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Mini Review

Biophysical and Molecular Biochemistry of the Dairy Production Chain: From Milk to Cheese

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Abstract

Understanding the bio-molecular and chemico-physical processes underlying food preparation plays a pivotal role. In fact, the stability and maintenance of food are fundamental to preserve its nutritional and organoleptic properties. Cooking in various forms, drying, salting, and freezing have been part of the human cultural heritage and daily experience for thousands of years. In this minireview, we give a condensed overview on the preparation of cheese, with some insights on aspects of food colloids. The biological/nutritional quality of the preparations, which must fulfill national and/or international standards, is obviously not taken into account. Finally, due to the conciseness of the work, we do not go into deeper details about the chemico-physical aspects of food processing. More extensive works on this subjects from our and other laboratories are found in the literature cited.

Introduction

General considerations on food preparation and food colloids

A great effort is invested in the optimization of food preparation and keeping. This is of fundamental interest because of the continuously expanding request of its availability. As a matter of fact, this has become a primary problem, often of difficult solution, in advanced as well as emerging and third-world socio-economical environments [1]. A number of preservation/keeping procedures have developed all over millennia of human history, possibly from the Neolithic Age up our days. Smoking, cooking, drying, salting, and freezing (although this is possibly a more recent achievement and limited to an industrial scale), are food maintenance procedures which are of common knowledge [2-5]. Very well-known long-keeping products are represented by anchovy paste, smoked- and dried stock fish, dried venison, and cheese [6,7]: they all constitute a very rich nutritional source of protein and fat. However, handcraft or "family" made finished products may result in uneven quality and stability often with a high propensity to rapid decay. Therefore, to assure quality, stability, and safety, the industrial food industry often resorts to the use of stabilizers [8]. Foodstuffs like ice creams and mayonnaise, for instance, are stabilized by addition of salts, lipids, proteins, and/or polysaccharides which are usually taste-neutral, fully biocompatible, and not expensive [9]. In any case, the features of stabilizers used in the food industry have many different chemical, biochemical, and physicochemical features as well as, peculiar rheological properties. This mostly imply their capability to adsorb onto surfaces [10]. Adsorbtion is the result of a number of interacting combined forces and effects: wrapping, steric, osmotic, and electrostatic effects, [reviewed in the classical work by Perutz and in the more recently reviewed by Israelachvili [11,12]. Assessing the effects due combinations of different forces is rather grueling, however we very briefly discuss here he role that some forces exert in food stabilization and food bio-colloids: this requires a very extensive illustration of fundamental aspects, interpreted according to the so-called Derjaguin-Landau-Verwey-Overbeek (DLVO) theory [13,14]. The theory DLVO combines attractive forces of van der Waals (vdW) type, and repulsive, double-layer (DL) forces. Refinements and modifications of the original theory are indeed available, but all in all, DL counteracts vdW terms and their combination regulates the interaction modes. Similarly charged surfaces undergo long range repulsions, and the energy barriers that keep them apart may be several KBT units high. This actually theory explains why food colloids remain dispersed, or coagulated, depending on the experimental conditions [15,16].

From Milk to Cheese: An Overview

Water is the basic component of animal and vegetable foodstuffs with vegetables being richer in the aqueous portion; solid moieties are macromolecules like proteins, fats, lipids, and small molecules like vitamins and salts. They may associate in supramolecular aggregates gel-like, liquid crystalline, amorphous, or semisolid matrices. The transformation of whole crude milk into cheese is controlled by the presence of fatty acids and glycerides existing as droplets; micelle-forming casein; coagulating enzymes, salts, and lactose. This latter is a main milk sugar component transformed in lactic acid during the process of cheese formation [17]. This process is completed when aggregation/gelation occurs and is led by heating, enzymic activity, changes in pH, presence of ions, and combinations thereof. Casein micelles are charged and, despite this, significant amounts of added salt, do not ensure coagulation to form cheese seeds [18]. 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 k-casein parts facing outward micelles. In the early cheese-making steps, pH activates/deactivates hydrolytic enzymes, whose activity also depends on temperature (Figure 1) [19].

Cheese and Yogurt

Two important dairy products in terms of economy and consumer satisfaction

Though they share the same raw product, that is milk, be this crude, pasteurized, whole o partially de-greased, significant differences occur between cheese-making and yogurt-making procedures. The former proceeds by controlling milk fermentation, to get a product with specific organoleptic requirements in terms of appearance, flavor, taste, and texture (Figure 2). In modern industrial cheese-making, the craft elements are retained to some extent, but there is more science than craft. The flip side of the coin, "family" cheese makers and craft-based factories operate on small scale and sell "handmade" products. The organoleptic features often are not uniform from batch to batch, as it commonly occurs in the treatment of other natural products (the

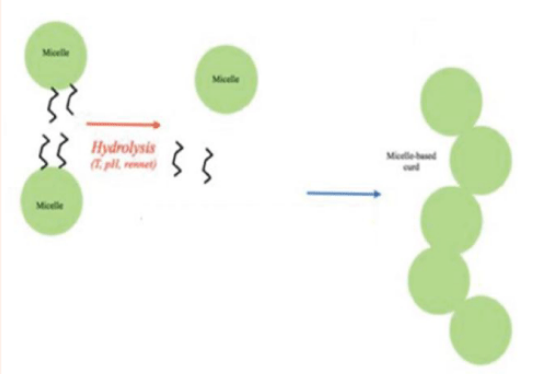

Figure 1: Left Panel: Steric stabilization of k-casein micelles due to proteins facing toward the bulk and keeping micelles apart which, therefore, cannot come into contact. Center Panel: Enzymes present in the rennet cut k-casein portions facing toward the bulk, which are cut by hydrolysis. Hydrolysis is due to temperature, pH and rennet. Right Panel: The final phase of cheese formation. Depleted micelles attract each other and coagulate in curds. The process of cheese formation and maturation is mediated by temperature, pH, and salt concentration.

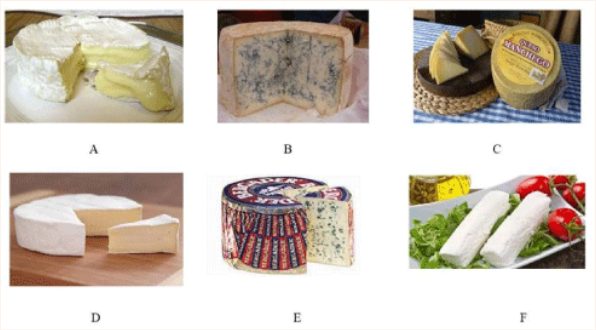

Figure 1: A: Mature French Camembert, B: Italian Gorgonzola, C: Spanish Queso Manchego (aged), D: French Brie (semi-fresh), E: German Bergader, F: Italian Caprini (goat-milk fresh cheese). Gorgonzola and Bergarder, as well as the world-renowned Roquefort, are known as blue cheeses, even though the Gorgonzola dues its color to a green mold.

Table 1: Consumption of cheese in a few countries in the European area.

Rank	Country	Kg per Capita
1	Denmark	28.1
2	Iceland	27.7
3	Finland	27.3
4	France	27.2
5	Cyprus	26.7
6	Germany	24.7
7	Suisse	22.2
8	Netherlands	21.6
9	Italy	21.5
10	Austria	21.1
11	Sweden	20.5
12	Estonia	20
13	Latvia	19.8
14	Norway	19.8
15	Israel	18.9

"peasant's wine or ham are typical examples of sometimes not fully successful food manipulations producing agreeable results).

Cheese

Organic cheeses are deliberately left to ferment under the action of spores (yeast, molds) and bacteria. This leads to niche products with a high market added value. But the semi industrial to industrial products follow invariably the same production chain: the cheesemaker feeds pasteurized milk to a temperature-controlled vat where the growth of microorganisms metabolizing lactose is favored. This sugar is transformed into lactic acid. Bacteria may be wild-type strains deriving directly from the non-pasteurized crude milk, or select strains added from a given culture. Also, the microorganisms may be either fresh, frozen or lyophilized concentrates (paste, powder, or pellets). The micro-organisms producing only lactic acid are defined as homofermentative; the ones producing CO_2, alcohol, aldehydes, ketones, are known as heterofermentative. Both processes, 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 an ensemble of enzymes obtained from the stomach of ruminants like sheep and calves. Rennet precipitates casein: it contains chymosin which converts casein to para-caseinate, the main component of cheese curd. Upon addition of rennet, milk is left to form curds for the required time (Table 1). After the formation of curd, milk fat is trapped into a casein matrix; at this point whey and water must be eliminated, in any case when cheese curds are formed, a partial, but 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. At this point cheese is pressed into wheels, removed from the molds and waxed or stored for maturation. In many cases, cheeses are, prior to waxing or coating with animal melted fat, pre-ripened in brine. Vacuum packing removes O_2 and prevents fungal growth during maturation, but this is seldom carried out on high quality product, if not in the immediate time before presentation to the market shelf. 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 more rapidly as compared to hard ones (weeks vs. months or years), because fungi are more active than bacteria; on the other hand, they are characterized by a shorter shelf life. Camembert and Brie are surface ripened by molds; Stilton is ripened internally and admits air to promote mold spore germination and growth, similar mold-mediated maturation occurs with Gorgonzola and in general blue cheeses. 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. Cheese can assume an incredible variety of tastes, textures, consistencies and, of course, flavors: all of them with different organoleptic properties. Some of them are not very well accepted by consumers because of their pungent aroma and taste. It is almost impossible to give a comprehensive view of the cheese variegated world: the reader should bear in mind that only in Italy (the Authors' home country) some 490 different kinds of cheese are present on the market-shelf, not to speak of France or Germany or Denmark that are also very important cheese producing countries. The high consumption of cheese all over the world makes this product one of the most appreciated commodities both under the nutritional and the economical (commercial) point of view. Figure 1 tries to depict the various aspects of both mature and semi-fresh to fresh cheese preparations, but unfortunately does not give any account of the flavor and taste.

Yogurt

The real origin of yogurt preparation procedures is unknown but reasonably dates to 6000 BCE. Its history is fascinating: the word allegedly comes from the term "yağ" meaning (oily, fatty) in the Kipchak language which was spoken by the ancient Turkish-Altaic populations. Subsequently, in modern Turkish it became "yoğurmak" which should mean "mixing fat" (or oil and/or butter). Yogurt 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, although goat milk variants do exist; it may be homogenized, strained (as in the case of Greek yogurt), or neither. Yogurt is produced by different bacterial organisms of the genus Lactobacillus and Streptococcus. Some of these bacterial strains like Bifidus actiregularis, are protected by industrial patents and are ingredients of probiotic processed milk derivatives which, strictly speaking are not yogurt. The genome analysis of Lactobacillus delbrueckii species indicates that the bacillus presumably found an optimal environment on the surface of a plant. Such plants may have been exposed to contact with milk, or bacteria transferred from domestic milk-producing animals. To produce yogurt, milk is heated to denature proteins so that they do not form curds. After cooling, the bacterial culture is added, and the temperature is maintained for

some hours to allow fermentation. The differences in chain production of "regular" cheese and yogurt should be evident at this point. The mixing of the fermenting milk inhibits the formation of clots, but it should be pointed out that in the apparatus form home yogurt making, mixing is never necessary.

Conclusion

Developments and optimization of food manipulation are in constant progress. Many methods are known for cheese- and yogurt-making. It is worth noting that the latter occurs in liquid 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. In any case, the hierarchy of active forces and their combination, causes different aggregation modes giving more or less complex conglomerates. These can be homogeneous or not, depending on the nature of the dispersed colloids. The supramolecular phases thus obtained are due to association of one colloid type with a different colloid matrix: uptake of fat droplets in cheese curds is a pertinent example. One final consideration is that milk is the principal (almost sole) ingredient of the transformation to cheese. Considering the multifaceted features of the several hundreds of cheeses known all over the world this should generate a sort of serendipity in all cheese lovers.

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