Bioguided Processing: A Paradigm Change in Food Production

Our understanding of biology derived through functional genomics will allow processing of foods to maintain or improve, rather than diminish, their nutritional and functional value. Milk is a good example.

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The effort to sequence the human genome, and subsequently to make the results publicly available, has created a new paradigm in the biological sciences. Now that we have access to the blueprint of our very structure and those of organisms from viruses to plants to animals, a mechanistic understanding of all the processes involved in our growth and metabolism is possible. Characterization of the genetic differences among us is underway, and should provide the information basis for a new approach to individual health. The challenge to the food industry will be to create product lines that will address individual dietary needs, and facilitate more personalized nutrition.

In the past few centuries, we have used our knowledge of the physical and biological sciences to improve the situation of our daily lives. In the 19th century, a fundamental knowledge of chemistry was acquired, and Mendeleev's periodic table began to take shape. This knowledge not only provided a deeper understanding of our world and ourselves, but also became applicable as the means to manipulate molecular structures to address specific end-points. Moving into the 20th century, knowledge of chemistry was used to affect and manipulate biology; examples of this activity range from pharmaceuticals to synthetic fertilizers to pesticides and food ingredients. These compounds not only changed the yields achievable on a given area of land, but also illustrated the ability to rationally modify agriculture and food production using scientific knowledge. However, chemical techniques have broad specificity in biological applications and thus do not offer precise control. In the 21st century, it will be our knowledge of biology and biological processes emerging from genomics which will guide our approaches to agriculture and food production toward sustainable, safe, nutritious, and delicious products.

Functional genomics is a term which has been applied to the field of discovering gene activities, and is a logical followup to genome acquisition. A beneficial by-product of functional genomics investigations will be the ability to use our mechanistic understanding of biology's structures and

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functions to manipulate a variety of organisms and biological materials to address specific food processing targets. As we become more familiar with the biochemical composition and structure of foods, with the metabolic needs of both pathogenic and commensal bacteria, and with the flow of biochemicals through metabolic pathways of plants, animals, and humans, we will acquire previously unknown dexterity in product development.

Bioguided processing refers to using our mechanistic understanding of biology to guide the processing of biomaterials for specific structures and/or functions as foods. More specifically, it incorporates biological structure and functional knowledge to process foods in such a way as to retain and concentrate their nutritive value, rather than using chemical and physical processes which eliminate the biological specificity of the raw materials.

Photo courtesy of Dairy Management, Inc.

Fig. 1—Milk is a good example of a food the synthesis and digestion of which are naturally bioguided. A mechanistic understanding of these pathways should improve the processing of milk and other products.

Traditional and Bioguided Processing

Contemporary separation operations in food processing schemes generally fractionate food constituents based on physical characteristics such as density, polarity, solubility, and size. Separation processes are monitored and streamlined on technological and not biological criteria. In fact, traditional food processing is largely designed to eliminate the unique properties of specific molecules. Instead, all biomolecules of a particular class, e.g., proteins, are exposed to substantial physical, thermal, and mechanical energy to make these properties uniform. This serves to restructure the material into more stable and/or more bioavailable food systems.

Processing replaces the biological complexity of biopolymers with the statistical average properties of their broad class, i.e., proteins, carbohydrates, and lipids. Such aggressive, nonspecific processing is designed to eliminate the deleterious and potentially antinutritive properties of food commodity organisms. Without these treatments, factors such as toxins, protease inhibitors, and hydrolytic or oxidative enzymes render the food unpalatable or, worse, overtly antinutritious. Unfortunately, such processing also eliminates potentially valuable structures and activities.

Bioguided processing, in contrast, is intended to account for the inherent biological composition of raw materials. Processes and separations are designed around specific organisms or biomaterials to utilize their unique properties and retain their biological and nutritive values.

Bovine and ovine milks are ancient components of the human diet, and many techniques developed to process and preserve these foods predate the Common Era. Through empirical observation, early humans learned to exploit the biochemistry of milk to produce more stable products such as cheese and butter. Brewing and winemaking are two other examples of early man's successful mixing of two processing streams (yeast and fermentable carbohydrate) to produce high-value, microbially stable end-products. In all these processing scenarios, part of the nutrition of the raw material is sacrificed in the short term (the whey fraction and lactose in cheesemaking, and fermentable carbohydrate in brewing and winemaking), so that a more-storable commodity is available long-term. Although calories of the fermentable carbohydrate are sacrificed for microbial stability, the utilization of this carbohydrate by the beneficial bacteria provides all the energy input needed to facilitate the product bioconversion.

Bioguided processing approaches have been discovered empirically and passed down as food technology through the centuries. A brewer cannot use raw barley to brew beer because the starch is physically inaccessible and therefore not fermentable by yeast. Instead, the germination process has been co-opted by maltsters to induce the catabolic enzymes responsible for endosperm conversion and starch hydrolysis.

As another example, while we are all familiar with the leavening effect yeast has on the dough during bread making, it is perhaps the reduction in phytic acid by microbial phytases which drove the development of this process by our ancestors. An inhibitor of mineral absorption, phytic acid represents 1% of wheat kernels, and thus is a substantial antinutritive factor in raw wheat. Yeast fermentations of dough carry out a highly specific biochemical conversion of the product, rendering it more nutritious, while consuming only a small amount of the fermentable carbohydrate and leaving the structure of the dough relatively intact.

Although our forebears had no knowledge of the underlying biochemistry employed in these conversions, they were able to discover, manipulate, and finally optimize these processes empirically in processing their raw materials. It is precisely the successes of foods such as cheeses, breads, fermented and cultured products, etc., that provide the promise of increased biological utilization for control of food processing. More than likely, the driver for the development of early biotechnology was the advantage that a community gained by storing nutritious food in times of plenty. Interestingly, the adaptation of these technologies to different territories and climates, and the subsequent optimization of the organoleptic properties of the resulting products has led to the array of styles of cheese, wine, and beer that are increasingly valuable even today.

However, taking such a perspective into new commodities and new foods requires that the specific details of both desired and undesired structures are known. In the past, trial and error (occasionally catastrophic error) repeated over generations yielded success. Now, trial and error will not suffice. Advances in analytics combined with genomics offer a new pathway to investigations into the composition and structure of raw materials. Newly acquired knowledge of biosynthetic pathways will serve as a starting point for material separation and processing. One of the obvious examples of how this science will proceed is provided by a biomaterial that was designed through evolution to be a processed food: milk (Fig. 1).

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Milk as a Model of Processed Food

A unique property of milks, in comparison with other foods consumed by humans, is that they have been guided during evolution expressly to be consumed and to supply nutrition to mammals. Through application of modern bioinformatic tools, the study of milk's synthetic genes, molecular physics of assembly in the epithelia, and digestive disassembly and nutritional targets in the neonate is providing an expanded view of nutrition (Ward and German, 2004). Such approaches will provide new principles and analogies to the bioguiding of raw materials for various food purposes.

The molecules and structures present in milk play roles in its secretion from the mammary gland, its delivery of nutrients to the neonate, and its other roles as a food. Furthermore, evolutionary selection for nutritional value was the guiding principle for all of milk's structures, constituents, and processes. For example, milk components stabilize reactive molecules, liberate constituents from larger complexes, and transport unstable molecules. They also hinder the transport of highly diffusible or volatile molecules, protect fragile items, and promote the self assembly and disassembly of multimolecular complexes.

However, the composition of milk remains constant neither in space nor time, nor across species nor maternal physiology. That milks differ markedly in composition across different species is a reflection of the varying nutritional needs of the newborns postpartum and the varying food properties necessitated by the lifestyles of different mammals. A thorough understanding of the structural results of the evolutionary pressure on milk and in particular the nutritional nanotechnology that has been incorporated into the structure of milk, will allow us to guide the synthesis and processing of new foods with unique health properties.

Although milk at one time was viewed only as an exogenous supply of nutrients for the infant, during the past few years bioactive components, with health effects disproportionate to their concentration, have continually been discovered and characterized (German et al., 2002). Milk is not simply a collection of nutrients appropriate for the neonate, but rather a dynamic structured material which has been designed by evolution to interface with a young mammal's biology. Breast-fed infants are less likely to have diarrheal disorders than infants fed formula (Keenan and Patton, 1995). In a particularly intriguing study, children drinking low-fat bovine milk were five times more likely to require medical care for acute gastrointestinal illness than children drinking whole milk (Koopman et al., 1984). This latter study implicates that it is the cream fraction, absent from skim milk, which may provide benefits to intestinal function and protection.

Milk is the product of mammalian genes, and the components of milk are the successes of evolutionary experimentation in nutrient vehicles. Therefore, lactation, and the subsequent milk produced, provides a distinct nutritional model for intensive deconstruction of a successful mechanistic system of food processing for nutrition. Uncovering the nutritional functions of the bioactive components of milk can be approached in unique ways by applying some of the emerging bioinformatic and knowledge tools of the genomics revolution.

For example, cross-species comparisons of the compositions and structures of mammalian milks with the level of development of the immune system and gastrointestinal tracts of the neonate is a point of access for investigations into milk bioactivity. Beyond the aggregate sum of bioactive nutrients, milk is processed, assembled (largely through guided self-assembly), and delivered in a compartmentalized fashion. Although the compartmental characteristics may well influence milk's various functionalities, they are largely unstudied, in part because it has been difficult to investigate as an isolated variable.

As scientists gain an understanding of the mechanisms by which these constituents function in the neonate, both individual nutrients and structural composite levels, they become potential beneficial dietary interventions for children and adults. Furthermore, a better understanding of the variation of individual metabolism proscribed by genotype will facilitate the design of diets to optimize personal nutrition and food processes to deliver them (Watkins et al., 2001).

Milk Fat Globular Membrane

In the epithelia of the mammary gland, fat globules are extruded from the surface of the cells into the alveolar lumen through a modified reverse endocytosis process. As a result of poorly understood events at the plasma membrane, all of the globules synthesized within the endoplasmic reticulum acquire a coat of apical plasma membrane from the host cell. Apart from certain viruses that share a similar excretion process, this mechanism of globule secretion from non-infected cells is unique in eukaryotic biology. Accordingly, much research on the milk fat globule membrane (MFGM) has centered on uncovering the biochemical mechanisms underlying its formation. Even when we have a better understanding of this unusual event, the process itself will not necessarily provide answers as to why it occurs. The MFGM certainly plays a role in stabilizing the fat globules against coalescence in the alveolar lumen, and it may confer some passive immunity to the nursling by binding enteric pathogens. Yet would these functions justify the evolutionary establishment of this unique process, considering the increased biosynthetic demand placed on the mother?

Infant mammals lack a fully developed immune system at birth, and considerable immune protection, both humoral and passive, is supplied by the milk of the mother. That MFGM is enriched in colostrums, the first milk expressed from the mammary gland, suggests that it may play a role in mediating immunity and development at this crucial junction. Since the gastrointestinal tract of a newborn human lacks the resident microflora of an adult, colonization must take place after birth to assure normal function and activity (McCracken and Lorenz, 2001).

Certain components of a mother's milk have been observed in scientific studies to selectively stimulate the growth of the beneficial microbes at the expense of others. Rueda et al. (1998) reported that supplementation of infant formula with gangliosides, a component of the MFGM, affected the intestinal microflora of preterm newborns by increasing the bifidobacteria content and lowering the level of *Escherichia coli.* Keenan and Patton (1995) suggested that a possible role for the MFGM is to simulate infant epithelial membrane in vivo, and thus serve as a decoy for intestinal pathogens.

Although the membrane makes up between 1 and 5% of the lipid fraction, the surface area in 1 mL of mature human milk is estimated to be 500 cm^2 (Ruegg and Blanc, 1981), and the proportion is higher in colostrum. Fig. 2 is a scanning electron micrograph of raw native bovine milk fat globules. It is evident from this image that there is a size distribution of globules from less than 1 µm to at least 5 µm, consistent with results from the literature.

Although the MFGM contains a significant portion of the compositional diversity of milk, and although its constituents are rich in micronutrient and trophic bioactivity, it is essentially processed out of the dairy products available today. Is it possible that this practice compromises some of the benefits that the native structure of the fat globule might confer?

In the current bovine milk-processing scheme, fluid milk is collected and then separated into the skim and cream fractions via centrifugation. Cream is added back to the skim fraction to create fluid milks of varying fat content. Other products, such as half-and-half, yogurt, and ice cream, are produced from mixtures of skim milk and cream, and excess cream is used to make butter. In the process reversing the oil-in-water emulsion of cream to a water-in-oil emulsion of butter, an aqueous by-product—buttermilk—is produced. The exact composition of this material is affected by both the composition of the cream and the processing conditions.

Traditional buttermilk, which is compositionally different from commercially available buttermilk, is rich in MFGM. When butter was produced at home, the aqueous phase remaining in the churn after removal of butter was often allowed to sour, and the resulting tangy liquid was sometimes used in baking. Anecdotal health claims have surrounded consumption of buttermilk for years. According to Irish lore, this liquid can assuage the symptoms of excess alcohol consumption and when heated with a clove of garlic serves as the cure for a variety of ailments. Pioneer women reputedly used buttermilk as a facial wash, and, not surprisingly, this material is rich in ceramide, a major skin lipid.

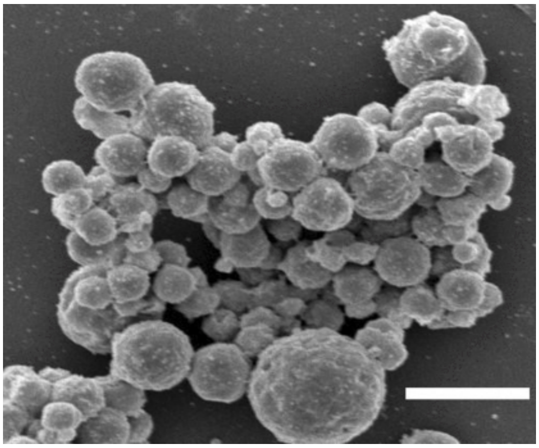

Fig. 2—Scanning electron micrograph of native bovine milk fat globules. Bar is approximately 5 μm.

As buttermilk had no clear endpoint application in early industrial butter production, like whey it was viewed in processing plants as a waste product and discarded. This process was wasteful and increased biological oxygen demand downstream from the processing plant. Buttermilk is now spray-dried and sold as buttermilk solids. Although the lipids from the MFGM are recoverable from buttermilk (Fig. 3), the polyunsaturated fatty acids of the phospholipids are labile to oxidation, and subsequent rancidity during spray drying thus compromises the nutritional and organoleptic properties.

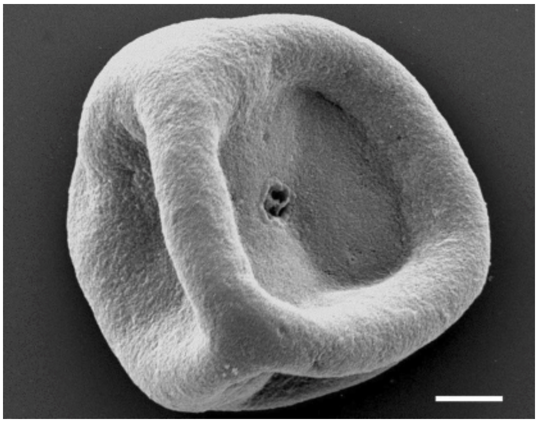

Fig. 3—Milk fat globular membrane. This material was recovered by ultrafiltration and supercritical extraction of the aqueous phase which results from butter production. Bar is approximately 1 μm. From Astaire et al. (2003).

As milk contains a myriad of beneficial components and structures, there is an opportunity for future bioguided processing to focus on creating dairy-based products designed to deliver specific nutritional benefits to consumers, while creating little or no waste.

Bioguided Approaches

Bioguided processing encompasses using the biology inherent in a raw material to design processing streams and endpoint applications. In designing such a scheme, factors affecting the precise composition of the raw material should be addressed first. Numerous studies have focused on the relation between a mother's diet and her milk composition, and this information may be useful in designing diets for the specific enrichment in milk of a particular chemical species associated with health. Alternatively, the elucidation of the metabolic pathways responsible for the ratios of different molecular species in milk and their subsequent manipulation through slight perturbations of metabolic pathway-flux may be used to produce milks with different compositions.

For example, human MFGM contains ten-fold more of the beneficial ganglioside GM1 than does bovine MFGM. Thus, a slight increase in the flux through the synthetic pathway of this molecule may result in bovine milk, or a product thereof, similar to the product evolutionarily designed for human consumption.

A further extension of this ideology is the manipulation of the intestinal flora of dairy cows to effect beneficial changes in the composition of their milk. Immune milk, produced by cows inoculated with human intestinal bacteria, has interesting bioactive effects in humans, such as a reduction of blood cholesterol in hypercholesterolemic patients.

A further understanding of the factors that affect the precise composition of mammalian milks should be provided by advances in bioinformatics and functional genomics, and should assist in the efficiency of the production of raw milk rich in bioactivity.

In bioguided processing, the inherent biological characteristics of a raw material are important not only in the determination of the endpoint applications of the isolates, but also in the processing pathways utilized in their isolation. Methods of processing based on biological specificity can be roughly broken into three categories: affinity-based, enzymatic, and microbiological. Thanks to advances in recombinant DNA, the technology to implement such processing schemes is currently available, and will continue to become more cost effective with time.

Surface recognition and non-covalent interactions are the basis for macromolecular associations within and between cells, and manipulation of these parameters affords great specificity in processing applications. Affinity-based binding and subsequent separation is a gentle separation procedure, as the chemical nature of the substrates is maintained. Removal of one specific component of raw milk may be achieved by a scheme analogous to affinity chromatography. Flowing the raw milk past surfaces to which affinity proteins are attached will allow for the specific removal of the species to which the tethered protein had affinity. The attached proteins may be immunoglobulins, lectins, or even enzymes engineered to retain substrate binding while being deficient in catalytic activity. In such a scheme, milk is diverted to surfaces containing these proteins until the binding sites are all occupied. The flow is then rerouted, and the adsorbed components are released by washing with a solution of different ionic strength.

Alternatively, in a process analogous to a "pull-down" assay, an affinity protein is attached to a small support molecule and dosed into a processing stream, where it binds specifically to its substrate. The complex is then recovered, and the target analyte collected. Affinity-based separations are most efficiently used to remove one or at most two molecules from a complex mixture. This process can be designed to create a final product minus one or two specific types of molecules, or, alternatively, the goal may be to isolate one or two specific molecules.

The use of enzymes in food processing facilitates the alteration or modification of the nature of raw materials, and it is effected with great specificity. Thanks to rDNA technology and expression systems and the expanding capabilities of biotechnology, enzymes are inexpensive, and will become less expensive as their prevalence grows. In recent years, the hydrolytic enzyme lactase, which breaks down lactose into glucose and galactose, has been used to produce dairy products for those who have trouble digesting milk sugar. Numerous enzyme activities may be utilized in removing, adding, or separating components of the MFGM.

Unlike affinity-based separations, enzymes catalyze the formation and lysis of covalent bonds and therefore alter the chemical nature of the substrates. This can be of use in processes designed to isolate or remove components from the MFGM. As non-covalent forces hold the membrane together, hydrolysis of any part of the amphipathic phospholipids leads to a change in their solubility. This scheme might be used to remove a specific component of the MFGM or to improve the emulsifying properties of ingredients made from the MFGM.

Enzymes are finding increasing usage in processing and manipulating lipid materials. Immobilized lipases are available for interesterification, which allows the production of triglycerides with specific functional properties.

Arguably, the lion's share of interest in functional foods involves the establishment and maintenance of intestinal health through the use of probiotics. The alliance of intestinal microflora and host epithelial cells is generally recognized as necessary for normal health, yet the mechanisms by which this alliance occur remain unknown (Vaughan et al., 1999). Nevertheless, research is underway to elucidate the biochemical basis of this symbiosis and should be facilitated by the application of molecular and bioinformatic tools.

Mollet (2001) observed the close phylogeny between organisms traditionally used in food fermentations and human microflora, and suggested that the relationship was established as a by-product of the awareness of the health-giving properties of food microorganisms and the history of their consumption. Sequencing of the genomes of food-grade bacteria is underway, and parallel developments in cloning techniques will lead to the availability of novel strains for future fermentations.

Treatment of milk fractions with commensal bacteria, or relatives thereof, will allow the development of new fermented products with novel applications. Furthermore, knowledge of the variation of metabolism of the microorganisms will allow the design of products in which the bacteria are optimally suited for survival in and subsequent colonization of the host. Bacteria might also be used in tandem, one to effect gross changes in the chemical composition of the raw material (e.g., drop in pH, hydrolysis of casein to form curd, etc.) and the second to act as a probiotic.

It is often stated that the genomic era will bring about dramatic changes in the understanding of human disease at the molecular level. This information will lead to the development of a new generation of pharmaceuticals designed to selectively target the genes responsible for disease, leading to an attenuation of symptoms. As these same developments will lead to the design of diets tailored for the individual, changes in food processing ideology are needed to provide the next generation of foods. Whereas in the current paradigm food production and processing are evaluated in terms of yield and efficiency, future processing schemes will be designed around effective delivery of the nutrients in food.

The processing of milk serves as an example for this coming paradigm change in food production. Milk is a complete food that evolved to provide nutrition to mammals. It provides biological functions deficient in the neonate, and milk's plasticity throughout lactation reflects the differing nutritional needs of the nursling. The application of genomic and informatic tools to the study of milk composition and function is leading to significant information on the molecular basis of nutrient–target cell interactions. As the biological functions of the constituents in milk are teased out, these molecules gain value as potential ingredients in functional food preparations.

Approaches in the design and production of functional fractions of milk can now begin with the inherent biology of the raw material. Bioguided processing takes advantage of the great specificity of biochemical reactions, and utilizes them to produce desired changes in the raw materials. Continued development in the production of biomaterials for processing, be it affinity-based, enzymatic, or probiotic, will drive increases in the creativity of food processing schemes and decreases in their costs.

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