desired or not, depending on the required product. By going through a series of maturation steps where temperature and humidity are controlled, the cheese-maker allows the surface *mold* to grow and mold ripening of cheese by fungi to occur. Mold-ripened cheeses mature quickly compared to hard ones (weeks vs. months or years), because fungi are
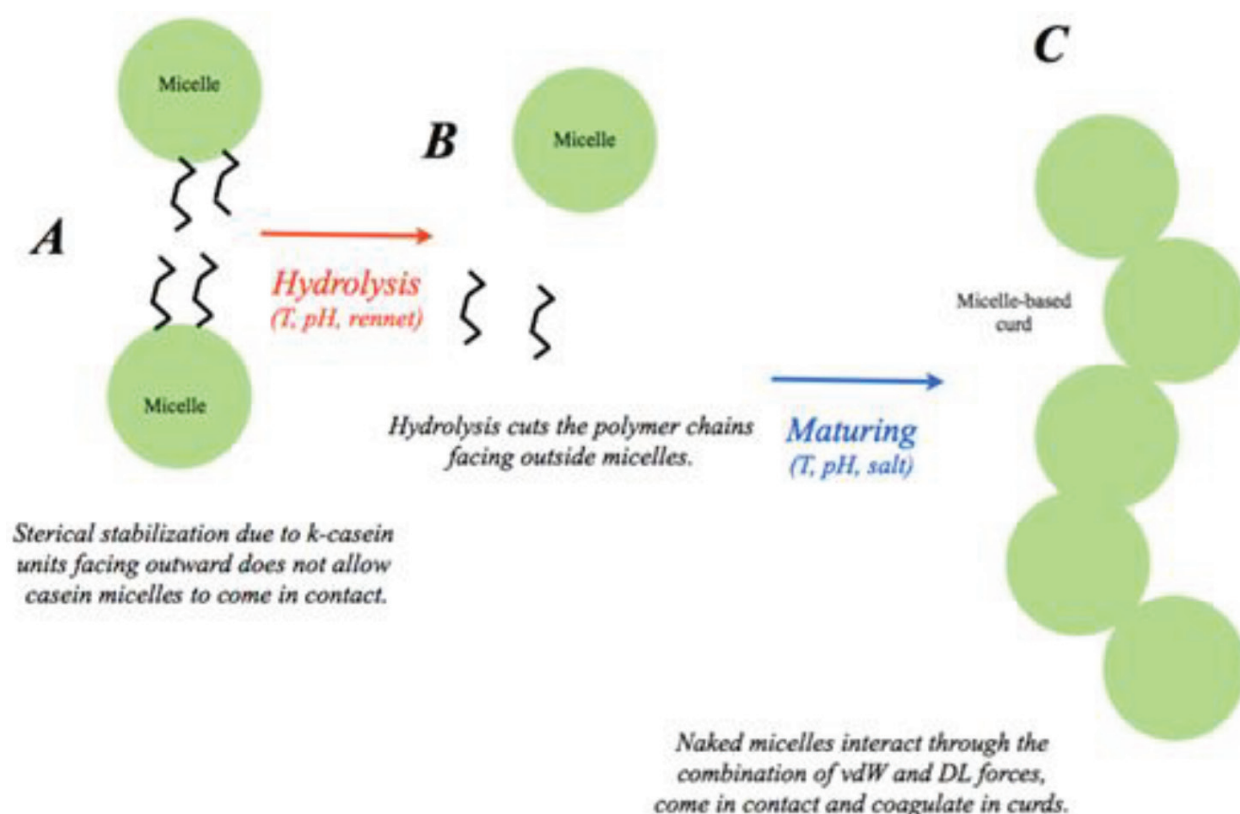

Figure 2. (**A**) Steric stabilization of casein micelles due to proteins facing toward the bulk and keeping micelles apart. (**B**) Enzymes present in the *rennet* cut *κ-casein* portions facing toward the bulk. (**C**) Depleted micelles attract each other and coagulate in curds. The process ends in cheese formation.

more active than bacteria. *Camembert* and *Brie* are surface-ripened by molds; *Stilton* is ripened internally and admits air to promote mold spore germination and growth. Surface ripening of some cheeses may be influenced by yeasts, contributing to flavor and coat texture. Others develop bacterial surface growths, giving characteristic colors and appearances.

Yogurt, conversely, is produced by bacterial fermentation of milk. Lactic acid acts on milk proteins and imparts yogurt its texture and flavor. Cow's milk is the common source to make yogurt; it may be homogenized or not. Yogurt is produced by *Lactobacillus delbrueckii* subsp. *bifidobacteria* (LDsB), *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* bacterial cultures. Genome analysis of LDsB indicates that the bacterium presumably originated on the surface of a plant. Milk may have been exposed to contact with such plants, or bacteria transferred from domestic milk-producing animals. The real origin of yogurt preparation procedures is unknown but reasonably dates back to 5000 BC. To produce along, milk is heated to denature milk proteins so that they do not form curds. After cooling, the bacterial culture is mixed in, and the temperature is maintained for some hours to allow fermentation.

Mayonnaise, conversely, is a very peculiar product in terms of origin, components, and physical state. On physicochemical grounds, it is a surface-stabilized oil/water (o/w) dispersion, whose quality is determined by the presence of adsorbed lecithins at the o/w interface. The stability of this dispersion is modulated by tiny amounts of acetic or citric acid, which impart phospholipids as a moderate and permanent charge. Stability of the dispersion is modulated by added electrolyte, such as NaCl. In addition, the o/w dispersion, as a whole, adsorbs significant volume fraction of air. Thus, a heterogeneous two-phase dispersion acts as air dispersant; the final result is a three-phase system stabilized by surface-adsorbed lipids.

3. Some aspects of food colloids

3.1. General considerations

Animal-based foods and most of our own body organs generally contain about 55–75% water; in vegetables it can be over 90 wt%. Solid moieties are proteins, fats, lipids, etc., associating in different forms to give gel-like, liquid crystalline, amorphous, or semisolid matrices. Most tissues result from colloid packing. From that evidence comes the generalization that animals and vegetables are made of several different colloid entities, nicely, but functionally, interconnected. In all these systems, disperse colloid particles coagulate. Coagulation does not occur when particles are similarly charged; that is, coalescence is prevented by electrostatic forces. This holds also in dispersions of oil droplets stabilized by a phospholipid layer. Low amounts of electrolyte ensure lipid-covered droplets to repel each other. If the surface charge density, σ, or the related potential, Ψ, is moderate, the energy barrier among particles, proportional to $ze\Psi$, is low, and there is a marked tendency to coagulation. The limit at which such phenomena occur is known as coagulation/flocculation threshold.

Further increase of salt reduces Ψ and ensures permanent coagulation. As σ approaches 0, the DL force is null, electrostatic effects vanish, and the whole energy coincides with the vdW one; thus,

particle-particle interactions become attractive. Similar conditions are met when food colloids aggregate in early manipulation stages and then redisperse as the pH or the ionic strength (I) varies. A simple case deals with oil droplets. The case of raw milk manipulation is substantially different from what is described above and ends in cheese formation. The whole process is controlled by the presence of fatty acids and glycerides existing as droplets; micelle-forming casein; coagulating enzymes, salts, and lactose (a milk sugar transformed in lactic acid) [30]; and so forth. The whole process is completed when aggregation/gelation occurs [31] and is governed by heating, enzymic activity, changes in pH, presence of ions, and combinations thereof.

Although casein micelles are charged, significant amounts of added salt do not ensure coagulation to form cheese seeds [32]. In fact, casein micelles are stabilized by steric effects, not allowing them to come in contact and coagulate. Steric stabilization counteracts attractive vdW forces and does not allow seed clustering. Such effects are minimized by the action of enzymes, cutting the *κ-casein* parts facing outward micelles. In the early cheese-making steps, pH activates/deactivates hydrolytic enzymes [33], whose activity also depends on T [34].

The presence of *rennet*, essential in the first stages of cheese curdling, is also relevant. Ion content and valence (calcium better suits compared to monovalent ions) favor casein aggregation in large micelles and, therefore, curd formation [35, 36]. To elucidate such aspects, we introduce below an approach to electrostatic stabilization and show that it, in combination with vdW forces, is relevant in food formulations, as indicated in **Figure 3**.

3.2. Electrostatic forces

Colloid entities are characterized by a given mass density and average size, can be more or less size polydisperse, and wear a surface electrostatic potential [37]. When dispersed in water, uncharged colloids readily coagulate, but surface charge density avoids that process. Irrespective of their nature and shape, colloid particles are covered with stabilizers, adsorbing thereon, and imparting them a permanent surface charge. In consequence of that, particles repel, depending on the modulus of Ψ, $|\Psi|$, which exerts a long distance effect and scales with kD (**Figure 3**).

The distance is D and 1/k is Debye's screening length. Repulsion occurs when particles are close to each other. The effect has the same meaning as that between planar surfaces of equal Ψ values (**Figure 4**).

Electrostatic potentials decay according to

$$\Psi(x) = \Psi^{\circ} \exp^{-kD} \tag{1}$$

where *D* is the distance from a charged surface of nominal potential equal to Ψ°. The meaning of k has been given above.

Another master equation for electrostatics refers to the interaction between two surfaces characterized by the same Ψ. It decays according to

$$\nabla^2\Psi = d^2\Psi/dx^2 + d^2\Psi/dy^2 + d^2\Psi/dz^2 = -\left(\varrho/\varepsilon\varepsilon^{\circ}\right) \tag{2}$$
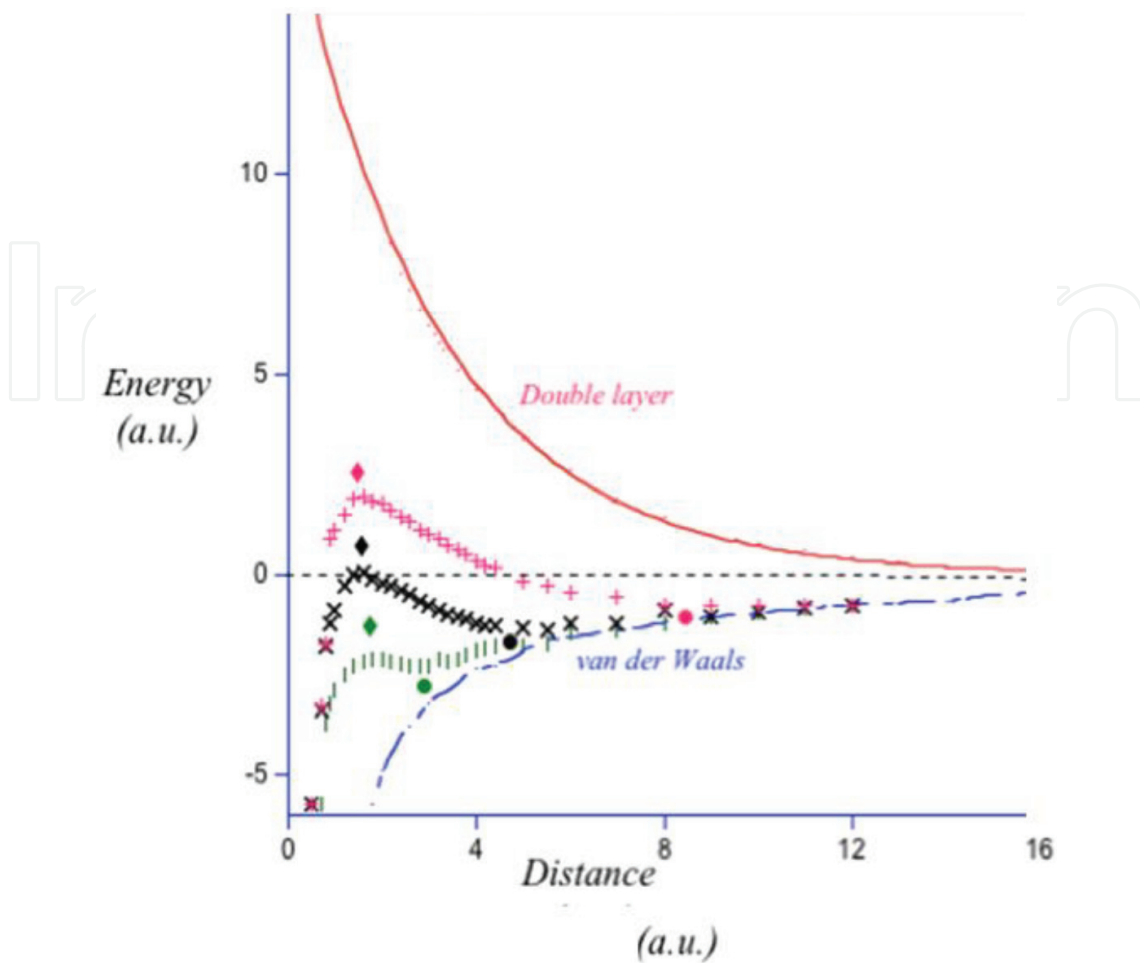
Figure 3. Combination of vdW and double-layer (DL) forces as a function of distance among two particles for high (red), medium (black), and low (l) surface charge densities. vdW terms are always attractive, i.E., E < 0; DL ones are always repulsive. Their combination results in energy vs. distance plots. The location of maxima, ♦, and minima, ●, depends on ionic strength, I. The primary minima at very short distances are not indicated. The maxima in the curves represent the location of energy barrier.

where ϱ is the ion number density of the medium and ε and ε° are the permittivity of vacuum and of the dispersant, respectively. The electric field is radial and its value does not depend on the direction; thus, we consider its components along only one axis, say x. And, Eq. (2) can be rewritten as

$$\nabla^2 \Psi = d^2\Psi/dx^2 = -\left(\varrho/\varepsilon\varepsilon^\circ\right) \tag{3}$$

Let us consider now the statistical energy terms. Boltzmann's law for the distribution of charged species in a given medium can be written as

$$c_i = c_i^\circ \exp^{-(ze\Psi/K_BT)} \tag{4}$$

where c_i is the local concentration of the ith ion, c_i° is its equilibrium value, $ze\Psi$ is the energy associated to the electric field for an ion of valence (z), and K_BT is the thermal one. Eq. (3) is
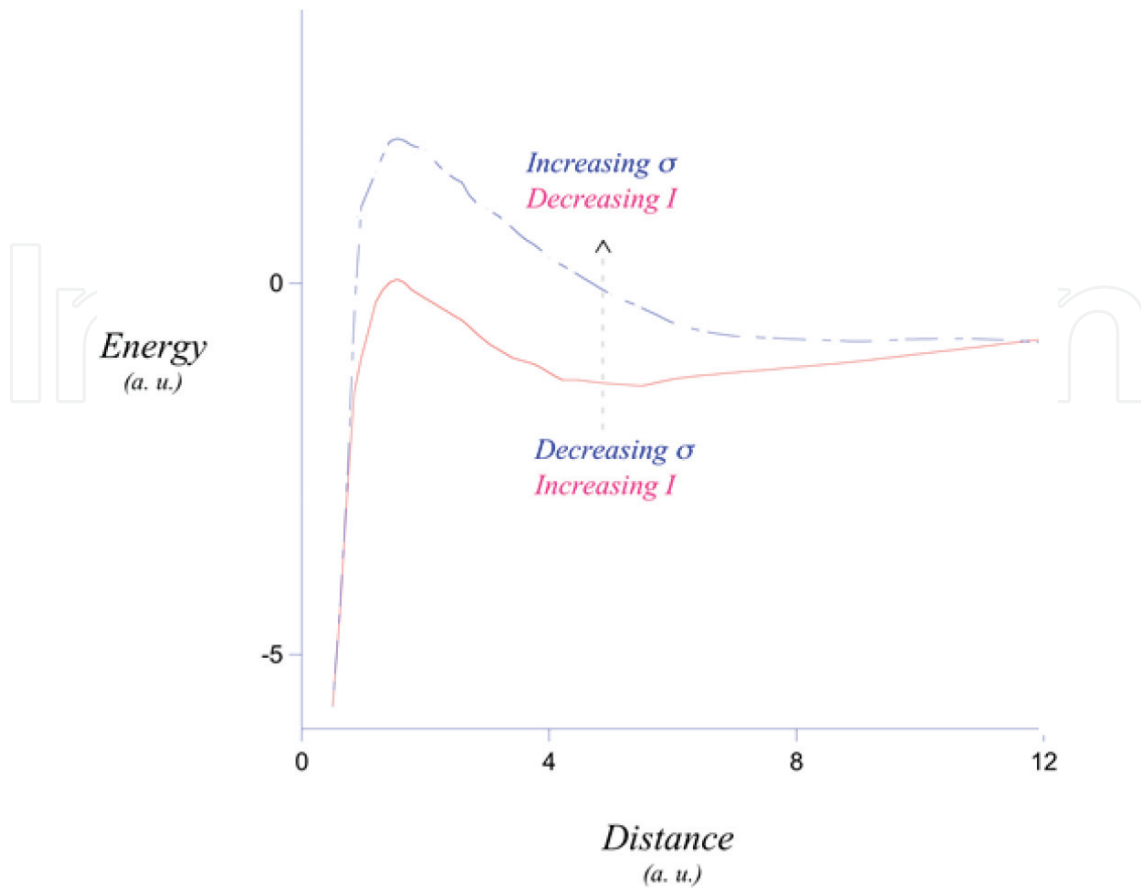
Figure 4. Relationship between ionic strength, surface charge density, and attractive/repulsive forces at fixed D. It is evident that repulsive forces turn to attractive, depending on I value.

modulated by the electrical to thermal energy ratio. The balance of such forces determines the spatial distribution of ions around a charged entity, depending on the electric field and thermal motions. Then,

$$\varrho = \varepsilon(c^+ - c^-) = \varepsilon c^\circ \left[\exp^{-(ze\Psi/KBT)} - \exp^{(ze\Psi/KBT)} \right] \tag{5}$$

In the above form, the equation (usually different from 0) represents the local charge density due to an ion in excess. If $|ze\Psi/K_BT|$ is <<1, the difference between exponents can be transformed in hyperbolic form ($\exp^x - \exp^{-x} = 2\sinh x$) and linearized. We assume $x = ze\Psi/K_BT$. Thus, when $x << 1$, Eq. (4) indicates a *linear* perturbation regime. Such conditions are currently used to determine the electrostatic energy contributions. Advantages due to linearization are substantial.

The charge density, ϱ, is related to the surface potential, σ, which, in turn, depends on Ψ. The links between ϱ, σ, and Ψ are expressed as

$$\sigma = -\int \varrho dx \tag{6}$$

$$\sigma = \left(2n^\circ \varepsilon K_B T / \sqcap \right)^{1/2} \sinh(ze\Psi/K_BT) \tag{7}$$

where ε is the dielectric permittivity of the medium. σ relates the system energy to electrical terms, according to

$$\Delta G = - \int \sigma d\Psi \tag{8}$$

Let us consider the role of electrostatic forces, favoring/disfavoring phase separation. In simple cases the focus is on the formation of mayonnaise and yogurt; subsequently, the more cumbersome case of cheese is described. vdW forces are present in all such cases. Significant differences arise when steric, osmotic, and DL contributions counteract vdW forces, do not allow adhesion, or shift the coagulation threshold to high concentrations. These features, observed in some cheese-making stages, are outlined below.

3.3. Electrostatic vs. vdW forces

In the classical formulation of DLVO theory, vdW forces are combined with DL ones. For bodies at constant T, the interaction energy (E_{int}) significantly depends on distance (D). At high D values, E_{int} is zero and all contributions vanish. Modulation of the above terms results in the presence of a primary and a secondary minimum. The first one occurs at very short distances, and the second one, at higher ones. The secondary minimum shifts to lower values in proportion to I; an energy barrier separates it by the primary minimum. The barrier height is related to the activation energy of coagulation (**Figure 3**).

The secondary minimum in **Figure 3**, some K_BT units high, shifts to lower distances in proportion to I. The tendency to coagulate is represented by the progressive overlapping of vdW and DL curves. The minimum at short distances is not indicated; the maximum is related to E_{att}. The role of ionic strength can be evidenced considering the electrostatic potential among two surfaces with a fixed number of charges per unit area which are shielded by increasing concentrations of salt (**Figure 4**).

In **Figure 5** we indicate how the electrostatic potential changes with I. In distilled water, $\Psi°$ rapidly increases with ion concentration. Neutral electrolytes (in the concentration range of 10^{-3} moles kg^{-1}) have a buffer effect on $\Psi°$. Since most foods contain substantial amounts of salt, the region where the effect of Ψ is significant ranges from 25 to 100 mV, in modulus [38]. For values $<|25|$ mV, samples tend to coagulate; above 100 mV counterion adsorption becomes large, thus minimizing electrostatic repulsions. Small changes in Ψ values are large enough to ensure dispersion, aggregation, or sedimentation in all such media. That is why a careful balance of surface energy terms is necessary.

The electrostatic contributions in real systems are measured by the so-called ζ-potential, a distance (d) apart from the colloid particle surface, at the slipping plane limit. ζ-potential is measured by electrophoretic mobility experiments or laser Doppler methods [39]. The decay of ζ-potential with pH and/or I is easily determined (see **Figure 6**).

Accordingly, surface charges are titrated, and coagulation or redispersion occurs (**Figures 5 and 6**). The salient point in the latter is the zero surface charge value, where precipitation
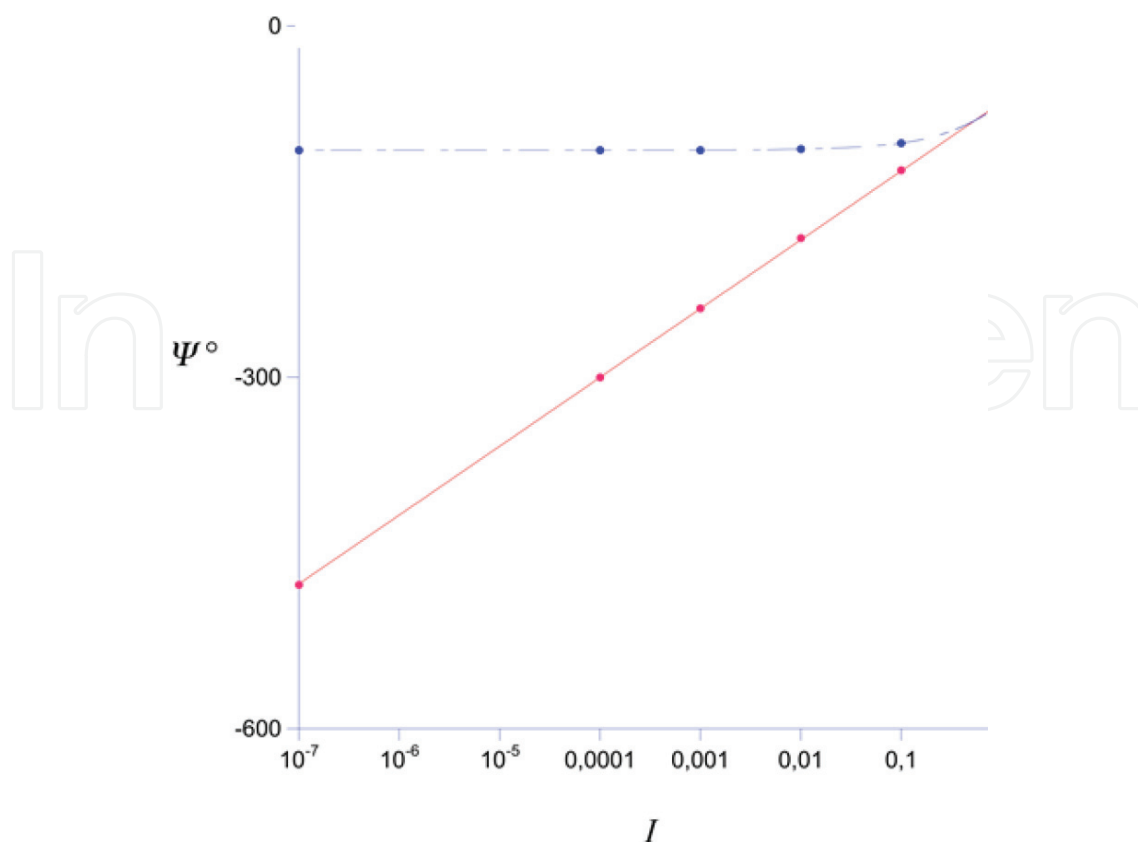
Figure 5. Effect of ionic strength, I, in moles kg^{-1}, on the electrostatic potential, $\Psi°$, of particles dispersed in water, red color line, and in 3.0×10^{-3} moles kg^{-1} MgCl$_2$, at 25.0°C. Note that I in bio-based systems can be significant; usually, the overall salt concentration is >5.0 $\times 10^{-3}$ moles kg^{-1}.

occurs. The electrostatic theory, thus, explains why salts screen repulsions, in direct proportion to valence and concentration. For instance, oil droplets covered by a charged lipid layer coagulate when NaCl content in the dispersing water-based medium reaches a critical value (some millimoles kg^{-1}).

Particle size, disparity, shape, and physical state (i.e., solid- or liquid-like) are immaterial. Although its value seems moderate, the surface charge density is relevant. σ of lipid-coated oil droplets (**Figure 5**) is about 1 unit charge/15 nm^2. And, despite such a relatively low value, stabilization is effective. Also, ion valence is relevant, as indicated by the relation $I = 1/2 \, \Sigma_{i = 1}$ $c_i z_i^2$. The coagulation concentration is concomitant to the secondary minimum of the curves in **Figure 3** and depends on z_i. The combination of such effects is also responsible for the stabilization effect due to proteins. Solving the above questions and taking into account protein stabilization are relevant in steric stabilization, as outlined in the forthcoming section.

3.4. Steric stabilization

This concept applies to entities covered by polymers or polyelectrolytes protruding outside the surfaces on which they are bound. Coatings consist of covalently linked (CL) or physically adsorbed (PA) polymers: the differences among two such classes are energetically significant.
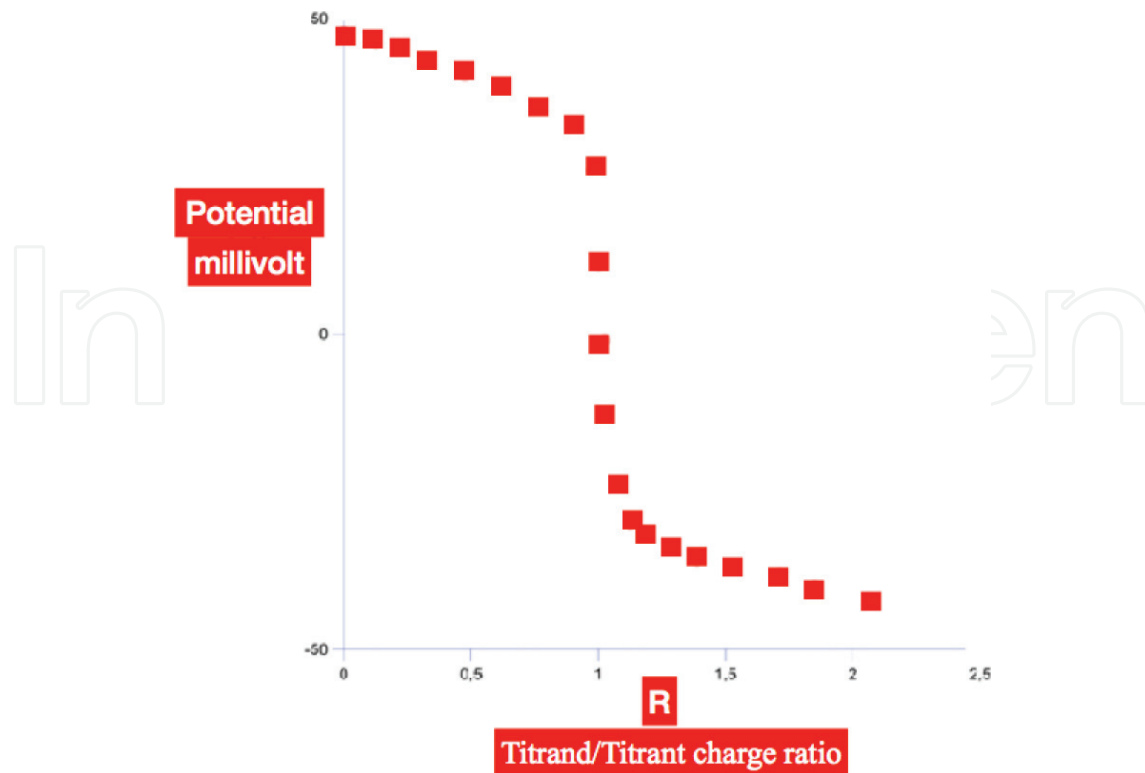
Figure 6. Dependence of ζ-potential, in mV, on the ratio titrant to titrand, R. When R ≅ 1, the ζ-potential approaches zero. The curve is symmetrical with respect to R. Data refer to 3.52×10^{-2} dispersions of sunflower oil (in volume fraction) with 2.3 mg/ml dipalmitoylphosphatidylcholine (DPDC) as dispersant. The solvent is 2.50×10^{-2} moles kg^{-1} NaCl and the temperature is 35.0°C. In this case the titrand/titrant ratio, R, depends on pH.

CL entities have permanent stabilizer/particle ratio. PA ones partition between particle surface and bulk, depending on the system composition and affinity; these composites are nonstoichiometric. PA surface adsorption energies are grossly one order of magnitude lower than those pertinent to covalent ones; indeed, both ensure substantial stability. The mentioned modes and energies have both advantages and drawbacks. CL polymers are depleted from the particles' surface by chemical reaction. This holds, for instance, when *κ-casein* is cut away from the surface of micelles by the combined action of *rennet*, *pH*, and temperature. Otherwise, casein micelles are stable for an indefinitely long time. The hydrolytic capacity of *rennet*, thus, is a prerequisite for effective coagulation. Once hydrolysis has gone to completion, PAs are detached from casein micelles and partition with the bulk. Unbalanced osmotic effects due to bulk polymer concentration in excess result in *depletion flocculation* [40]. That is, PAs are released and no longer stabilized micelle clusters of casein and calcium phosphate coagulate, and phase separation occurs to give cheese seeds and clusters.

Steric stabilization overlaps with other effects, jointly tending to keep particles dispersed. The result is modulated by the presence of charges on the protruding polymers. Osmotic, electrostatic, steric, and hydration forces sum each other and counteract vdW ones (**Figure 7**).

It is clear, thus, why particles may remain dispersed even when DL contributions are minimized. In some instances, the terms due to the aforementioned forces may be noticeable and
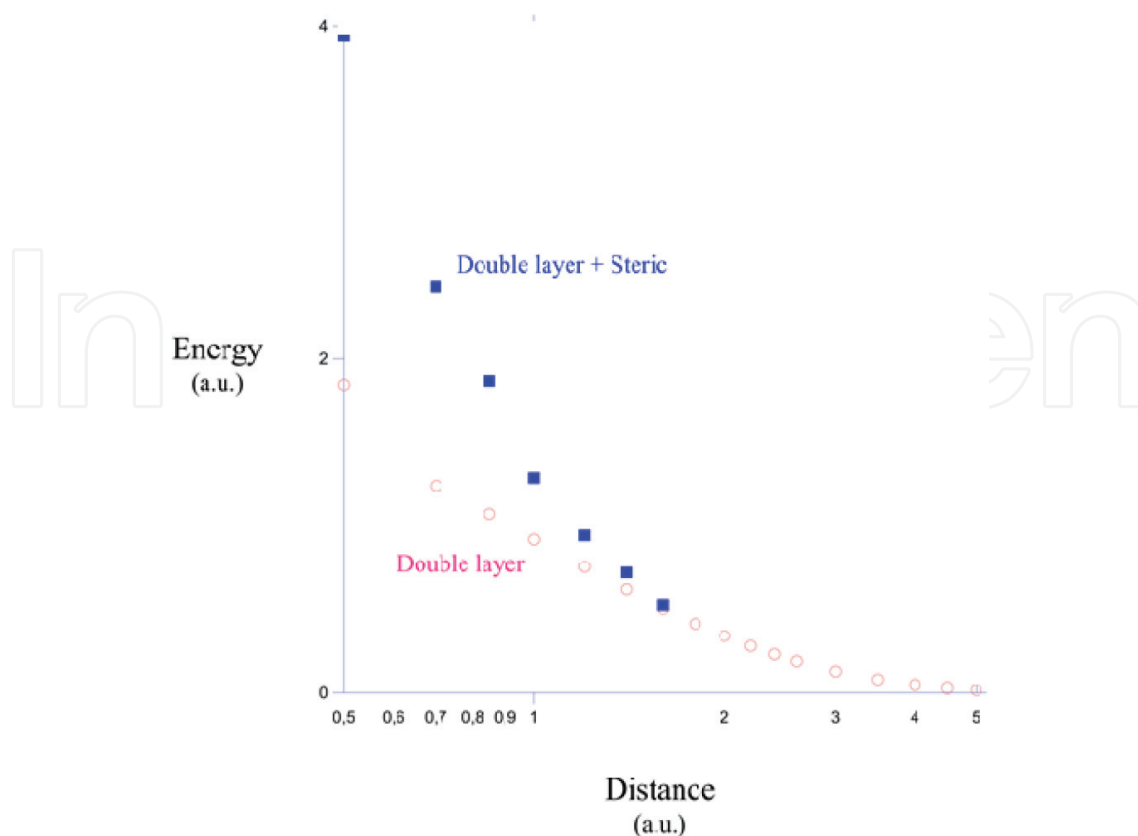
Figure 7. Dependence of the interaction energy, E, in arbitrary units, on the normalized distance among particles, D, in case of double layer, red, or when double layer and steric contributions overlap.

favor the dispersed state with respect to coagulation. For instance, consider the possibility to add to the plot in **Figure 7** osmotic and hydration forces.

We may combine all forces effective in a given medium in the generalized relation:

$$E_{tot} = \sum_{i=1} E_i \exp^{-k_iD} \tag{9}$$

where E_i is a given energy mode, D is the distance, and $1/k_i$ is the related screening length. Expectedly, forces decay exponentially, even though this statement is not to be generalized. Most forces, in fact, scale as $1/D^n$ (with $n \geq 3$). Eq. (8) conforms to short-distance decay modes and indicates that repulsion rapidly decreases with distance [20]. Attraction, conversely, is governed by vdW terms, responsible for phase separation. In this regard, the differences between yogurt and cheese coagulation clarify which forces govern the onset of such materials.

4. Conclusions

Forces responsible for attractive/repulsive interactions among food colloids are discussed. One must be aware that some of them, i.e., DL ones, are ubiquitous, although not always stabiliz-ing. This ineludible fact is due to the presence of ions in most media. That is why association or

phase separation is common as the ionic strength increases. Salting/desalting methods are responsible for cheese- and yogurt-making, among others. It is worth noting that the latter occurs in media which *per se* contain ions in the original matrix. It is also worth mentioning that calcium is present in significant amounts; that is why aggregation is relatively easy. Additional effects counteracting vdW ones arise from steric and osmotic contributions. It was suggested how to face with such processes and how to estimate from simple considerations stabilizing/destabilizing effects. It must be pointed out that DL effects significantly reduce on increasing the ionic strength.

The hierarchy of active forces and their combination ensure slightly different aggregation modes, giving more or less complex conglomerates. These can be homogeneous or not, depending on the nature of dispersed colloids. The supramolecular phases thus obtained may be due to intertwingled association of one colloid type into matrices made up by another. The uptake of fat droplets in cheese curds is a pertinent example. More effects, mostly due to surface adsorption, may significantly affect the final quality and appearance of the mentioned *soft matter* food.

Perspectives are governed by the continuous developments and optimization of food manipulation processes. These usually optimize former know-how, not disregarding the maintenance and quality of classical produce. In this regard it is safer to rely on craft-tempered goods produced by advanced technologies.

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