Elevating optimal human nutrition to a central goal of plant breeding and production of plant-based foods

David C. Sands[1,*], **Cindy E. Morris**[1,2], **Edward A. Dratz**[3], and **Alice Pilgeram**[1]

[1] Plant Sciences and Plant Pathology Department, Montana State University, Bozeman, MT, USA

[2] INRA, Unité de Pathologie Végétale UR407, F-84140 Montfavet, France

[3] Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT, USA

Abstract

High-yielding cereals and other staples have produced adequate calories to ward off starvation for much of the world over several decades. However, deficiencies in certain amino acids, minerals, vitamins and fatty acids in staple crops, and animal diets derived from them, have aggravated the problem of malnutrition and the increasing incidence of certain chronic diseases in nominally well-nourished people (the so-called diseases of civilization). Enhanced global nutrition has great potential to reduce acute and chronic disease, the need for health care, the cost of health care, and to increase educational attainment, economic productivity and the quality of life. However, nutrition is currently not an important driver of most plant breeding efforts, and there are only a few well-known efforts to breed crops that are adapted to the needs of optimal human nutrition. Technological tools are available to greatly enhance the nutritional value of our staple crops. However, enhanced nutrition in major crops might only be achieved if nutritional traits are introduced in tandem with important agronomic yield drivers, such as resistance to emerging pests or diseases, to drought and salinity, to herbicides, parasitic plants, frost or heat. In this way we might circumvent a natural tendency for high yield and low production cost to effectively select against the best human nutrition. Here we discuss the need and means for agriculture, food processing, food transport, sociology, nutrition and medicine to be integrated into new approaches to food production with optimal human nutrition as a principle goal.

1. Introduction

An obvious but rarely stated goal of food production is to provide the human species with the proper nutritional factors to allow each person to reach their full intellectual and physical potential. However, in practice the two most important drivers of food production are optimal yield and minimal cost. The goals predicted for agriculture in the future forecast

*corresponding author: Plant Sciences and Plant Pathology Department, 119 Plant BioScience Building, Montana State University, P.O. Box 173150, Bozeman, MT, USA 59717-3150, tel: +1 (406) 994 5151, fax: +1 (406) 994 7600, dsands@montana.edu.

more of the same; i.e. that plant production will target supplying adequate calories for an ever increasing population, while also addressing the growing concern about the impact of agricultural practices on the environment [1], but not the need for superior nutritive value to enhance human health.

There has been increasing interest over the past several decades in improving the nutritional quality of crops. The wide-scale release of lines of quality protein maize (QPM) now being cultivated in Africa, Asia and Latin America [2] is one of the fruits of concerted efforts of breeders to improve nutritional quality of crops. The upcoming releases of biofortified crop varieties planned by programs such as HarvestPlus (www.harvestplus.org) and AgroSalud (www.agrosalud.org/) also illustrate the increasing interest in enhancing nutritional quality of crops. Nevertheless, enhancement of nutritional value of crops is high on the list of goals or accomplishments of rather few plant breeders, even though improved nutritional value is of critical importance for human well-being. In a hungry world, where the rate of increase of food availability trails behind the rate of increase of the demand for food, it is unlikely that enhanced nutritional value of crops can be a goal in itself. This will be particularly true when goals for enhanced nutritional value are for single nutrient traits rather than overall nutritional quality of crops. In this paper, we propose fundamental changes in current strategies of plant breeding, crop production, and food and feed processing to better facilitate the goal of production of more highly nutritious plants and plant-derived foods.

To develop commercial varieties of crops with enhanced nutrition, it may be essential to link nutrition to a commercial driver such as yield. In the case of crop lines biofortified for mineral nutrients, yield increases can go hand in hand with nutritional quality [3]. Unfortunately and inadvertently, high yield, pest resistance and low cost can also select against the best human nutrition, which we have called "The Breeder's Dilemma" [4]. In this paper we will point out more examples of this conflict and describe their origins. The exciting challenge for the future is to adjust research priorities in the way plant selection and plant breeding are accomplished to overcome this conflict. The ultimate goal is to create a sustainable food-production system that meets the needs of the global population in terms of calories and concomitantly addresses important needs for balanced amino acids, balanced essential fatty acids, optimal trace and macro- minerals, and vitamins, with foods low in toxins or anti-nutrients. The food production methods will need to account for the potential conflicts between optimal nutritional quality for humans and agricultural animals and the physiological needs of plants for growth and defense against pests and disease.

2. Evolutionary context of plants as food

Cereal grains are archetypical of the role of staple crops in the diet of modern humans and the effort invested in their breeding. Three cereal grains: wheat, maize (corn), and rice, constitute 75% of the world's total caloric intake. Over 50% of all the protein consumed by humans worldwide is low nutritional quality protein provided by wheat, maize, rice, barley, sorghum, oats, rye and millet [5]. From an evolutionary perspective, our modern reliance on grains is a dramatic shift away from the diet to which humans have evolved. Earlier than 15,000 to 100,000 years ago, *Homo sapiens* survived via hunting, fishing, and foraging. After about 10,000 - 15,000 years ago, during what might be considered as possibly the first 'agricultural revolution', a confluent series of factors contributed to domestication of plants including cereal grains as sources of food. The advantage of such grains was that they could be stored over several years, thereby being a reliable source of food during unfavorable conditions.

Some ethnological and archeological studies suggest that almost immediately after transition to grain-based diets, humans experienced reductions in stature, increases in infant mortality,

a shrinkage of life spans, increases in infectious diseases and multiple nutritional deficiencies, including iron deficiency anemia and mineral disorders impacting both bones and teeth [5-7]. It has been argued that widely differing assumptions about different preagricultural regimens have little impact on the estimated dietary essential omega-6/omega-3 fatty acid ratios, for example, which have major health impacts as discussed later [8]. We should note that there are other detailed ongoing studies of the Neolithic demographic transitions of instances where human populations rose 200 fold in a short period of time illustrating the disagreement about aspects of the Paleolithic diet [9]. However, it is well accepted that our foods have changed at a considerably greater rate than our inherent physiology and the underlying genes, which have changed rather minimally in the last 10,000 years [10,11] and essentially not at all in the past 40-100 years [12]. Modern progress in hygiene and developments in medicine have led to reduced infant mortality and longer average life spans, especially where cereal grain based diets could be supplemented with other sources of nutrients. Nonetheless, many of the negative consequences of the transition to grain-based diets remain with us today. The inadequacies of high carbohydrate and grain-based diets have been summarized at length [5,7,13]. Furthermore, over the last 200 years, as the population has become more urban, there has been a striking separation of food production from food consumption. Transportation of food away from its source of production has amplified the roles of marketing, intermediary distribution, and processing; and shelf life considerations have influenced the choice of varieties grown, thereby leading to reduced importance of traditional local foods.

The inadequacy of cereal grains as a primary food for humans arises from the fundamentals of plant physiology. Plant seeds are packed with compounds stored as sources of energy for supporting germination. Their carbohydrate, protein and lipid profiles reflect the specific requirements for seed and seedling survival. This nutrient profile, especially after selection during domestication [14], is far from optimal for human or animal nutrition. For example, the seeds of most cultivated plants contain much higher concentrations of omega-6 fatty acids than omega-3 fatty acids than is desirable for human nutrition [15,16], with few exceptions such as flax, camelina (*Camelina sativa*) and walnuts. Temperature is an important regulator of the ratios of the unsaturated fatty acids in plants, with growth at colder temperatures in temperate climates favoring the production of the omega-3 fatty acids in seed oils and moderate to warm temperatures favoring expression of the omega-6 and saturated fatty acids [17-19]. Seed germination during cold temperatures requires higher concentrations of polyunsaturated triglycerides that are in liquid form even at sub freezing temperatures. Cold tolerant crops such as camelina and flax contain seed oils that are high in omega-3 oils, possibly enabling germination in cold soils. However, the major oil crops, such as corn, soy, sunflower, safflower, and canola are warm- and hot-season crops that are high in omega-6 oils. High dietary intake of omega-6 oils impedes the desaturation and elongation of the omega-3 oils to produce the long-chain omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [16,20,21]. Higher intakes of omega-3 fatty acids and lower intakes of omega-6 fatty acid are correlated with decreased all-cause mortality, coronary heart disease, stroke mortality, bipolar depression, postpartum depression [22] and the reduction in the incidence of a number of cancers [23,24], and these issues will be discussed in more detail later in this paper.

In addition, many seeds contain compounds such as phytic acid which tightly binds zinc and iron to assure sufficient reserves of these minerals. Phytic acid-bound zinc and iron are insoluble and unavailable for assimilation by animals and humans, even though they are present in a diet [25]. And finally, certain cereal grain proteins can be antigenic. Between 0.5% to 1.0% of Western, Arabian, and Indian populations are genetically predisposed to celiac sprue syndrome, an autoimmune type of inflammatory disease of the small intestine triggered by the ingestion of wheat gluten (alpha-gliadin) [26]. Clearly, seeds have not

evolved to be ideal foods for animals and humans, and in fact they are anything but ideal foods for us. Likewise, other of our staple crops that are high in rapidly metabolized carbohydrates, such as potatoes or crops used as sources of sugars, are far from ideal sources of nutrition for humans (for a detailed overview of this subject see Taubes [13]). Breeding strictly for yield can exaggerate these nutritional inadequacies. We have described this problem as a breeder's dilemma, in that selection strategies for yield are frequently in conflict with selection for nutritional quality [4].

3. The Green Revolution

The green revolution was very successful in raising overall yields of foods, and in particular cereal grains. The breakthroughs in breeding came first in wheat and rice. Dwarfed lines were selected for high yield and agronomic characteristics, such as insect and disease resistance. These relatively quick technical fixes, along with increased fertilizer and herbicide use, tripled the yields of cereal grains as well as other staple crops such as potatoes. The increased yields helped those nations on the brink of starvation traverse to a period of relative food security. These spectacular results in increased food production were due to the visionary leadership of a small core group of agriculturists who clearly defined the principal limiting factors in cereal grain production and organized a global cadre of scientists, economists and politicians to carry out their mission of increasing crop yield (This unprecedented accomplishment of Dr. Norman Borlaug and his colleagues was recognized with a Nobel Peace Prize in 1970). Generous funding by the Rockefeller and Ford Foundations and later by a wide range of donors (primarily Western governments) facilitated implementation of the Green Revolution project and global adoption of high yielding grain varieties [27].

However, the predominant breeding and production strategies engendered by the Green Revolution gave us more high-carbohydrate, low-quality-protein cereal grain diets. These diets in Asia and South America have reduced the percentages of poor and malnourished (in rising populations), although they have not been as effective in Sub Saharan Africa, where the percentages of malnourished are increasing. We find it hard to ignore the fact that many people (more than 1 billion) are severely malnourished according to estimates reported in an FAO press release from June 19, 2009 (http://www.fao.org/news/newsroom-home/en/). The increased yields of Green Revolution cereals led farmers to switch from legumes to cereals. The high-yielding cereal crops lack balanced essential amino acids, balanced essential fatty acids, vitamins, minerals and additional quality factors. Apparently, the priority to fend off starvation did not give sufficient consideration to nutritional quality, perhaps for technical as well as strategic reasons. In retrospect, inadequate nutrition may have contributed to downward cycles of poverty and health [28].

Malnutrition handicaps its victims with fatigue, lethargy, susceptibility to pathogens, and a range of health complications, thus limiting their ability to care for themselves, to obtain and benefit from education, and to contribute to society. Efforts to document the costs of malnutrition for human health and for society provide powerful testaments to the extent of these losses [29,30]. Malnutrition has multiple causes including lack of food (production), lack of resources to procure food (poverty and infrastructure), and impairment of ability to absorb nutrients (disease) [31]. When malnutrition is due to problems of food availability or procurement it is also often associated with limited food diversity and nutritional quality. In fact, lack of access to a diversity of foods is an indicator of poverty, and conversely dietary diversity has been proposed as an indicator of food security [32]. Diets that are heavily based on cereal grains or on a limited number of other staple crops may lack sufficient amounts of certain essential nutrients. In contrast, diets based on a diversity of foods tend to contain more complete nutrient complements. Thus, overwhelming reliance on cereal grains

intensifies malnutrition and complicates delivery of adequate nutrients to modern humans. The deficiency of many of the staple crops and lack of dietary diversity particularly aggravates malnutrition during weaning of children [33]. On the other hand, many chronic diseases are on the rise in developed countries that depend less on staples for essential nutrients. In this case the nutritional quality of staple crops used as animal feed effects the nutritional quality of the animal products consumed by people. In developed countries there is a profound concern about the increasing medical and social costs of chronic diseases. Improving the nutritional quality of staples and animal products consumed, especially their pro-/anti-inflammatory properties, arguably has great potential for having beneficial effects on the health and well being of the population, as well as reducing the costs of medical care, as will be developed further in this paper.

4. Law of the Minimum

Justus von Liebig's two hundredth birthday passed in 2003 with little fanfare, compared to that of Darwin. Nevertheless, Liebig imparted a rule-based paradigm that is one of the foundational principles of agriculture: Liebig's Law of the Minimum. In biological systems, certain inputs are essential. Optimal growth and/or performance will be limited by the least available of these essential inputs. For example, plants require light, water, nitrogen, potassium, phosphorous, and sulfur. Among these requisite elements, one is usually the limiting factor. If the plant lacks enough sulfur to grow, further addition of any of the other elements will not really help. Extra potassium cannot compensate for the missing sulfur. Addition of sufficient sulfur will allow the plant to grow until one of the other elements becomes limiting. Accordingly, agronomic efficiency and optimum plant growth is best obtained by balanced fertilization relative to water and light availability. For instance, adequate nitrogen fertilizer is required for high protein production in plants that do not fix their own nitrogen from the atmosphere, but the addition of nitrogen need not outpace other limitations.

The same Law of the Minimum paradigm can be applied to human nutrition. Calories, proteins (i.e. essential amino acids), essential fatty acids, minerals, or vitamins can limit growth and cognitive development. Beriberi (vitamin B1 deficiency), scurvy (vitamin C deficiency), and pellagra (niacin deficiency) are classic examples of how a single nutrient deficiency can have severe consequences on human health. In complex human diets, the limiting factor or imbalance of nutrients can be elusive and may well vary from individual to individual. Furthermore, diets can be deficient in multiple nutrients simultaneously [34,35]. These deficiencies can be partially masked by our ability to store excess caloric intake as fat, which is often misinterpreted as an indication of adequate nutrition. Obesity can result from consumption of an overabundance of calories or consumption of an unbalanced diet, so that much of the excess consumed carbohydrates and fats are stored. The human body strives to regulate blood sugar levels, and carbohydrates in excess of what can be burned for energy or stored as glycogen must be converted to fat [13].

Zinc is an example of an element that can commonly be limiting for both plant production and human development. Zinc can be deficient in soils due to geological and pedological history of a region or its deficiency can be caused or compounded by continuous grazing and cultivation of land that eventually depletes essential elements from the soil. The calcareous soils in much of the Middle East, Africa and Australia exhibit a low availability of zinc. This deficiency carries through to the animal and human populations because not only is yield reduced, but forage and grain from the stunted plants are also deficient in zinc. Symptoms of zinc deficiency in humans range from stunted growth in children, to geophagia in pregnant women, and abnormal behavior ranging from lethargy to violence in young adults [36-38]. It is no surprise that zinc is important in biological systems since zinc has many catalytic,

structural and regulatory functions in cells. For example, about 100 enzymes are dependent on zinc as a catalyst. The multitude of references on the structural, regulatory and catalytic roles of zinc are brought together on a university website (http://lpi.oregonstate.edu/infocenter/minerals/zinc/) Thus, a deficiency in zinc will affect numerous enzyme functions, greatly reducing cellular efficiency. A coordinated research effort in Turkey supported by NATO successfully addressed this zinc deficiency in soils and mandated zinc sulfate amendment of 300,000 tons of NPK fertilizer [39]. This effort has resulted in considerably higher crop yields in the Central Anatolian Plateau, and we might presume that zinc soil amendments resulted in more zinc availability in cereals, vegetables and animal products from the region, thus providing adequate zinc in foods to the consumers there.

5. Single Component Targets of Enhanced Nutritional Value

A review of the research on nutritional enhancement of crop plants reveals the focus on enhancement of single nutritional traits, such as provitamin A in Golden Rice [40] or increased lysine in wheat [41] or barley [42]. A number of other approaches to enhancing plant nutrition have been recently summarized [43]. While these efforts are certainly commendable, they are piecemeal approaches, frequently using plant species for experimentation that do not meet widespread agricultural needs, and have been focused on rather conventional physiological limits, with certain exceptions (viz. Golden Rice).

The complex biochemistry involved when a human body assimilates, metabolizes, and utilizes nutrients is now a much more tractable science than even a decade ago. The genes, protein isoforms, signaling networks, and the microbiome that influence these processes are becoming much better understood at the cellular level, as illustrated in part by the Human Metabolomics Database (http://www.hmdb.ca/), for example. Furthermore, so-called "diseases of civilization" in modern humans are being better characterized [44] and changes in gene expression hence in basic physiology due to the transition from a physically-active to a more sedentary way of life are being elucidated [45]. It is widely recognized that "systems biology" has the potential to increase the understanding of how nutrition influences metabolic pathways and homeostasis, how this regulation is disturbed in diet-related diseases, and to what extent individual genotypes and epigenetics contribute to such diseases [46]. These insights are being complemented by a re-evaluation of optimal human nutrition from the perspective of evolutionary adaptation and by comparison of the apparent needs of humans with those of other primates [47,48]. It is evident that numerous human diseases are linked to or aggravated by nutritional choices and those habits that we attribute to life-style. These go beyond some of the better understood afflictions that are clearly caused by diet and, in particular, by the nature of plant foods, such as favism [49], neurolathyrism [50], goiter and cretinism [51], and pellagra [52]. As genetic components of disease are uncovered, the often dominant role played by diet and lifestyle in health, and the interaction of diet and genes is becoming increasingly evident (examples include Type 2 diabetes [53], coronary heart disease [16], psychiatric disorders [54], Alzheimer's disease [55], celiac sprue disease [56,57], death from all causes and depression [22], and etc.). Furthermore, the link of human behavior – violence and aggression in particular – to nutrition is becoming more evident [58]. Given this current trend of discovery, and the relatively modest role that genetics appears to play in complex diseases (www.cdc.gov/genomics/public/index.htm), it seems likely that in the coming decades the connections between health and nutrition will grow very substantially and will be much more fully recognized. The nutrients that we selected for discussion below come from this contemporary perspective.

5.1 Protein quality

The most limiting essential amino acid in cereal grains tends to be lysine, followed by methionine, tryptophan, isoleucine, and threonine and thus these essential amino acids tend to be low in vegetarian (or mostly vegetarian) diets. The lack of the full complementation of essential amino acids reduces the effective protein content of plant-based foods. If essential amino acids are limiting in a diet, the rate of overall protein synthesis will be limited, especially in children.

The biological basis of protein limitation in seed-based foods appears to be the result of evolutionary strategies that plants use to build storage proteins. Seed storage proteins have evolved to store amino nitrogen polymerized in compact forms, i.e. in storage proteins such as zein in maize, gluten in wheat and hordein in barley. As the seed germinates, enzymes hydrolyze the storage proteins and the plant is able to use these stored amino acids as precursors to re-synthesize all of the twenty amino acids needed for de novo protein synthesis. In contrast, animals are unable to synthesize eight of the amino acids commonly found in proteins (lysine, methionine, threonine, isoleucine, leucine, valine, tryptophan, and phenylalanine), and a balanced supply of these essential amino acids must be obtained from food sources on a daily basis for optimal protein synthesis. In addition, some infants and small children, possibly due to epigenetic fetal imprinting [59], cannot synthesize sufficient arginine and possibly histidine in addition to these eight essential amino acids. Therefore, it is not surprising that plant seed storage proteins are far from ideal sources of protein nutrition for animals or humans. In particular, most cereal, rice and legume seed storage proteins contain high proportions of nonessential amino acids. Essential amino acids constitute less than 15% of cereal grain protein currently [60]. Zein, the principal storage protein of maize, contains no tryptophan, the precursor of serotonin, an antidepressive neurotransmitter necessary for mental stability [61]. We can hypothesize that there might have been behavioral effects of tryptophan deficiency on populations such as the Aztecs, Zulu and Balkan people who predominantly ate maize. In Desert locusts, *Schistocerca gregaria*, increased serotonin levels were linked to the transition from a solitary behavior to a swarming, gregarious phase [62]. Whereas zein contains no tryptophan and very low levels of lysine, rice protein is very low in leucine, and wheat and barley are low in lysine.

As a breeding strategy, high total protein content is not in itself a sufficient goal. An extremely urgent priority for improved human nutrition is to alter seed proteins to adequately supply the five most limiting essential amino acids, listed above, at the expense of non-essential amino acids. With recent current price increases in nitrogen fertilizers (2-3 fold, linked largely to fuel cost increases), this priority for a higher proportion of essential amino acids will be even more important, as the protein percentage of cereal grain seed weight is correlated with soil nitrogen availability. Perhaps rotation schemes of cereals with nitrogen fixing pulses, such as beans and lentils, will become more prevalent, reversing the present trend for predominance of cereals.

The most well-known efforts to improve protein quality in staple crops are the development of Quality Protein Maize (QPM) varieties containing enhanced levels of lysine and tryptophan [2]. Varieties with acceptable agronomic qualities were developed after over 30 years of classical breeding efforts, based on the *opaque-2* mutation that alters endosperm quality. The breeding effort was slowed in part by the enhanced susceptibility of the high-protein lines to ear rot and storage pests. But these deficiencies were eventually overcome by selecting for lines that dried more rapidly and that had less cracking of the pericarp.

In the laboratory of the first author we have taken two different approaches to improving the protein quality of crops. First, we successfully selected a series of high lysine wheat cultivars over a period of twenty years, by standard breeding methods [41]. This selection

involved screening a mutagenized M-2 population of spring wheat by placing embryos on embryo rescue agar dosed with lysine and threonine, as previously described [42]. This led to effectively selecting for constitutive production of lysine and for absence of the aspartokinase enzyme that is repressed by either lysine or threonine or both. Surviving embryos consistently had elevated levels of lysine relative to parental populations and the seed produced from these embryos also had increased levels of lysine. The increased nutritional value of these lines, however, carried a cost in terms of lower total yield. A striking result was that grasshoppers, aphids, rats and deer preferentially feasted on the foliage of these high lysine wheats in the field, rather than on neighboring conventional low lysine wheats. The highest lysine wheat had the highest predation and subsequently the lowest yield (D.C. Sands, unpublished field observations). In more controlled experiments, to validate evidence for the impact of nutritional quality of plants on their attractiveness to insects, we have observed that when L-lysine or L-methionine are added to the soil of potted wheat plants, grasshoppers quickly congregate on the plants with the added amino acids (D.C. Sands, unpublished data). Thus, we are led to the hypothesis that selection for insect resistance may have inadvertently resulted in the selection for lower nutritional value, as we have described in more detail [4]. Genetic engineering approaches, using seed-specific promoters for high lysine and methionine storage proteins, rather than elevating free amino acids in the leaves, might obviate the foliar predation.

Our second approach to improving the protein quality of plants was to design *ab initio* a "super protein" gene module high in essential amino acids [63]. The amino acid content of the super protein was designed to elevate its essential amino acid content to an even higher level than animal food proteins such as egg albumin, or infant food proteins such as milk lacto albumin (Table 1). This designed nutritional protein had 6-fold more lysine than the highest comparable cereal seed storage proteins. The levels of methionine, tryptophan, threonine and isoleucine were similarly elevated. The super protein gene was first expressed in *Escherichia coli* and then was transformed into potato, where there was a problem with genetic stability. This group of researchers then redesigned a more stable artificial seed storage protein, modeled after corn zein proteins, with about 80% essential amino acids consisting of 20-amino acid helical repeating units [64]. To modernize this approach, the sequence of the "super" storage protein could be improved to take into account codon usage frequencies, allergenicity, protein folding, digestibility, solubility and heat sensitivity, as well as sensory and organoleptic properties. Rao and Shewry in 2009 reviewed newer techniques of plant protein design including combinetorics, gene shuffling and rational design using site specific mutagenesis [65]. These methods have led to improvement in seed storage proteins and their expression in plants [65,66]. The level of synthesis of such proteins will be dependent on *in situ* availability of an adequate pool of free amino acids, itself a complex problem. If well-designed super proteins meeting the design parameters mentioned above are incorporated into cereal grain seeds and synthesis can be accomplished at substantial levels, the nutritional benefits could be enormous.

Manipulating the free amino acid content of plants should be done with caution, given that not all free amino acids are desirable at high levels in foods. Some amino acids are toxic or are precursors of toxins. For example, asparagine is the precursor of the toxic compound acrylamide, a carcinogen that is found in many foods after heating or baking [67]. Amino acids can also act as neurotransmitters, chemical messengers between cells in the brain and the nervous system, and in excess can lead to damaging effects on the brain under some common circumstances [68,69]. When an impulse arrives, neurotransmitters are released from the axon, diffuse across a short gap, and bind with an empty receptor on the surface of another neuron. Normally, the bound neurotransmitters are rapidly sequestered or inactivated. However, if the receptors are over-activated with an excess of neurotransmitter, a process called excitotoxicity can occur and nerve cells can be damaged or killed [68-70].

The amino acid glutamate is the excitatory neurotransmitter in about 80% of the brain synapses and stimulate calcium flow into the neurons [69]. When the glutamate concentration around the synaptic cleft cannot be sufficiently decreased and reaches excessive levels, the neuron damages or even kills itself (excitotoxicity). Some crucial parts of the brain (such as the hypothalamus) are not protected by the blood-brain barrier, the blood-brain barrier is ineffective in fetal and infant brains [71,72], is damaged in stroke and other diseases [73], can be damaged by blows to the head, the blood-brain barrier decreases with age [74], and hypoglycemia magnifies the sensitivity of the brain to glutamate excitotoxicity [75]. Aspartic acid is also an excitatory neurotransmitter [76,77]. Ingested proteins are hydrolyzed relatively slowly and the buildup of free amino acids in the bloodstream is minimized, whereas the ingestion of monomeric amino acids, small peptides, or prehydrolyzed proteins can lead to excitotoxicity, especially in sensitive individuals. Excitotoxicity is also the main damage mechanism in stroke, and contributes strongly to brain damage in traumatic brain injury, spinal cord injury, alcoholism, and neurodegenerative diseases (multiple sclerosis, Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), Parkinson's disease, and Huntington's disease) [77], which may be aggravated by excessive free amino acids in the diet. There are also well-known examples of neurological disorders induced by the consumption of toxic, free amino acids found in certain plant species. Neurolathyrism is a disease that leads to the permanent crippling of legs due the action of 3-N-oxalyl-L-2,3-diaminopropanoic acid (a structural analogue of glutamine) contained in grass pea (*Lathyrus sativus*) [50,78]. This drought-tolerant legume is consumed mostly in Ethiopia and the Indian subcontinent [50], but there is renewed interest for this crop in Europe [78]. The Western Pacific amyotrophic lateral sclerosis-parkinsonism-dementia complex (also known as Guam disease) is also suspected to be due to action of an amino acid found in cycads that is structurally similar to the neurolathyrism agent [79]. In the Pacific Islands cycad seeds are used to make flours.

5.2 Carbohydrate quality

A second breeding priority concerns the quality of carbohydrates. Gregor Mendel crossed several genotypes of peas (*Pisi sativum*), including wrinkled seed types and round seed types, which provide an interesting example. The wrinkled peas store carbohydrates as amylose, (unbranched and "resistant" starch) [80]. Conversely, the plump, round-seed types store carbohydrate as branched starch (amylopectin). Branched starch is more rapidly digested resulting in rapid sugar release (higher glycemic index) [81]. Foods with high glycemic indices are generally not recommended for sufferers of juvenile and adult onset diabetes (Types I and II respectively). Excessive carbohydrates must be turned into fat by the body, and excessive consumption of high glycemic index foods is correlated with the development of many chronic diseases, including type 2 diabetes, high blood pressure and heart disease [13]. Many crops including potato, cassava, maize, wheat and peas are packed with the rapidly hydrolysable branched starch (up to 80%) [82]. Breeding and selection of these modern crops is biased towards plump seeds and tubers that have a much higher abundance of branched starch. Selection against starch branching enzymes can be accomplished, either by observing recessive phenotypic traits such as shrunken or wrinkled seeds, or by use of one of several commercially available amylose/amylopectin assay kits. Increased awareness of the health benefits and availability of plants and foods with low glycemic indices might make considerable contributions to human health.

5.3 Fatty acid profiles

The lipid contents and even more importantly, the fatty acid profiles of foods are important for human nutrition. Lipids are largely composed of fatty acids that vary in carbon chain length and degree of unsaturation. Different fatty acids have different effects in humans. Omega-6 and omega-3 fatty acids are essential nutrients. Omega-6 fatty acids are precursors

to eicosenoids that are pro-coagulatory and largely pro-inflammatory. Inflammation and coagulation are requisite responses to disease and injury, but in excess inflammation appears to be a root cause of many types of chronic diseases, as will be discussed. Omega-3 fatty acids, in contrast reduce the production of and decrease the activity of pro-inflammatory eicosenoids, are a critical element in resolution of (recovery from) inflammation [83], and higher levels of omega-3 fatty acid are implicated in prevention of many types of diseases [15,16,22,84], especially at lower levels of omega-6 fatty acids [85]. Adequate levels of omega-3 fatty acids are also essential for optimal brain development in infants and young children [21], for most effective learning and memory[86], for reduction of violent behavior and to oppose depression [22]. The health benefits of omega-3 fatty acids are linked to adequate consumption of a relatively balanced ratio of omega-6 and omega-3 fatty acids. This is easier said than done. Linoleic acid, an omega-6 fatty acid, is the predominate fatty acid in most grains (Table 2) and in most vegetable oils (Table 3). Omega-3 fatty acids are considerably less available in modern foods. There are three major omega-3 fatty acids (ALA (alpha-linolenic acid, C18:3ω3) and longer chain omega-3 fatty acids (EPA (eicosapentaenoic acid, C20:5ω3) and DHA (docosahexaenoic acid, C22:6ω3)). The primary dietary sources of DHA and EPA are fish (although there are significant amounts of DHA in eggs of ALA-fed chickens and grass-fed meats) and the primary modern dietary sources of ALA are vegetable oils such as soy, flax, walnut, and camelina.

Small amounts of ALA occur in all green plant chloroplasts and in algae, and this ALA is concentrated by grass-fed animals and by fish. Humans can inefficiently elongate ALA to the more active DHA and EPA, however, high levels of omega-6 fatty acids in the diet inhibit the conversion of ALA to EPA and DHA [16,21,86,87]. A key point in understanding essential fatty acid nutrition is the fact that the desaturation of dietary omega-6 and omega-3 fatty acids (required for elongation of the hydrocarbon chains) compete for the same enzymes [85,88]. Estimations of the omega-6: omega-3 fatty acid ratio of the average western diet range from 10:1 up to 25:1, although the ratio indicative of a healthy diet appears to be closer to 2:1 [87,89]. Reductions in inflammatory diseases [90] (for example, coronary heart disease [15], asthma [91], arthritis [92] Alzheimer's disease [84], and cancer [23,24]) in developed nations are correlated with elevated omega-3 and reduced omega-6 intake (see reviews [16,22,86,93]). DHA and possibly EPA have further benefits including promoting cognitive development in babies [86,94]. Human breast milk is high in DHA, but only recently has infant formula been supplemented with DHA. In the US non-supplemented infant formula is sold along side the more expensive supplemented formula and DHA should be required in all infant formulas. Improved fatty acid nutrition would result from increased consumption of fish from cold waters, reduction of consumption of high omega-6 oils (corn, safflower, sunflower and cottonseed) and increase in consumption of omega-3 oils (flax, camelina or walnut), increases in the production and consumption of grass-fed, flax-fed or camelina-fed animal products (including dairy products [95]) or by development of plant sources high in DHA and/or EPA. The distribution of high omega-3 eggs from Camelina-fed chickens has been blocked in Montana by regulatory government action, thwarting this attempt to distribute healthier foods. The desaturase and elongase genes needed to convert ALA or SDA (stearidonic acid (C18:4ω3)) to the longer chain omega-3 fatty acids have been cloned from algae and fungi and expressed in common oilseeds (soybean [96,97], linseed [98] and canola [99]). None of these transgenic crops are currently under commercial production. Omega-3 fatty acids are preferentially destroyed by hydrogenation to improve shelf life of products, which also produces trans-fatty acids. In efforts to reduce trans-fatty acids in foods, low omega-3 soy is being developed to reduce the products of hydrogenation (the low omega-3 soy has a omega-6/omega-3 ratio of ~50:1, as opposed to traditional soy which has an omega-6/ omega-3 ratio of 7:1, as shown in Table 3).

5.4 Minerals and vitamins

The term micronutrients is used to refer collectively to the inorganic (minerals) and organic (vitamins) nutrients that are required by the body in small quantities and that must be obtained from foods. A recent report by United Nations System Standing Committee on Nutrition [28] estimated that more than half of the world population suffers from micronutrient undernourishment. This situation is attributed to crop production in soils depleted of essential elements or to consumption of (staple) crops with inherently low levels of bioavailable minerals, compounded by a lack of fresh fruit and vegetables or fish and animal products in the diet [100]. Cereal grains have especially low concentrations or low bioavailaility of many micronutrients for humans, and it is notable that deficiencies in iron, iodine, zinc, and vitamin A rank among the most widespread. Each of these deficiencies could be overcome by food diversification or fortification (food supplementation), biofortification (crop fertilization or variety selection), or variety improvement. Food fortification with minerals or vitamins is common in developed nations (for example, iodized salt or vitamin-D fortified milk). However, fortified foods are not universally available, as many of the world's poor and malnourished are subsistence farmers and thus a large fraction of people in the world do not benefit from this nutrition strategy. A promising solution is to enhance the content or the bioavailability of micronutrients in staple crops (i.e. biofortification) [101]. This is the premise on which the HarvestPlus program was founded [3,102].

Crop fertilization for enhancing plant nutrient content can be costly and not economical in many regions of the world, although technologies of applying micronutrients on the seeds used for sowing have lowered costs. The minerals added to seeds may increase plant growth, but do not necessarily lead to accumulation of the nutrient in question in the consumed portions of the plant (grain, fruit, and seed) or the minerals may be bound in a form that cannot be readily absorbed, i.e. which are not bioavailable. It has been suggested that breeding efforts should focus on plant varieties that produce high yields and accumulate minerals in spite of low concentrations or low bioavailability of minerals in the soil [103]. Mineral bioavailability would be improved by selecting varieties with reduced levels of mineral chelating agents such as phytate or oxalate. Phytate and high levels of oxalate limit absorption of phosphate and cations, especially iron and zinc in the gut of monogastric animals [104] even though these elements may be abundant in the cereal seed. Food processing such as milling or polishing may further increase the deficiencies in iron, zinc and iodine. Increased consumption of certain organic compounds and vitamins (ascorbate (vitamin C), β-carotene (pro-vitamin A)) will increase absorption of some mineral elements [103].

Vitamin deficiencies are also widespread, disproportionately impacting populations who rely on cereal-based diets. For example, most animals can synthesize their own vitamin C (or ascorbic acid), but humans, and many other primates, lack an enzyme necessary for such synthesis and must obtain vitamin C through their diet [105]. All plants synthesize ascorbic acid, although the content is highly variable between plant species as well as within plant organs. Grains contain minimal ascorbic acid [106]. Historically, scurvy was a common disease among sailors and soldiers who consumed diets lacking perishable fruits and vegetables [107]. This disease is prevented or reversed by consumption of vitamin C, but the level of vitamin C that prevents scurvy is not sufficient for optimum health. In industrialized societies, ascorbic acid deficiency is avoided simply by consumption of fresh fruit and vegetables [108] (information also available on a university website http://lpi.oregonstate.edu/infocenter). The availability of ascorbic acid in grains can be increased by engineering plants with appropriate genes encoding biosynthetic enzymes [106]. Such intervention could generate plants with increased resistance to oxidative stress,

as well as fruits and vegetables with longer shelf life and higher nutritional value for humans [109].

Vitamin A deficiency is the leading cause of preventable blindness in children and deficiency increases the risk of disease and death from severe infections (reviewed in [110]). This essential vitamin is especially deficient in Africa and Southeast Asia. The dietary sources of vitamin A are preformed vitamin A (commonly found in meats and dairy) and provitamin A carotenoids (β-carotene, α-carotene, and β-cryptoxanthin) (found in yellow and orange-fleshed fruit and vegetables and in dark-green leafy vegetables) [111]. The efficiency of uptake of provitamin A and other essential carotenoids from the diet is enhanced by fat in the meal. The vitamin A concentration in breast milk is dependent upon the nutrition and health of the mother [112]. Provitamin A is found in a number of fruits and vegetables [113]. The content and bioavailability is highly variable between species and within varieties [114]. For example, white–fleshed sweet potatoes (*Ipomoea batatas*) do not accumulate β-carotene. In contrast, orange-fleshed sweet potatoes) are a rich source of β-carotene. Rice and wheat grains contain only trace levels of β-carotene [113]. The micronutrient content of rice is further decreased by dehusking because the provitamin A carotenoids are concentrated in the outer aleurone layer [115]. Golden Rice was developed by inserting two genes into rice that turn on β-carotene production in the endosperm [116,117]. The intensity of the golden color of the rice is indicative of the concentration of β-carotene in the endosperm. The response to golden rice has been mixed, ranging from ovation for its potential impact on world nutrition, to condemnation for development and promotion of a transgenic crop [118], which is unfortunate due to the health benefits that could result from its adoption.

Efforts to biofortify a wide range of crops are multiplying and include the HarvestPlus and AgroSalud programs mentioned above as well as programs funded by the Bill and Melinda Gates Foundation Program, "Grand Challenges in Global Health" (http://www.grandchallenges.org/). The productivity of these programs will depend on strong collaborations and relatively unencumbered exchanges of germplasm in order to identify and mobilize genes to increase nutrition, maintain yield and to counter pests. Genetically combining or even linking improved agronomic traits with nutritional traits could enable market competitiveness of nutritional crops. The scope of what remains to be done is vast and other sources of funding and additional research teams, particularly for enhancing protein and fatty acid qualities, need be deployed to better meet the nutritional needs of peoples in underdeveloped as well as in developing and developed nations.

6. Methods for Enhancing Nutritional Traits in Plants

Do breeding and selecting for nutritional quality require approaches that are not already part of those currently employed to enhance crop quality? Here we argue that there is a critical need for innovation to surmount such paradoxes [4] that tend to decouple improved nutrition from yield. Overcoming this dilemma is critically important for nutritious cultivars to be competitive with other high yielding cultivars.

Firstly, it should be noted that progress has been made with traditional approaches to improving crop nutritional quality. The original selections of high protein mutants of maize that led to QPM were identified in a mutagenized population using traditional breeding techniques [119,120]. The resulting breeding lines had higher lysine and tryptophan relative to the wild type, had altered endosperm and proved to be devoid of two storage proteins (19 and 22 kD alpha zeins). In wild type maize, the major seed storage proteins are deficient in lysine and tryptophan, which contribute to the poor nutritional quality of the seed. The extensive screening that has also led to the identification of low phytate lines of maize,

barley, wheat and rice is feasible for other crops such as sorghum and millets, as reviewed previously [121].

More recently, molecular and transgenic methods have been used to develop plant lines with increased nutritional value [43]. Concerning protein quality, the content of essential amino acids can be raised by engineering with feed-back insensitive biosynthetic enzymes and by inhibiting their normal catabolism [122], by displacement of low quality endosperm proteins (i.e. zein, hordein) with higher quality endosperm proteins, or by decreased degradation of amino acids [123]. For example, the lysine content in soybean or rapeseed transformed with bacterial genes encoding feedback insensitive lysine synthesis genes is nearly double the lysine content of the parental lines [124]. In a further attempt to enhance lysine concentration in grains, a feedback insensitive dihydrodipicolinate synthase from maize has been expressed in rice [125]. Higher lysine plants can also be engineered by gene suppression techniques. Maize was generated with reduced levels of expression of zein protein in the endosperm [122,126]. The plants compensate by increased production of non-zein proteins, which resulted in an improved amino acid balance, but also in higher levels of the free amino acids, asparagine, aspartate, and glutamine (see section 5.1 for the down-side of high levels of free amino acids in human nutrition, but which might well be beneficial for animal feeding). High lysine maize plants were also generated by decreasing expression of the gene encoding lysine ketoglutarate reductase, an enzyme that catabolizes free lysine in maize kernels [127]. Efforts have also been made to identify plant proteins that are inherently rich in lysine and to increase their lysine content where possible. For example, mutagenized forms of genes for the barley chymotrypsin inhibitor 2, that code for proteins with increased frequencies of lysine residues compared to the wild type protein, have been incorporated in vectors for transformation of sorghum [121]. Direct transfer of a lysine-rich pollen-specific protein from potato, without subsequent mutations, led to an increase of protein content and up to a 50% increase in lysine content of maize [128]. Likewise, phytases or proteins that confer the capacity to accumulate micronutrients have been transferred to grains from micro-organisms or from other plant species in efforts to exploit transgenics for crop improvement [121]. Enhancing the nutritional quality of plants will often require modifying complex synthetic or metabolic pathways rather than single gene systems. In light of this, a method of combinatorial nuclear transformation has been developed to generate a diversity of combinations of transgenes for a complex metabolic pathway in a plant. This novel technique has been exploited to generate a library of metabolic phenotypes for the carotenoid pathway in maize [129].

Screening of varieties with altered nutritional quality can also be accomplished via classical chemical analyses. Most chemistry laboratories in universities have gas chromatographs and high performance liquid chromatographic equipment necessary to quantify levels of fatty acids, amino acids, and other nutrients and anti-nutrients. The problem of cost per sample sometimes necessitates preliminary screening, however high throughput methods can foster screening of populations of thousands of M-2 mutant plants, as has been done in searching for plants with an unusually high level of omega-3 fatty acids [130]. Bioassays of seeds, using nutrient-dependent microbes, can be developed for almost any nutrient, and these can be used as an inexpensive first screen for nutrient content (as described below). In cassava breeding for elevated provitamin A carotenoids, initial screening has been based on visual assessment of root color [131].

Innovative approaches to selecting for highly nutritious crops might be simple and counterintuitive. Deploying predators might be one example. Insects [132] and certain rodents [133] have an uncanny ability to find plants that have increased content of essential amino acids such as lysine, methionine and threonine. Plant breeding lines unusually attractive to herbivores might also have unusually high levels of desirable amino acids. Baby

chicks develop a high preference for a diet containing lysine only three days after hatching [134]. We are aware of a maize breeder who uses rodents in his barn as a preliminary screen to detect which lines are higher in lysine (Dave Christensen, private communication). Leaf bioassays with the lysine auxotroph *Leuconostoc mesenteroides (Pediococcus cerevisiae* (ATCC 8043)), in conjunction with the appropriate amino acid assay medium, can be used to verify the content of amino acids [135]. The *Leuconostoc* agar plate assay of half seeds can also be used to select for seeds with higher lysine content, as in the case of wheat and safflower seeds.

Predation might also be used to select varieties with decreased antinutritive content Cassava is cultivated throughout the tropics for its starchy roots that provide food for over 500 million people, as well as for livestock feed and industrial products [136]. There are over 5000 known phenotypically distinctive cassava varieties. All contain varying concentrations of the cyanogenic glucosides linamarin and lotaustralin, which are hydrolyzed to hydrogen cyanide (HCN) by endogenous linamarinase, when the tissue is damaged. The Tukanoans of northwestern Amazonia cultivate high-cyanogenic potential cassava (Kii) in preference to low-cyanogenic cassava (Makasera) [137]. Processing of the more toxic Kii is labor intensive relative to the Makasera. However, Kii produces significantly higher yields in comparison to Makasera. Insect predation is more intense on the less toxic Makasera, perhaps explaining the lower yields. Morant and colleagues [138] have demonstrated that the level of glucoside in plants can be increased by genetic engineering. Such plants have increased resistance to predation. Alternatively, gene silencing can be used to reduce the levels of glucosides resulting in plants with reduced content of anitnutritive compounds.

Insect preference might also be used to select for other nutritional attributes or possibly to introduce desirable traits into a population. For example, honey bees show a preference for oilseed pollen with high caloric value [139]. In an unrelated study, bee foraging preference was correlated with essential amino acid content [140]. These preferences might facilitate transfer of linked traits into outcrossed populations.

Another counterintuitive approach would be to use plant pathogens to select for plant lines that enhance the growth of debilitated (for example, auxotrophic) strains of the pathogens. Plant pathogenic bacteria and fungi are often capable of growth on minimal media without amino acids. However, mutants of *Ustilago* or *Pseudomonas* auxotrophic for a particular amino acid can be used as bioindicators of amino acid levels in a breeder's population. Genetic libraries of plant pathogens (e.g. *Ustilago maydis, Pseudomonas syringae*, and *Ralstonia solanacearum*) provide a wide range of auxotrophic strains to choose from. The usefulness of this method has not yet been demonstrated.

7. Beyond Crop Improvement: Enhancing Plant-Product Nutritional Quality

Horticultural practices and harvesting crops at the optimal state of maturity are obvious considerations for optimizing nutritional quality of plants [141]. In addition, post harvest processing of plant tissues has been used for millennia to enhance nutritional quality via either the release of nutrients that may otherwise be bound by plant tissues or by elimination or destruction of toxins and anti-nutrients. These include cooking (thermal processing), fermentation, germination, milling, and soaking of plant parts - and of seeds in particular. For example, thermal processing is well-known to enhance the nutritional quality of grain legume proteins by inactivating trypsin and haemagglutinins [142]. Heating can also destroy anti-nutrients such as goitrogens, thiaminases and phytates [143]. Germination of seeds (sprouting) exploits the natural breakdown of food reserves for plant growth and is accompanied by an increased activity of many enzymes such as amylase, protease, phytase and lipase that can enhance nutritional value of seeds as food for humans [142]. However,

post harvest processing is not always advantageous for nutritional quality. A Pan American Health Organization publication summarizes the lability of fats, proteins, amino acids and vitamins with regard to conditions of food processing [144]. Salt, heat, alkaline or acidic conditions, light, and copper cooking utensils can decrease concentrations of vitamins A, C, B_1 and B_2 and of folate and carotenoides, for example. The digestibility of some proteins, such as kafirins (prolamin storage protein) in sorghum, can be reduced via heating [145]. Nevertheless, post harvest processing is an important strategy for balancing the physiological constraints of plants for their health and vigorous growth with the nutritional needs of humans. We focus below on the particularly interesting possibilities offered by fermentation.

7.1 Expanding the use and capacities of microbial fermenters

Fermentation of plant tissues is used throughout the world to produce a myriad of beverages, breads, pastes, sauces, pickles, and textured meat substitutes [146]. Traditionally, fermentation results from the action of micro-organisms naturally present on the starter materials or by adding agents to promote fermentation. The action and proliferation of fermentation agents can be enhanced by salting, acidification or alkalization. These processes may enhance nutrient quality by, for example, solubilizing iron, improving bioavailability of vitamins, and hydrolyzing proteins. Fermentation can foster longer shelf life by inhibiting spoilage and the growth of human pathogens and can render foods more palatable and flavorful [146].

Fermenter strains could be modified or selected to be more efficient, better adapted to climate conditions for fermentation, to produce additional nutrients not available in the starter plant materials, and to minimize potentially negative effects of fermentation. The fermenter strains could be combined with a wide variety of cultivars of a given plant species to complement or replace plant breeding efforts. Modifying fermenter strains might be more cost-effective (and more rapid) than creating nutritious plant lines/cultivars.

An example of the application of modified fermenter strains comes from the work of the first author to fortify fermented wheat breads, using lysine-excreting sourdough bacteria (*Lactobacillus fermentum*) [147]. This work was carried out for baladi bread production, a traditional sourdough flatbread in Egypt. At the end of the day, any unsold bread is collected and added to poultry feed. The price of baladi bread is subsidized by the government, whereas the price of poultry feed depends on market value, and thus this bread could be more profitable as poultry feed than as human food. Baladi bread made with a lysine-excreting heterofermentative strain of *Lactobacillus fermentum* not only had increased levels of free lysine, but it also produced lactic and acetic acids and CO_2, thus requiring much less yeast. The baladi bread made with the enhanced fermentation agents also had a longer shelf life than traditional loaves of baladi bread [148]. Thus, the enhanced value of the bread was maintained for a longer period of time. Another potential application of modified fermenter strains, not yet attempted as far as we know, would be to make bread with oils from flax or camelina in combination with engineered yeasts that are capable of elongating the alpha-linolenic fatty acid (C18:3ω) to the long chain omega-3 fatty acids EPA and DHA. Transgenic yeast expressing EPA and DHA have been constructed [149]. The folic acid content of bread can also be increased by fermentation with selected strains of yeast resulting in a 3-5 fold increase [150] or by transgenic strains [151]. The folic acid contribution of the yeast could be further increased by selection for resistance to toxic analogs, a selection technique that has already been successful with plants [152]. One practical advantage of this approach is that it would be relatively straightforward to replace several of the strains of conventional yeast, of which there are only a few varieties, with new improved EPA/DHA-producing or folic acid-producing strains. Secondly, these same strains could be utilized for production of many different types of bread.

Fermentation with improved yeast strains might be the fastest way to increase the vitamin, micronutrient, and mineral content of breads and other fermented foods. This approach presumes that such microbes can be selected or constructed and that the nutritive-adding yeast can be produced and distributed through existing commercial channels. To date this has not happened. In the case of leavened bread, the bread baking industry will need to buy into the technology to enable broad distribution of the fortified products. Or governments could mandate their use, just as they mandated iodized salt, etc. Fermentation microbes might be beneficial to improve nutrition of traditionally fermented foods in other ways besides lysine, folate or omega-3 fatty acid enhancement. For example, fermentation organisms such as *Lactobacillus sanfranciscoensis* with phytase activity would release phosphate and zinc that are bound by inositol hexaphosphate (phytic acid) and thereby unavailable for absorption by humans and animals [153]. Bread yeasts capable of deaminating asparagine, a precursor of acrylamide, might prevent accumulation of acrylamide, a carcinogen, during baking. As mentioned above, acrylamides are wide-spread in foods and innovative efforts are needed for their elimination [67]. Microbial detoxification is also considered as a potentially effective means for reducing mycotoxin contents of foods [154].

The concept of uncoupling plant varietal selection from nutritional enhancement by treatment with selected microbes has the additional advantage that this would free plant breeders to focus on important agronomic fitness traits. Furthermore, mixed fermentation inocula could be conceived for the production of multiple nutrients that could not be achieved by plant breeding or by pure cultures of micro-organisms. The efforts of microbiologists could be linked to those of plant breeders through the selection of crop varieties that are better substrates for fermentation or that harbor, as epiphytes, the desired strains of micro-organisms for fermentation thereby facilitating natural fermentation processes.

8. Toward Humanistic Nutrition

Many of us who have become agricultural scientists were motivated by the challenge to 'solve the world food problem' or to 'feed the planet'. This goal is evolving beyond the objective of providing caloric diets for bare existence. Today the crucial challenge is to more optimally nourish humans, to provide optimal fuels and building blocks so that sub-optimal nutrition does not impair the functioning of human minds and bodies and prevent the attainment of full human potential.

We have described some of the initial efforts to improve the nutritional value of crops as food for humans, however, we believe that there is tremendous potential for significantly more nutritional enhancement of crops. But this enhancement will not occur as long as maximum and stable yield is the over-riding driver of the economic competitiveness of plant varieties. Some more powerful driving force is needed to trigger a change from scattered, piecemeal improvement of individual nutrients in a multitude of foods to creating more complete foods that provide the highest overall balance of necessary nutrients. This is especially important for peoples with access to a limited diversity of foods, be it for reasons of availability, culture or choice. One driver for enhanced nutritional value of crops may be the self interest of subsistence farmers who largely grow crops for their own consumption, which places them outside the influence of quality standards set by marketing boards. It should also be kept in mind that the nutritional value of staple crops fed to animals influences the nutritional value of the animal products utilized for human food, which is an extremely important factor for the nutritional state in developing and developed countries.

To meet the challenge of optimally nourishing human potential, the ties among the scientific and socio-economic disciplines related to food and diet - including agriculture, food processing, food transport, sociology, nutrition, and medicine – need to be much more integrated into approaches to food production that address key attributes of the human condition. One of these key attributes is that human potential is in large part defined by optimum biological functioning of the human body. A second of these key attributes is the human desire for self-determinism. 'Humanistic nutrition' is the name we propose for a food production system that addresses and integrates these attributes of the human condition. The tools available to implement such a humanistic nutrition project are partly the products of basic research in genetics, plant physiology, biochemistry, and the emerging new integrative field of systems biology [46,155]. The traditional fields of research have diverged into disparate and often poorly-communicating scientific disciplines such as food science, plant genetics and breeding, plant pathology, soil science, nutrition, toxicology, food microbiology, epidemiology, human/animal/plant biochemistry, and various fields of medicine. The goals of each discipline have largely become compartmentalized and most research, publication and funding mechanisms tend to reflect this stratification. The US National Science Foundation has recently increased funding for multidisciplinary approaches, to encourage investigators to overcome the tendency of scientists to stick with ever narrower subjects, with which they are comfortable. To advance 'humanistic nutrition' these all disciplines must somehow also be brought to interact more effectively with the health and medical sciences, as well as with the socio-economic sciences and government bureaucracies. Much greater understanding of optimum human nutrition in health, disease prevention and disease must be emphasized in all medical training and the medical establishment must move from a system of treatment to a system of prevention and health optimization. Farm subsidies or food aid programs, directed principally or even exclusively toward crops with more optimal nutrition, might be drivers for the increased interaction of these disciplines. It should also be kept in mind that the nutritional content of staples that are fed to domestic animals has a very large bearing on the nutritional value of the animal products for human consumption.

Solving the world food problem has been focused virtually entirely on the noble goal of alleviating hunger. The second half of the problem, optimally nourishing humans, and hence their potential, amounts to elevating the overall quality of existence in human health and productivity. If the elevation of nutrition is even partially achieved, this will likely have beneficial impacts on attainments in education, will enhance economic development, and will foster the pursuit of happiness and furthermore, do all of this in a sustainable manner. The elevation of human health will reduce the need for medical interventions, enhance the functioning of healthier immune systems, and promises to lower the cost of medical care. Thus, the stakes are very high.

The impediments to enhancing overall nutritional quality of plants are also numerous. These include lack of will and insufficient collaboration among many relevant scientists, patent thickets that hinder collaborations, the reduction of private foundation support during times of economic recession, sudden increases in the cost of phosphate and nitrogen fertilizers with fluctuations in the price of oil, higher disease and pest susceptibility of the most nutritious crops, the lack of enthusiasm for genetically engineered crops in some quarters, and a thicket of government regulation. However, the challenges of creating plant varieties and plant-food production systems in support of humanistic nutrition, and awareness of the critical importance of nutrition for enhancing the quality of life of people across the planet, might well attract a new breed of committed scientists, more focused on tackling novel interdisciplinary paradigms for linking crop and human development. It follows that new policies and approaches to achieve comprehensive training of talented scientists are clearly needed. Better training of health professionals about the importance of nutritional impacts

on minimizing inflammation and chronic disease could also provide a means to help turn the economic drivers toward better health through better food.

Part of the challenge in creating a plant-based humanistic nutrition will come through the effort required for validation of the impacts of enhanced nutritional quality of foods on human potential. Such validation will require increasing the linkage and interactions between guiding the production of improved plants with the needs of the biomedical medical sciences. Via this link, humanistic nutrition might play an important role in elevating the attractiveness of the plant and agricultural sciences for young researchers and to more clearly illustrate the importance of these disciplines to addressing world class problems. Validating the benefits of enhanced nutrition on animal health is relatively straightforward, in that diet can be controlled and computer driven formulations are commonly used. Validating the benefits of enhanced nutrition on human potential will involve epidemiological approaches and some intervention to confirm the improved nutritional status of populations, who benefit from improved foods. This type of validation is planned in the context of the HarvestPlus program (www.harvestplus.org) and has also been realized for populations benefiting from QPM (for example, [156]). With criteria for positive validation of health effects, foundation and government grants could require a human nutrition component to plant breeding research. Benchmark physiological assays for effects of nutritional factors on human health could include serum levels of essential amino acids and fatty acids, assaying for a "master regulator" that starts a cascade of events when any one amino acid becomes deficient [157] as well as assays for zinc and other mineral deficiencies (measured in hair). Indicators of basic physiological response could be eventually coupled to socio-economic and public health indicators such as cognitive capacities, frequency of acts of violence, rates of infectious disease and responsiveness of the immune system, for example [30,158]. These are just a few of the possible impacts of enhanced nutrition on society. Malnutrition is clearly not the only limiting factor in attainment of full human potential and nominally well fed people could also benefit from optimized human nutrition. Thus, a concerted multidisciplinary effort to promote humanistic nutrition in an unprecedented fashion sets an attractive stage to investigate the complex interactions of human nutrition, genetics, health and behavior under diverse environmental and cultural contexts. Mechanistic understanding of biology has been growing at an exponential rate in the "post-genomic era". As scientists, working in concert, we have before us an opportunity on an unprecedented, never before known in human history, to actively promote the development of health, well being and human potential for the benefit of all.

Acknowledgments

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Table 1

Essential amino profiles of Super-protein, lactalbumin, and hordein [63]

Protein	% of total amino acids					% of total protein
	Lysine	Isoleucine	Methionine	Threonine	Tryptophan	
Lactalbumin	9.1	6.2	2.5	5.2	2.2	25
Hordein	3.4	4.3	1.4	3.4	1.3	14
Super-protein	21.6	8.1	16.3	10.8	10.8	68

Table 2

Nutritional data for a range of grains and oil crops.

Grain	%Protein	% carbohydrate	% Fat	Omega-6 fatty acid (mg/100g)	Omega-3 fatty acid (mg/100g)	~omega-6: omega-3 ratio
Barley[a]	12.5 g	63.6	2.3	999	110	9:1
Wheat[a]	13.7	60.0	1.9	738	38	19:1
Maize[a]	6.9	69.0	4	1706	53	32:1
Oats[a]	16.9	54.7	6.9	2424	111	22:1
Rice[a]	7.1	78.5	0.7	146	31	5:1
Millet[a]	11.0	64.4	4.2	2015	118	17:1
Sorghum[a]	11.3	68.3	3.3	1305	65	20:1
Rye[a]	14.8	55.2	2.5	958	157	6:1
Soy[a]	34.5	25.6	20.6	10281	1378	7:1
Camelina[b]	23.8		43.3	6408	16367	2:5
Flaxseed[a]	18.3	28.9	42.2	5911	22813	2:7

[a] Information available at a variety of sources including www. nutritiondata.com.

[b] [159]

Table 3

Fatty acid profile of common vegetable oils and cod liver oil.

Oil	SFA	MUFA	Omega-6 FA	Omega-3 FA				~Omega-6: Omega-3 ratio
				ALA	EPA	DHA	Total	
Olive[a]	13.8	73.0	9.8	0.7	0	0	0.7	13:1
Soy[a]	15.6	22.8	50.4	6.8	0	0	6.8	7:1
Canola[a]	7.4	63.3	18.6	9.1	0	0	9.1	2:1
Corn[a]	12.9	27.6	53.5	1.2	0	0	1.0	44:1
Sunflower[a]	22.5	42.5	14.3	0	0	0	0	-
Camelina[b]	9.7	32.8	16.0	38.1	0	0	38.1	2:5
Flax[a]	10.0	18.5	16.8	55.0	0	0	55.0	2:7
Cod Liver[a]	22.6	46.7	1.0	1.0	6.9	10.9	19.7	1:19

[a] Information available at a variety of sources including www. nutritiondata.com.

[b] [159]