Fertilizing Crops to Improve Human Health: a Scientific Review

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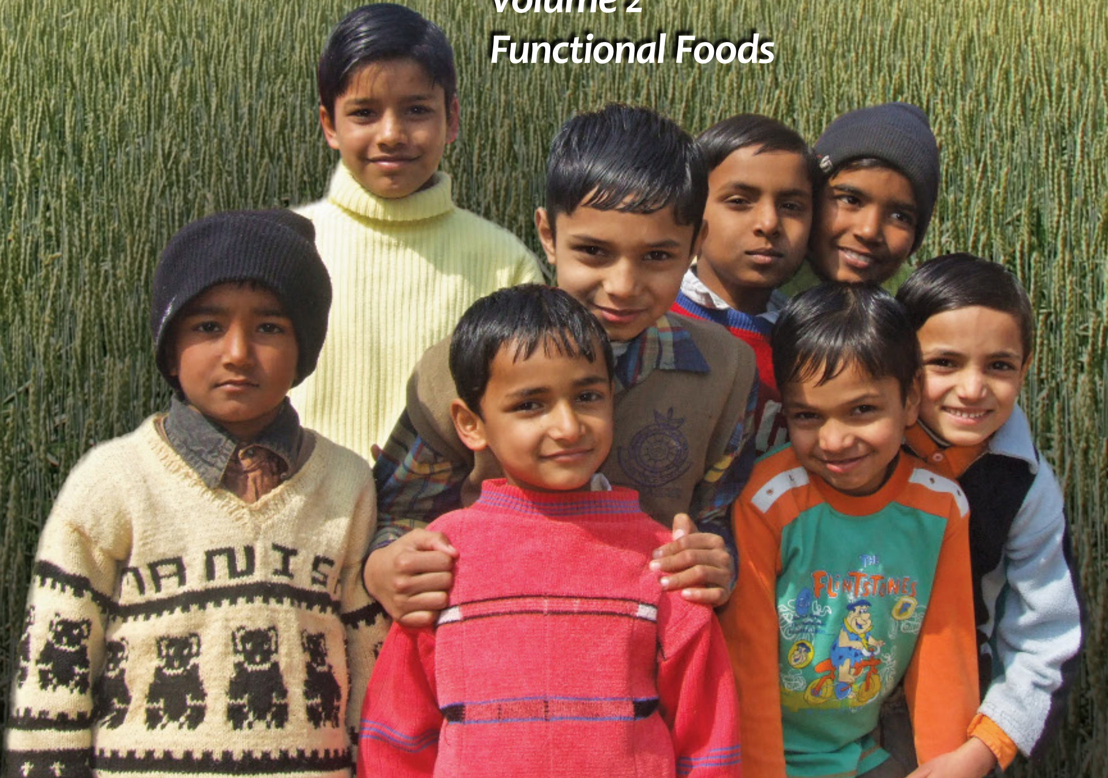
Volume 2
Functional Foods



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Abbreviations and symbols commonly used throughout this publication

Al	Aluminum
B	Boron
C	Carbon
Ca	Calcium
$CaCO_3$	Calcium carbonate
CaO	Calcium oxide
$CaSO_4 \cdot 2H_2O$	Calcium sulphate (gypsum)
CH_4	Methane
Cl^-	Chloride
Cu	Copper
$CuSO_4$	Copper sulphate
F	Fluorine
Fe	Iron
Fe^{2+}	Ferrous iron
Fe^{3+}	Ferric iron
H^+	Hydrogen ion
HCO_3^-	Bicarbonate
H_2O	Water
I	Iodine
K	Potassium
KCl	Potassium chloride (also muriate of potash or MOP)
K_2O	Oxide form of K, used in trade to express K content of fertilizer
K_2SO_4	Potassium sulphate (also sulphate of potash or SOP)
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
Na	Sodium
NaCl	Sodium chloride
N_2	Dinitrogen
NH_3	Ammonia
NH_4^+	Ammonium

Ni	Nickel
NO_2^-	Nitrite
NO_3^-	Nitrate
NO_x	Nitrogen oxides (nitric oxide and nitrogen dioxide)
N_2O	Nitrous oxide
O_2	Dioxygen
P	Phosphorus
PO_4^{3-}	Phosphate
P_2O_5	Oxide form of P, used in trade to express P content of fertilizer
S	Sulphur
Se	Selenium
Si	Silicon
SO_4^{2-}	Sulphate
Zn	Zinc

Chapter 5

Calcium, Magnesium, and Potassium in Food

Forrest Nielsen[1]

Abstract

The biochemical and physiological functions and consequences of deficient intakes, which show the nutritional importance of Ca, Mg, and K for humans, are reviewed. The dietary recommendations and food sources for these essential mineral elements for humans are presented. Factors that can influence the dietary intake and availability of these minerals for humans are discussed, including plant nutrition, and thus fertilization, impacts. Calcium, Mg, and K are essential for plants, in which they are widely distributed and have biochemical roles similar to those in animals and humans. Thus, foods of plant origin always contain measurable amounts of these minerals because of their need for growth and development. Increasing the amount of Ca to the root increases the amount of Ca in plants. Magnesium preferentially accumulates in grain when soil availability is low, but when Mg supplies approach adequacy, vegetative structures become storage sinks for Mg. As a result, Mg in foods can vary depending upon the environment in which they were grown. Increasing K to roots increases the K content of all organs of plants except seeds and grain. Thus, increasing soil K through fertilization may increase the K content of fruits and vegetables but not cereal grains. The preceding indicates that plant nutrition, which is impacted by fertilization, influences the amount of Ca, Mg, and K provided by foods of plant origin towards their requirements by humans.

Introduction

Calcium, Mg, and K are essential macro mineral nutrients for animals and humans. The essential functions of these mineral elements in animals and humans

Abbreviations specific to this chapter: AI = Adequate Intake; ATPase = Adenosine Triphosphatase; CRP = C-reactive protein; DRI = Dietary Reference Intake; EAR = Estimated Average Requirement; FAO/WHO = Food and Agriculture Organization/World Health Organization; μ*M* = micromolar; mmol = millimoles; NHANES = National Health and Nutrition Examination Survey; RDA = Recommended Dietary Allowance; RNI = Recommended Nutrient Intake; UL = Tolerable Upper Limit.
For abbreviations and symbols used commonly throughout this book see page v.

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are similar to those in plants. Animals and humans require much larger amounts of Ca than do plants, owing to its role in skeletal growth and maintenance. Because of this difference, Ca may be considered a micro mineral nutrient for plants, although it is most often classified a secondary macro nutrient. Calcium, Mg, and K have major metabolic functions throughout animals and plants, and thus are consistently found in food. Thus, in addition to assuring optimal crop production, fertilization practices may influence the meeting of Ca, Mg, and K requirements for humans.

Calcium

Nutritional Importance for Humans

Biochemical and Physiological Functions. Calcium has three major metabolic functions. Calcium is a second messenger that couples intracellular responses to extracellular signals, an activator of some functional proteins, and indispensable for skeletal function.

In its role as a signaling or messenger ion, Ca^{2+} mediates vascular contraction and vasodilation, muscle contraction, nerve transmission, and hormone action. In response to a chemical, electrical, or physical stimulus, extracellular Ca^{2+} enters the cell and increases intracellularly through release from internal stores such as the endoplasmic or sarcoplasmic reticulum (Awumey and Bukoski, 2006; Weaver, 2006). Increased intracellular Ca^{2+}, often in the form of a Ca receptor protein called calmodulin, stimulates a specific response, for example, activation of a kinase to phosphorylate a protein that results in a physiological response (Weaver and Heaney, 2006a).

A number of enzymes, including several proteases and dehydrogenases, are activated or stabilized by bound Ca independent of changes in intracellular Ca^{2+} (Weaver and Heaney, 2006a). These enzymes include glyceraldehyde phosphate dehydrogenase, pyruvate dehydrogenase, and α-ketoglutarate dehydrogenase (Weaver and Heaney, 2006a).

About 99% of total body Ca is found in bones and teeth. Bone crystals have a composition similar to hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], which contains about 39% Ca. The crystals, which have the ability to resist compression, are arrayed in a protein matrix, which has the ability to withstand tensile loads. Alterations in either the inorganic (hydroxyapatite) or organic (protein matrix) components can result in changes in bone strength (Rubin and Rubin, 2006). The skeleton must undergo continuous remodeling throughout life (it is replaced every 10 to 12 years) to adapt its internal microstructure to changes in its mechanical and physiological environment. Additionally, bone is renewed continuously to repair micro damage to minimize the risk of fracture.

Consequences of Deficient Intakes. The maintenance of extracellular Ca^{2+} by mobilization of skeletal Ca stores means that nutritional Ca deficiency almost never manifests itself as a shortage of Ca^{2+} in critical cellular or physiological processes

(Heaney, 2006). However, a low Ca intake may increase circulating 1,25(OH)$_2$-vitamin D to a point that it opens Ca channels in some cells (e.g. muscle and adipocytes), resulting in increased intracellular Ca (Weaver and Heaney, 2006a). The increased intracellular Ca may contribute to the development or severity of disorders such as those associated with obesity. However, for most healthy individuals, the main concern about Ca intake is an amount that will maintain bone health. If bone renewal during remodeling or turnover is slower than bone loss, osteoporosis may occur. If bone repairing is slower than micro damage accumulation, stress fractures may occur. In a large case-controlled study of hip fracture risk in women in Europe, fracture risk declined until Ca intake rose to an estimated 500 mg (12.5 mmol)/day (Dawson-Hughes, 2004). Calcium supplementation alone of individuals consuming more than 500 mg (12.5 mmol) Ca/day apparently does not decrease fracture risk (Dawson-Hughes, 2004; Shea et al., 2002; Jackson et al., 2006; Cumming and Nevitt, 1997). This finding is consistent with reports that the Estimated Average Requirement (EAR) for adults, based on well-controlled balance studies, is between 700 and 800 mg/day (Hunt and Johnson, 2007; Uenishi et al., 2001), but lower than the recent EAR of 1,000 mg/day established by the U.S Food and Nutrition Board (2010). The EAR is an estimated intake that will meet the requirement of 50% of individuals in a life stage and gender group. Most studies with adults showing a positive influence of high dietary Ca in decreasing bone loss or fracture risk also had supplemental vitamin D as an experimental co-variable.

Dietary Recommendations

Calcium intake recommendations vary widely worldwide (Looker, 2006), with the United States among the highest where the Recommended Dietary Allowance (RDA) for adults aged 19 to 50 years was recently set at 1,200 mg (25 mmol)/day (Food and Nutrition Board, 2010). The RDA is an average daily intake that is sufficient to meet the requirement of nearly all (97% to 98%) individuals in a life stage and gender group. The official United States RDA is similar to the RDA of 1,035 mg (25.8 mmol)/day for adult men and women suggested by an analysis of primary Ca balance data from tightly controlled metabolic feeding studies (Hunt and Johnson, 2007). The Ca Dietary Reference Intake (DRI; reference values that can be used for planning and assessing diets for healthy populations) in the United Kingdom is much lower; 700 mg (17.5 mmol)/day for adults aged 19+ years (Francis, 2007). In India, the recommended dietary allowance for Ca is only 400 mg (10 mmol)/day for adults (Harinarayan et al., 2007). Several countries and organizations, including the United States and European Community have established 2,500 mg (62.4 mmol)/day as the Tolerable Upper Limit (UL) for Ca (Looker, 2006). The UL is the highest daily intake that is likely to pose no risks of adverse health effects to almost all individuals in the general population. DRIs established by the U.S. Food and Nutrition Board (2010) for adolescents and adults are shown in **Table 1**.

The Food and Nutrition Board of the U.S. Institute of Medicine (2010) recently concluded that, with a few exceptions, most North Americans are consuming

Table 1. U.S. Daily Estimated Average Requirement (EAR), and Recommended Dietary Allowance (RDA) or Adequate Intake (AI) for Ca, Mg, and K (Food and Nutrition Board, 1997; 2005; 2010).

Life Stage Group	Ca, mg		Mg, mg		K, g
	EAR	RDA	EAR	RDA	AI
Males, age					
9-13	1,100	1,300	200	240	4.5
14-18	1,100	1,300	340	410	4.7
19-30	800	1,000	330	400	4.7
31-50	800	1,000	350	420	4.7
51-70	800	1,000	350	420	4.7
>70	1,000	1,200	350	420	4.7
Females, age					
9-13	1,100	1,300	200	240	4.7
14-18	1,100	1,300	300	360	4.7
19-30	800	1,000	255	310	4.7
31-50	800	1,000	265	320	4.7
51-70	1,000	1,200	265	320	4.7
>70	1,000	1,200	265	320	4.7

enough Ca. Based on intake data (Looker, 2006), most Europeans also are consuming enough Ca. However, inadequate Ca intakes may be a significant concern in other countries where the diet is refined grains or grains low in Ca (e.g. rice), and very little milk products are consumed. Examples of these countries are Bangladesh (Combs et al., 2005) and Nigeria (Thacher et al., 2009).

Human Calcium Intake Factors

Calcium Content in Foods. The most Ca-dense foods in Western diets are milk products; they contain about 300 mg (7.5 mmol) Ca per serving (e.g. 8 ounces of milk or yogurt; 1.5 ounces of cheddar cheese). Grains are not particularly rich in Ca, but when consumed in large quantities, they can provide a substantial portion of dietary Ca. For U.S. adults, dairy products provide 78%, grain products about 11%, and vegetables and fruits about 6% of dietary Ca (Weaver and Heaney, 2006a). The Ca contents of some foods of plant origin are shown in **Table 2**.

Interactions with Other Nutrients. In addition to content, bioavailability of Ca from foods is an important consideration for the provision of adequate needs. About 32% of Ca in milk and dairy products is absorbed (Weaver et al., 1999). Calcium bioavailability from foods from plants is typically lower than milk be-

Table 2. Calcium, Mg, and K (mg/100 g) content in selected foods as served of plant origin.

Food	Ca	Mg	K	Food	Ca	Mg	K
Fruits[†]				**Vegetables[†]**			
Apple	6	5	107	Lettuce	18	7	141
Orange	40	10	181	Celery	40	11	260
Banana	5	27	358	Tomato	10	11	237
Peach	6	9	190	Carrots	33	12	320
Strawberry	16	13	153	Onions	23	10	146
Pear	9	7	119	Peppers	10	10	175
Grapes	10	7	191	Potato[§]	8	20	328
Plums	6	7	157	Lima Beans[§]	32	74	570
Grapefruit	12	8	139	Navy Beans[§]	69	53	389
Cherries	13	11	222	Peas§	27	39	271
Avocado	12	29	485	Soybeans	145	60	539
Grains[‡]				**Nuts[†]**			
Barley	50	150	470	Almond	264	268	705
Corn	30	140	370	Brazil nut	160	376	659
Oats	70	140	440	Cashew	370	292	660
Rice, white	30	120	150	Pecan	70	121	410
Rye	70	140	520	Pistachio	105	121	1,025
Wheat	40	160	420	Walnut	98	158	441

† *Values from USDA Nutrient Database (2010)*

‡ *Values are for whole grains (not food as served) as reported by McDowell (1992)*

§ *Boiled, with salt*

cause of the presence of oxalate and phytate. For example, only about 5% of Ca in spinach, which is high in oxalic acid, is absorbed (Heaney et al., 1988). However, plants from the *Brassica* genus are unusual in that they do not use oxalic acid to detoxify excess Ca that would cause cell death. Thus, fractional absorption of Ca from vegetables such as broccoli (61%), bok choy (54%), and kale (49%) is higher than from milk (Weaver et al., 1999). These foods also are moderate sources of Ca with reported values (µg/g) of 493 for broccoli, 718 for kale and 929 for bok choy (Weaver and Heany, 2006b).

Phytic acid is the storage form of P in seeds. The amount of phytic acid or phytate in seeds, which only modestly inhibits Ca absorption, depends upon the P content of the soil that is growing plants (Weaver and Heaney, 2006b). Only foods with high phytate content, such as wheat bran and dried beans, significantly reduce Ca absorption (Weaver and Heaney, 2006b). Interestingly, food products from soybeans, rich in oxalate and phytate, have relatively high Ca bioavailability (Heaney et al., 1991). In addition, a study with Nigerian children indicated that Ca absorption was enhanced by increased phytate in a meal containing maize porridge (Thacher et al., 2009).

Because Ca and Na share the same transport system in the kidney proximal tubule, Na can have a negative effect on Ca metabolism. Every 1,000 mg (43 mmol) of Na excreted by the kidney results in an additional loss of 26.3 mg (0.66 mmol) of Ca (Weaver, 2006). This additional loss apparently is not offset by changes in Ca absorption because a high Na intake results in bone loss (Weaver, 2006).

Dietary protein increases urinary Ca loss, apparently through an increased urinary acid load caused by the presence of acids from the breakdown of S-containing amino acids (Cao and Nielsen, 2010). However, dietary protein does not decrease Ca retention because of offsetting changes in the absorption of Ca (Cao and Nielsen, 2010). Dietary P in forms other than phytate, such as that found in meat and carbonated beverages, also increases urinary Ca loss, apparently through increasing urinary acid load (Cao and Nielsen, 2010). However, similar to increased S amino acid intake, the increased P does not decrease Ca balance or retention (Heaney, 2008; Cao and Nielsen, 2010).

High intakes of Al, such as those resulting from the consumption of Al-containing antacids, can increase Ca loss. Therapeutic doses of such antacids can increase daily urinary Ca excretion by 50 mg (1.25 mmol) or more (Heaney, 2008).

Some non-digestible oligosaccharides (e.g. inulin) enhance Ca absorption and bone mineralization (Coudray et al., 1997; Abrams et al., 2005).

Plant Nutrition Impacts on Food Calcium

Nutritional Importance for Plants. Although usually classified as a secondary macronutrient, Ca may be considered a micronutrient for plants because their requirement for this element is small (Wallace et al., 1966; Marschner, 1995). The Ca requirement is much lower for monocotyledons than dicotyledons. For example, in well-balanced flowing nutrient solutions, maximal growth was achieved with 2.5 μM by ryegrass and with 100 μM by tomato, a factor of 40 difference (Marschner, 1995). Calcium is essential for the maintenance of plasma membrane integrity that facilitates ion uptake. Calcium also functions as a structural component in cell walls and as a second messenger in cellular signaling (Marschner, 1995). Calcium deficiency rarely occurs in non-legume plants grown on soils with appreciable cation-exchange capacity and a pH higher than 5.3 because the amount of Ca in soils is large compared to plant requirements (Barber, 1984). However, inadequate Ca for growth may occur on soils that are highly weathered, low in pH, and low in cation-exchange capacity. Crops with high Ca requirements, such as legumes, may need a high pH for some soils to supply sufficient Ca (Barber, 1984).

Soil solution and exchangeable Ca are the main forms that are absorbed by plant roots. Calcium in soil solution is balanced by soluble anions such as sulphate and carbonate. Calcium is the dominant exchangeable cation in many soils. Exchangeable Ca is in equilibrium with soil solution Ca (Barber, 1984). Alkaline soils containing Na, acid soils high in H^+ and Al, and serpentine-derived soils high in Mg have other dominant exchangeable cations (Barber, 1984). Exchangeable Ca is increased in acidic soils by liming, which precipitates out Al. Adding soluble Ca to acidic soils is not suitable for increasing exchangeable Ca because it displaces Al from cation exchange sites, resulting in an increase in Al solubility and toxicity, instead of precipitating Al into an insoluble form.

Factors Affecting Calcium Content in Foods from Plants. Assuring Ca requirements for plant growth assures that foods from plants will always contain some Ca, which can vary by soil Ca availability. Increasing the availability of Ca to the root increases the amount of Ca in plants. Increasing the amount of the cations Mg, K, NH_4^+ decreases the uptake of Ca by plants. However, plant species variation in uptake of Ca is the most significant factor in amount of Ca provided by foods from plants. For example, when exposed to similar solution Ca concentrations, uptake of Ca by tomato was the greatest; soybean and lettuce were intermediate, and wheat was lowest (Halstead et al., 1968). In general, soybeans, nuts, and *Brassica* foods are high in Ca, some legumes and vegetables are moderate sources of Ca, and cereal grains, especially without the bran component, and fruits are poor sources of Ca. The variation in the Ca content of foods from different plant species is displayed in **Table 2**.

Magnesium

Nutritional Importance

Biochemical and Physiological Functions. Magnesium is needed for enzymatic reactions vital to every metabolic pathway (Rude and Shils, 2006; Volpe, 2006). These reactions include those involving DNA, RNA, protein, and adenylate cyclase synthesis, cellular energy production and storage, glycolysis, and preservation of cellular electrolyte composition. Magnesium has two functions in enzymatic reactions. It binds directly to some enzymes to alter their structure or to serve in a catalytic role (e.g. exonuclease, topoisomerase, RNA polymerase, DNA polymerase). Magnesium also binds to enzyme substrates to form complexes with which enzymes react. The predominant role of Mg is involvement in ATP utilization. An example of this role is the reaction of kinases with MgATP to phosphorylate proteins. Magnesium exists primarily as MgATP in all cells. Magnesium at the cell membrane level regulates intracellular Ca and K, and thus, is a controlling factor in nerve transmission, skeletal and smooth muscle contraction, cardiac excitability, vasomotor tone, blood pressure, and bone turnover.

Consequences of Deficient Intakes. Based on dietary intake recommendations, subclinical or marginal Mg deficiency (50% to <100% of requirement) commonly occurs throughout the world (Nielsen, 2010). Yet, pathological conditions attributed specifically to dietary Mg deficiency alone are considered rare. However, epidemiological and correlation studies indicate that a low Mg status is associated with numerous pathological conditions associated with aging, including atherosclerosis (Ma et al., 1995; Abbott et al., 2003), hypertension (Ma et al., 1995; Touyz, 2003), osteoporosis (Rude et al., 2009), diabetes mellitus (Barbagallo et al., 2003), and some cancers (Dai et al., 2007; Leone et al., 2006).

The pathological conditions associated with a low Mg status have been characterized as having a chronic inflammatory stress component (Hotamisligil, 2006; Libbey, 2007). Human studies indicate that a low Mg status often is associated with increased inflammatory and oxidative stress. C-reactive protein (CRP) is a well-documented indicator of low grade or chronic inflammation (Ridker, 2007). Several studies have found that Mg intake was inversely related to elevated serum or plasma CRP (King et al., 2005; King et al., 2007; Bo et al., 2006; Song et al., 2007; Chacko et al., 2010, Nielsen et al., 2010). Low serum Mg concentrations also have been associated with elevated CRP (Rodriguez-Morán and Guerrero-Romero, 2008; Almoznino-Sarafian et al., 2007). Animal experiments, however, suggest that Mg deficiency in humans may play more of a contributory role than a primary causative role in pathological disorders characterized by chronic inflammation (Nielsen, 2010). Although severe Mg deficiency (feeding less than 10% of requirement) results in an inflammatory response (Mazur et al., 2007), moderate-to-marginal or subclinical Mg deficiency alone apparently does not markedly affect variables associated with chronic inflammatory stress in animal models (Vormann et al., 1998; Kramer et al., 2003). However, animal experiments indicate that moderate Mg deficiency can enhance the inflammatory or oxidative stress induced by other factors (Nielsen, 2010). Thus, based on the dietary recommendations given below, Mg deficiency may be a significant nutritional concern under conditions that cause oxidative or inflammatory stress, such as obesity and high dietary intakes of sucrose or fructose, that lead to chronic diseases associated with aging (Nielsen, 2010).

In addition to contributing to the risk for some chronic diseases, controlled metabolic ward studies indicate that subclinical or marginal Mg deficiency also can affect physical performance and heart function. Heart rate and oxygen consumption increased significantly during sub-maximal exercise when untrained postmenopausal women were fed 150 mg (6.17 mmol) compared to 320 mg (13.16 mmol) Mg/day (Lukaski and Nielsen, 2002). Postmenopausal women fed marginal Mg-deficient diets also exhibited heart arrhythmias and changes in K metabolism (Nielsen, 2004; Nielsen et al., 2007 Klevay and Milne, 2002).

Dietary Recommendations

The lack of usable data has been the basis for the difficulty to establish sound recommendations for Mg by various policy groups. The Mg RDAs for adolescents and adults set by the U.S. Food and Nutrition Board (1997) are shown in **Table 1**. The RDAs for adult men and women between ages 30 and 60 years were set at 420 and 320 mg (17.28 and 13.16 mmol)/day, respectively (Food and

Nutrition Board, 1997). These RDAs are consistent with the recommendation of 6 mg (0.25 mmol)/kg body weight/day suggested by Seelig (1981) and Durlach (1989). The U.S. RDAs were based almost exclusively on findings from one poorly controlled balance study performed in 1984 (Lakshmanan et al., 1984). In that study, subjects consumed self-selected diets in their home environment and were responsible for the collection of their urine, feces, and duplicate diet and beverage samples used in the balance determinations. The study design resulted in the finding of much overlap in Mg intakes that gave negative and positive Mg balances. Because of the tenuous nature of the data used, the North American Mg RDAs have been appropriately questioned. The Food and Agriculture Organization/World Health Organization (FAO/WHO, 2002) concluded that evidence was lacking for nutritional Mg deficiency occurring with the consumption of diets supplying a range of Mg intakes sometimes considerably less than the RDAs for the United States and Canada. Thus, the expert consultation subjectively set Recommended Nutrient Intakes (RNIs) for Mg at 220 and 260 mg (9.05 and 10.69 mmol)/day for women and men respectively.

There are reports suggesting that the RNIs set by the FAO/WHO are more appropriate than the RDAs of the United States and Canada. These include the findings of impaired physical performance and energy use and heart arrhythmias in postmenopausal women fed slightly less than 200 mg (8.23 mmol) Mg/day under controlled metabolic ward conditions (Lukaski and Nielsen, 2002; Klevay and Milne, 2002), which suggest that intakes less than the RNIs set by FAO/WHO probably would result in Mg deficiency. Balance data from 27 different tightly controlled metabolic ward studies found that neutral Mg balance, without considering surface losses, occurred at an intake of 165 mg (6.79 mmol)/day with a 95% prediction interval of 113 and 213 mg (4.65 and 8.76 mmol)/day (Hunt and Johnson, 2006). These latter findings suggest that adults should strive for dietary Mg intakes of over 220 mg (9.05 mmol)/day. The U.S. Food and Nutrition Board (1997) determined that Mg ingested as a naturally occurring substance in food would not exert any adverse effects. Thus, the adult UL for Mg was set at 350 mg (14.6 mmol) of *supplementary* Mg.

In the U.S., data from the 2005-2006 National Health and Nutrition Examination Survey (NHANES) indicated that about 60% of all adults do not meet the Mg RDA set by the Food and Nutrition Board (1997). It is estimated that about 10% of adults older than 19 years have Mg intakes from food and water that are about 50% of the RDA, an intake that may be insufficient according to balance data from controlled metabolic unit studies (Hunt and Johnson, 2006). In either case, intake data suggest that a significant number of adults do not consume adequate amounts of Mg.

Magnesium Intake Factors

Magnesium Content in Foods. **Table 2** shows that green leafy vegetables, whole grains, legumes, and nuts are rich sources of Mg (Volpe, 2006). Milk and milk products provide moderate amounts of Mg. Fruits, tubers, meats, and highly refined cereal grains are poor sources of Mg. Corn flour, cassava and sago flour, and polished rice flour are very low in Mg.

Interactions with Other Nutrients. Several dietary substances, including Ca, P, Zn, protein, vitamin B_6, and short-chain oligosaccharides may affect Mg metabolism. High dietary P was found to decrease Mg absorption (Rude and Shils, 2006). The decreased absorption may have been caused by the formation of insoluble Mg-phosphate. However, the decreased absorption was counterbalanced by decreased excretion such that Mg balance did not change. Magnesium absorption also may be decreased through binding with phosphate groups of phytate in high-fiber foods (Coudray and Rayssiguier, 2001). An increase in renal acid load, which might be induced by a high P or protein intake, may decrease Mg retention through increased renal loss (Rylander et al., 2006). On the other hand, a low protein intake also results in decreased Mg absorption and retention (Schwartz et al., 1973), which is consistent with the finding of decreased Mg balance (Hunt and Schofield, 1969) with a low protein intake. High Zn intakes of 142 mg (2.17 mmol)/day (Spencer et al., 1994) and 53 mg (0.81 mmol)/day (Nielsen and Milne, 2004) decreased Mg balance in adult males and postmenopausal women, respectively. Young women depleted of vitamin B_6 exhibited negative Mg balance because of increased urinary excretion (Turnland et al., 1992). Inulin (Coudray et al., 1997) and two fermentable polyols (Coudray et al., 2003) were found to increase the intestinal absorption of Mg.

Plant Nutrition Impacts on Food Magnesium

Nutritional Importance for Plants. Magnesium is an essential nutrient for plants, in which the relative abundance of Mg is less than N, K, and Ca and is similar to S and P (Wilkinson et al., 1990). The Mg requirement for optimal plant growth is in the range of 0.15 to 0.35% of the dry weight of the vegetative parts (Marschner, 1995). Magnesium is the central atom of chlorophyll and is needed for the aggregation of ribosome units for protein synthesis. Similar to animals and humans, plants have many enzymes that require Mg or have MgATP as a substrate. Thus, Mg is involved in DNA, RNA, protein, lipid, and carbohydrate formation and/or function and cellular energy production and storage. Intense cultural practices may increase the frequency of Mg deficiency in crop production, and the concentration of Mg in various parts of plants is affected by Mg fertilization (Wilkinson et al., 1990).

Magnesium is taken up by plants in the water soluble form (Mg^{2+}) from soil solution. The presence of this form is influenced by the exchangeable fraction of Mg in soils. The availability of Mg in acidic soils is reduced by Al and Mn and in alkaline soils is reduced by Ca, K, and Na (Wilkinson et al., 1990). Acid-forming N fertilizers (NH_4^+) are antagonistic to Mg uptake by plants, and may increase soil acidity, which increases exchangeable Al to compete with Mg (Wilkinson et al., 1990). Most Mg deficiencies in cultivated crops are the result of excessive K fertilization or concentrations in the soil (Wilkinson et al., 1990). Magnesium deficiencies also commonly occur in plants grown in severely weathered, wet, acid, or sandy soils (Wilkinson et al., 1990). The first symptom of Mg deficiency in plants is loss of chlorophyll (chlorosis), in which Mg is critical for its light-gathering function for photosynthetic C reduction.

Factors Affecting Magnesium Content in Foods from Plants. Because Mg is involved in many cellular functions, it is distributed throughout the plant. About 10% is bound to chlorophyll, 75% is associated with the structure and function of ribosomes, and 15% is bound to enzymes and other cation-binding sites (Wilkinson et al., 1990). The concentration of Mg in the food portions of plants is influenced by factors that affect the availability of Mg from the soil and Mg fertilization. For example, Mg fertilization (134 kg/ha) of sweet corn increased Mg in grain by 33%, and of snapbeans increased Mg in pods by 31% (Than, 1955). Fortunately for animal and human nutrition, Mg apparently preferentially accumulates in grain when availability of Mg to plants is low. When Mg supplies approach adequacy, vegetative structures then become storage sinks for Mg (Wilkinson et al., 1990). These findings indicate that the values for foods from plants, especially foods made from vegetative plant parts, can vary depending upon the environment in which they were grown.

Potassium

Nutritional Importance

Biochemical and Physiological Functions. Potassium is an activator or cofactor in some enzymatic reactions. These reactions include pyruvate kinase in carbohydrate metabolism that yields ATP, and Na^+, K^+-ATPase that is responsible for the active transport or pumping of Na^+ and K^+ in opposite directions across plasma membranes. This pump results in K being the major intracellular cation and Na the major extracellular cation. Potassium is the ion that neutralizes high concentrations of intracellular anions (e.g. proteins, phosphates, and Cl^-). In addition, a major function of K is membrane polarization, which depends upon the concentrations of intracellular and extracellular K (Preuss, 2006). The roles of K result in it being involved in acid-base regulation, osmotic pressure maintenance, nerve impulse transmission, muscle contraction, and carbon dioxide and oxygen transport (National Research Council, 2005; Preuss, 2006).

Consequences of Deficient Intakes. Because of its role in membrane polarization, the major effects of both hypokalemia (<3.5 mmol/L plasma) and hyperkalemia (>5.5 mmol/L plasma) involve changes in membrane function, which are particularly significant in neuromuscular and cardiac conduction systems (Sheng, 2006; Preuss, 2006). Potassium deficiency caused by low dietary intake rarely occurs because K is usually consumed in amounts required for obligatory losses and maintenance of tissue levels. Depletion occurs only when intake is inadequate during prolonged fasting or with severe dietary restriction (Sheng, 2006). Adverse effects of hypokalemia include cardiac arrhythmias, muscle weakness, and glucose intolerance. Cardiac arrest caused by abnormal electrical conduction is the most serious clinical manifestation of hyperkalemia.

Chronic low K intakes not resulting in hypokalemia have been associated with hypertension and its related cardiovascular disorders such as stroke. Numerous studies have shown that K supplementation may lower blood pressure, especially in salt-sensitive individuals (Suter, 1998; He and MacGregor, 2001; Food and Nutrition Board, 2010). A low K diet may cause Na retention and augment hypertension in hypertensive individuals (Krishna and Kapoor, 1991), and increase blood pressure in healthy individuals (Krishna et al., 1987). However, hypertensive individuals are more likely to respond to K supplementation than non-hypertensive individuals (Siani et al., 1991).

Chronic low K intakes not resulting in hypokalemia also have been associated with bone loss. This association is thought to occur through inadequate K intakes resulting in a disordered acid-base metabolism. Modern diets generally are high in acid-producing NaCl, P, and proteins (contain acid-producing S amino acids), and low in fruits and vegetables containing acid-balancing K and bicarbonate, which results in a metabolic acidosis. This acid-base imbalance has been associated with bone loss that may lead to osteoporosis (New et al., 2004; MacDonald et al., 2005). The suggestion that K deprivation adversely affects bone maintenance through disordered acid-base balance is supported by the finding that K citrate prevented increased urine Ca excretion and bone resorption induced by a high NaCl diet in postmenopausal women (Sellmeyer et al., 2002). However, some K supplementation trials have not shown significant positive effects on bone maintenance in older men and women (Dawson-Hughes et al., 2009; MacDonald et al., 2008). These conflicting findings indicate that further studies are needed to determine the significance of K intake in the relationship between metabolic acidosis and bone maintenance.

Dietary Recommendations

The U.S. Food and Nutrition Board (2005) were not able to establish an EAR or a RDA for K because of insufficient dose-response data. Instead, an Adequate Intake (AI) of 4.7 g (120 mmol)/day was set for all adults. The AI is set when there are insufficient data to set a RDA and based on estimates of an average intake by a group of healthy people. The Food and Nutrition Board (2005) stated that available evidence indicated that the AI should help blood pressure, reduce the risk of kidney stones, and possibly reduce bone loss. The Food and Nutrition Board (2005) noted that the beneficial effects of K mainly were associated with forms of K with bicarbonate precursors, which are the forms found naturally in foods such as fruits and vegetables.

Dietary intakes of K of adults in the U.S. and Canada generally are lower than the AI (Food and Nutrition Board, 2005). The median intake in the U.S. was found to be about 2.9 to 3.2 g (74 to 82 mmol)/day for men and 2.1 to 2.3 g (54 to 59 mmol)/day for women, and in Canada, 3.2 to 3.4 g (82 to 87 mmol)/ day for men and 2.4 to 2.6 g (62 to 66 mmol)/day for women (Food and Nutrition Board, 2005). Based on NHANES III data, the K intakes of only 10% of men and less than 1% of women in the U.S. are ≥ the AI (Food and Nutrition Board, 2005).

Potassium Intake Factors

Potassium Content in Foods. Because K is the principal intracellular cation in animals and plants, it is widely distributed in foods. Thus, a severely K-deficient diet is very unlikely. The richest plant sources of K are fruits and vegetables. Milk and meat products also are good sources of K. In one population study (Rafferty and Heaney, 2008), fruits and vegetables provided 44%, and dairy, meat, and cereal grains provided 56% of the K in the diet. Refined sugars and fats are very low in K. The K contents of some foods of plant origin are shown in **Table 2**.

Interactions with Other Nutrients. The beneficial effects of K supplementation usually are found when K is associated with bicarbonate precursors or organic anions such as citrate and malate (Demigné et al., 2004), which results in greater alkalizing potency; these are the major forms of K in fruits and vegetables. The K in cereals and animal products is chiefly associated with phosphate and Cl^- (Demigné et al., 2004), which are acidogenic; these foods also are high in acidogenic S amino acids (Food and Nutrition Board, 2005).

As indicated above, K interacts with Na and Cl^- to maintain acid-base balance and electrical and chemical gradients in the body. Also indicated above, is the fact that K blunts the effect of NaCl on blood pressure. The relationship between K and Na suggests the need for K may be increased by high intakes of Na.

Magnesium status also can affect the K metabolism. Magnesium depletion can cause hypokalemia (Whang et al., 1994). Both severe (Shils, 1980) and moderate (Nielsen et al., 2007) Mg deprivation increases urinary K excretion. Intracellular K is decreased during Mg depletion, which is enhanced by the inability of the kidney to conserve K (Rude and Shils, 2006). Repletion of the K deficit caused by Mg deprivation by supplemental K does not occur unless Mg is simultaneously supplemented (Rude and Shils, 2006).

Plant Nutrition Impacts of Food Potassium

Nutritional Importance for Plants. Next to N, K is the mineral nutrient required in the largest amount by plants (Marschner, 1995). Potassium in plants activates or stimulates numerous enzyme reactions, including pyruvate kinase, phosphofructokinase, starch synthase, and membrane-bound proton-pumping ATPases. Potassium is involved in protein synthesis, photosynthesis, and osmoregulation (Marschner, 1995). The K requirement for optimal plant growth is in the range 2 to 5% of dry weight of vegetative parts, fleshy fruits, and tubers (Marschner, 1995).

Potassium in soil occurs in soil solution, as exchangeable K, difficultly exchangeable K and as a mineral (Barber, 1984). Soil solution K is considered the primary source of K absorbed by the plant root. Soil solution K varies with weathering, past cropping practices, and fertilization (Barber, 1984). Exchangeable K held by negative charges on clay and organic matter usually ranges from 40 to 500 mg/kg soil; 150 mg/kg is considered enough to ensure optimal plant growth

(Barber, 1984). Because plants may absorb a large fraction of available soil K, and K movement from the difficultly exchangeable pool to the exchangeable pool does not readily occur in some soils, K fertilization of crops is important for production. Growth of plants is retarded in K deficiency, and in severe deficiency, plant leaves and stems become chlorotic and necrotic. Loss of turgor and wilting are symptoms of K deficiency when the soil water supply is limited. Potassium deficient plants are more susceptible to lodging, frost damage, and fungal attack (Marschner, 1995).

Factors Affecting Potassium Content in Foods from Plants. Increasing the supply of K to roots increases the K content of all organs of plants except grains and seeds, which maintain relatively constant K content of 0.3% of the dry weight (Marschner, 1995). Soybeans may be considered an exception, with K contents as high as 1.9% on a dry-matter basis. Cereal grains are the only plant food group that consistently yields noncarbonic acid precursors in excess of bicarbonate precursors (Food and Nutrition Board, 2005). Thus, increasing soil K to plants apparently will have limited impact on the amount and form of K in foods based on seeds or cereal grains. However, increasing K to roots may increase the K content of fruits and vegetables, which are characterized by having K associated with alkalizing bicarbonate precursors.

Summary and Conclusion

Calcium, Mg, and K are essential nutrients for humans. Foods of plant origin are good sources to help meet human requirements for these elements. Legumes or pulses (especially soybeans), nuts and some vegetables can provide significant amounts of Ca, which may be increased by available soil Ca. Green leafy vegetables, whole grains, legumes and nuts are rich sources of Mg. The Mg content in these foods can vary depending upon the environment in which they were grown. The richest sources of K are fruits and vegetables, but grains may provide significant amounts to the human diet. Increasing the supply of K to roots increases the K content of all organs of plants except grains and seeds. Because the Ca, Mg, and K contents of foods of plant origin vary with the amount and availability of these minerals in the soil, fertilization practices may have an impact on how well these foods contribute to meeting the requirements for Ca, Mg, and K. FCHH

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Chapter 6

Protein, Carbohydrate, and Oil Composition of Food Crops

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Abstract

Fertilizer use in crop production is often focused toward optimizing crop yield and profitability for the producer. However, it also affects the chemical composition of crops, potentially influencing the quality of food products made from them. Major crop quality factors include protein, carbohydrate, and oil; both the relative amounts and their specific composition and bioavailability can be important. To produce cereal grains with high levels of valuable protein, N must be made available at levels higher than those necessary for optimum yields. Management tools such as late foliar applications or controlled-release technologies can increase N availability for protein production while keeping losses of surplus N to a minimum. Applying other nutrients, particularly S, in balance with N is important to optimize protein for bread-making quality. Nitrogen applications increase the hardness of rice grains, reducing breakage during milling, and improving the amino acid balance from a nutritional standpoint. However, moderate rather than high levels of N optimize starch composition for improved cooking and eating quality. Higher levels of N increase protein in maize and potato, but reduce its biological value owing to lower ratios of the limiting amino acids lysine and tryptophan. However, opaque-2 cultivars of corn bred for high quality protein maintain high biological value at higher levels of N. Adequate K can help minimize acrylamide formation in fried potatoes. Enhancing S supply to soybeans during grain-filling can improve protein composition by increasing the ratio of the limiting amino acids methionine and cysteine. Where S limits yield in canola, the depressed yield can lead to increased oil concentration. In general, fertilizing for optimum yields does not differ greatly from fertilizing for optimum quality for most of the world's major food crops. In the long term, ensuring that soil fertility is maintained is important to avoid the major declines in crop yield and nutritional quality that can be seen when crops are grown on highly depleted soils.

Abbreviations specific to this chapter: QPM = Quality Protein Maize; LMWG = low-molecular-weight glutenins; HMWG = high-molecular-weight glutenins; CVD = coronary vascular disease; ALA = α-linolenic acid; GI = glycemic index.
For abbreviations and symbols used commonly throughout this book see page v.

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Introduction

Effective nutrient management is critical to optimize crop yield and profitability and to reduce the risk of negative environmental effects from fertilizer use. However, nutrient management also plays an important role in crop quality. Major crop quality factors include protein content and composition, oil content and fatty acid profile, and carbohydrate composition. The relative importance of these factors depends on the final end use of the crop. This chapter discusses the influence of fertilizer management on the composition of the major food crops rice, maize, wheat and potatoes, and the oilseed crops, soybean and canola (rapeseed), and how such changes in composition influence the nutritional and functional quality of the crops.

Quality Considerations for Food Crops

The three major staple food crops grown in the world are the cereals rice, maize and wheat (FAOSTAT, 2010). Cereals are an important source of energy, carbohydrate, protein and fiber in the human diet (McKevith, 2004). However, cereals are often consumed in a processed form, such as in breads and pasta, so quality of the product is often defined on the basis of the effect on the functional properties for processing, rather than solely on nutritional quality (Shewry, 2009).

Potatoes also make a major contribution to human nutrition, ranking fourth in world production volume after rice, wheat and maize (FAOSTAT, 2010). While considered primarily a source of starch, a potato crop can produce as much as 800 kg protein/ha, and its protein has been shown to have high nutritive value in comparison to other sources of plant protein including wheat, rice, maize, bean and soybean (Wang et al., 2008).

The two major annual oilseed crops produced in the world are soybean (*Glycine max* L.) and rapeseed (canola) (*Brassica spp.*), while palm oil, a product of the perennial oil palm tree (*Elaeis guineensis)* ranks between these two annual crops in terms of global oil production. Cottonseed, sunflower, peanuts and maize (corn) are also important sources of edible oil (USDA-FAS, 2010). In crops such as canola, soybean, sunflower, or flaxseed where the primary end product is oil, optimizing the concentration of extractable oil in the seed is desirable. The fatty acid composition of the oil is also important, affecting stability and health aspects. The meal that remains after the oil is removed is also important as a source of protein, and therefore, protein content is also a quality consideration for the oilseed crops. Soybean dominates the market both as a source of oil and meal and is a major protein source for vegetarian diets and in some Asian countries, including Japan.

Protein

Protein content and composition affects both the nutritional and functional properties of foods. In the UK, cereals contribute about 23 to 27% of the dietary intake of protein (McKevith, 2004), with the proportion likely to be higher in countries where animal protein consumption is lower. In wheat, protein is mainly stored as gliadins and glutenins, in rice as glutelin (oryzenin) and in maize as prolamin (zein) (Dewettinck et al., 2008). The nutritional quality of a protein source depends largely on the essential amino acid content as well as the concentration of protein in the processed grain (Gatel, 1994). Bioavailability or digestibility of the protein is also a factor. Cereal proteins in general tend to be low in essential amino acids such as lysine and to a lesser degree threonine, but contain relatively high amounts of cysteine and methionine. However, the glutelins in rice tend to have higher lysine content than the prolamins in maize and wheat, increasing its nutritional value (Juliano, 1999; Souza et al., 1999).

Depending on the end-use, cereals often undergo milling to extract the endosperm, leading to removal of the seed coat and aleurone layers, which contain much of the trace element content of the seed (**Figure 1**). However, proteins are located around the starch granules in the endosperm (Piot et al., 2000) as well as in the outer layers of the kernel, and although protein content decreases with milling, the decrease is not as great as that for the trace elements and vitamins (Batifoulier et al., 2006; Dewettinck et al., 2008; Greffeuille et al., 2005).

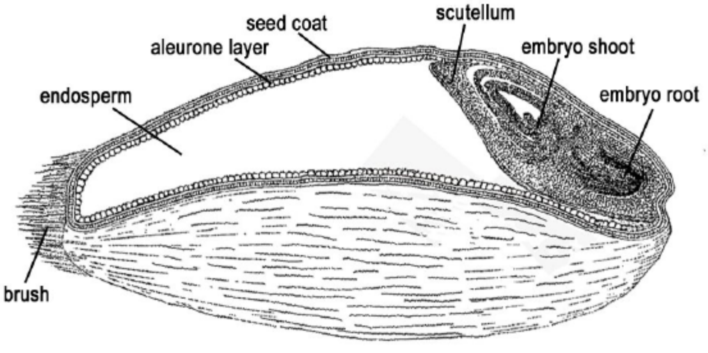

Figure 1. General structure of a cereal grain (from WHEAT: THE BIG PICTURE, http://www.wheatbp.net/cgi-bin/display.pl?image=Graindiag; accessed 20 January 2011).

Oilseed crops may also contribute to protein composition of the human diet, either directly, as a food source, or indirectly after utilization as a livestock feed. The meal left after oil is extracted from soybean or canola is high in protein and is commonly used as a protein supplement. Soybean is also used in the human diet, mainly as a protein source in infant formula, meat substitutes, soy milk, tofu and baked goods, being especially important in vegetarian diets. Soybean has a relatively well-rounded amino acid profile for a plant protein, containing adequate quantities of most of the essential amino acids, with the exception of methionine and cysteine. Soybean is particularly high in lysine, and combining cereals such as rice, wheat or maize with soy protein improves the protein composition of the diet (Erdman and Fordyce, 1989; **Table 1**).

Table 1. Concentration (g/100g) of dry matter, protein and selected amino acids in soybean meal, wheat, maize and rice.

	Soybean (meal) †	Tofu‡	Wheat‡	Maize‡	Rice‡
Dry matter	92	30	88	89	88
Crude protein	44	16	14	9	7
Amino acids:					
Methionine	0.59	0.20	0.22	0.20	0.17
Cysteine	0.67	0.22	0.33	0.17	0.15
Lysine	2.70	1.04	0.35	0.27	0.26
Threonine	1.72	0.64	0.39	0.35	0.26
Tryptophan	0.60	0.25	0.17	0.07	0.08
Arginine	3.29	1.05	0.60	0.47	0.59
Isoleucine	2.02	0.78	0.52	0.34	0.31
Leucine	3.39	1.20	0.95	1.16	0.59
Valine	2.11	0.80	0.62	0.48	0.44

† from Fontaine et al. (2000).

‡ USDA-ARS (2010). National nutrient database values for tofu (raw, firm), wheat (mean of durum and hard red), maize (yellow), and rice (raw, white, long-grain).

Carbohydrate

Carbohydrates comprise about 40 to 80% of the energy in the human diet, with the greatest proportion occurring in the developing countries. Starch accounts for about 20 to 50% of the energy in countries where the total carbohydrate intake is high (FAO, 1998). Cereal crops provide over 50% of the carbohydrate consumed, followed by sugar crops, root crops, fruits, vegetables, and pulses. Carbohydrates are classified according to their degree of polymerization into three major groups, sugars, oligosaccharides and polysaccharides (**Table 2**).

Carbohydrate quality is affected by a number of factors, with glycemic index (GI) being of particular importance. The GI measures the rate at which starch is digested to release energy, with low GI associated with a more prolonged supply of energy and a more stable blood sugar level over time. Glycemic response is affected by the nature of the monosaccharide components, the nature of the starch, the cooking and processing that the food undergoes, and other food components present in the diet.

As well as being a source of energy, carbohydrates provide an important source of dietary fibre in the form of inulin, cellulose, hemicelluloses, resistant starch and other components (Gebruers et al., 2008). Dietary fibre is associated with a reduced risk of chronic diseases such as cancer, coronary heart disease, and diabetes.

Table 2. The major dietary carbohydrates (adapted from FAO, 1998).

Class (Degree of Polymerization)	Sub-Group	Components
Sugars (1-2)	Monosaccharides	Glucose, galactose, fructose
	Disaccharides	Sucrose, lactose, trehalose
	Polyols	Sorbitol, mannitol
Oligosaccharides (3-9)	Malto-oligosaccharides	Maltodextrins
	Other oligosaccharides	Raffinose, stachyose, fructo-oligosaccharides
Polysaccharides (>9)	Starch	Amylose, amylopectin, modified starches
	Non-starch polysaccharides	Cellulose, hemicellulose, pectins, hydrocolloids

Oil

In crops such as canola, soybean, sunflower, or flaxseed where the primary end product is oil, optimizing the concentration of extractable oil in the seed is desirable. A secondary consideration is the effect on oil quality. In oils destined for human consumption, such as canola, maize, sunflower or soybean oil, it is desirable to maximize the mono-unsaturated fatty acids to improve the health aspects.

Oil is important to human health, particularly in developing countries, where it is in short supply and can potentially limit the absorption and retention of critical nutrients like vitamin A. While they noted that the amount of dietary oil required for optimal bioavailability of carotenoids from plants is not clearly defined, Brown et al. (2004) found that salad dressings containing canola oil markedly improved carotenoid absorption from salads consisting of spinach, romaine lettuce, cherry tomatoes, and carrots.

High levels of saturated fatty acids in the diet are associated with an increased risk of coronary vascular disease (CVD). In contrast, omega-3 polyunsaturated fatty acids ($n-3$ fatty acids) have been associated with a reduced risk of coronary heart disease. While fish oils are among the best sources of preformed long-chain omega-3 polyunsaturated fatty acids, α-linolenic acid (ALA), an essential shorter-chain omega-3 fatty acid, is present in flaxseed, soybean, and canola oils and is also of interest for CVD prevention (Jung et al., 2008).

Wheat

Wheat is a major component of the human diet throughout much of the world. Approximately 95% of wheat grown in the world is hexaploid bread wheat (*Triticum aestivum,* L.), used for a wide range of baked goods, including bread, cookies, cakes and biscuit, with most of the remaining 5% being tetraploid durum wheat (*Triticum durum*, Desf.) commonly used for pasta and noodles, couscous and bulgar. Protein content and composition is a critical quality factor in the production of these products (Shewry, 2009).

Protein

The protein content of wheat grain varies between approximately 10% and 18% of the total dry matter (Belderok, 2000). Nutritionally, wheat is a good source of many of the essential amino acids, although the prolamins that are the major storage protein in wheat are deficient in lysine, threonine and tryptophan (Shewry, 2009; **Table 1**).

Nitrogen is the major nutrient influencing protein concentration in grain. Nitrogen is a major component of protein (Olson and Kurtz, 1982), with approximately 17% of protein being composed of N. Numerous studies over the years have demonstrated the relationship between increasing soil N concentration and increasing grain yield and protein concentration (Fowler, 2003; Kindred et al., 2008; Miao et al., 2006; Miao et al., 2007; Olson et al., 1976; Terman et al., 1969). Nitrogen assimilation by the grain is more source-limited than dry matter yield (Gooding et al., 2007). If the amount of available N from the soil through the growing season is low relative to the crop yield potential, crop yield and protein concentration usually both increase with application of N fertilizer (Campbell et al., 1997; Fowler, 2003; Gauer et al., 1992; Halvorson and Reule, 2007). If crop yield is increased by factors such as beneficial weather, improved crop cultivars, or improved management practices, while the N supply remains constant, protein concentration often decreases by biological dilution (Fowler, 2003).

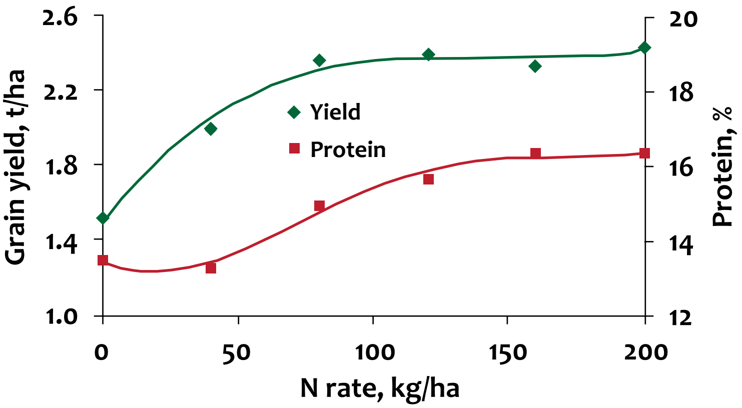

Figure 2. Yield and protein of wheat respond to applied N fertilizer.

The pattern of response of yield and protein content to N dose reflects the supply of available N relative to the crop yield potential. If N supply is restricting to yield, low doses of N fertilizer tend to increase grain yield preferentially over protein (Fowler, 2003). There may be a decrease in protein concentration with small applications of N, as the yield increase dilutes the protein with biomass (**Figure 2**). After an initial lag, protein concentration increases to an optimum—usually greater than the optimum for yield—and then levels off.

In semi-arid climates, where moisture is frequently the environmental factor that limits yield, moisture supply interacts with N in affecting protein concentration. Where moisture is ideal and yield is increasing substantially in response to N applications, protein increases are moderate until higher N rates are applied and the yield response begins to decline. With moderate moisture and N supply, both yield and protein increase with increasing N application rate. With moisture stress, yield is restricted and the major effect of increasing N application is to increase protein (**Figure 3**). In moisture-limited environments, changes in moisture supply essentially shift the protein-N response curve, so that with increasing moisture supply a greater amount of available N is required to produce a specific protein concentration in the grain (Gauer et al., 1992). As the available N increases relative to the crop yield potential, protein assimilation per increment of N decreases and the N use efficiency of the crop decreases. Therefore, applying N at rates greater than those required for optimum crop yield in order to increase protein content may lead to lower NUE and increased potential for N losses.

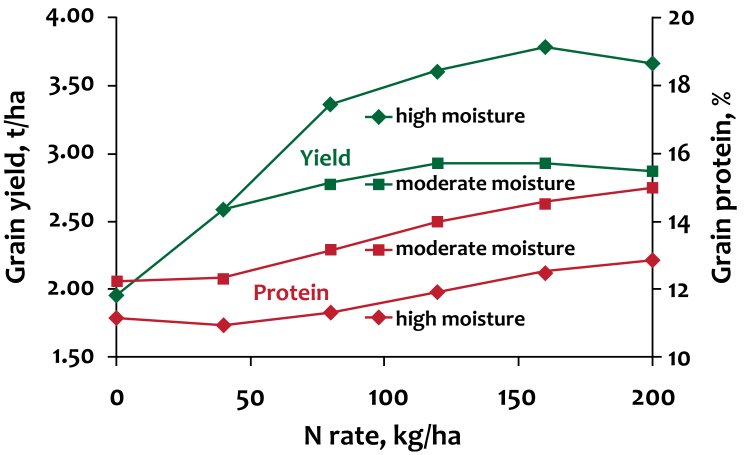

Figure 3. Grain yield and protein concentration of hard red spring wheat under moderate and high moisture conditions (adapted from Gauer et al., 1992).

Timing of N supply also influences grain protein concentration. Nitrogen available to the plant early in the growing season tends to stimulate vegetative growth and increase crop yield, while later applications have a smaller effect on yield but a larger effect on protein concentration (Fowler, 2003; Fowler et al., 1990). Yield potential is largely affected by pre-anthesis conditions that determine the grain sink in terms of spikelets and florets, and the biomass of stems and leaves that provide a source for subsequent re-translocation of N (Peltonen, 1993). Application of N after anthesis normally has a greater influence on protein accumulation than on starch accumulation (Souza et al., 1999; Wuest and Cassman, 1992). Nitrogen in the grain comes from post-anthesis uptake and from remobilisation and transport of N from the vegetative parts of the plant to the grain. In wheat, as much as 70 to 100% of N uptake may occur by heading (Boatwright and Haas, 1961; Malhi et al., 2006). Often, the N in wheat grain is primarily derived from translocation from leaves, stems and chaff rather than from further absorption from the soil.

With winter wheat, application of N in the spring or split with a higher proportion applied in the spring tends to produce higher protein concentration than fall application (Grant et al., 1985; Kelley, 1995; Vaughan et al., 1990). In no-till winter wheat grown in Saskatchewan, Canada, early spring applications of ammonium nitrate and urea-based fertilizers were effective at enhancing grain yield. Delaying the application by three weeks reduced grain yield response and grain protein yield, but increased grain protein concentration (Johnston and Fowler, 1991). Delays in the availability of N as a result of late spring applications of N on winter wheat or prolonged dry periods following spring fertilization can limit grain yield potential (Fowler et al., 1990). Then, if accessed later, the fertilizer N may be in excess of requirements for optimal yield, so grain protein concentration can increase. Use of enhanced-efficiency fertilizers, such as polymer-coated products, that delay the release of N into the soil solution may be effective at increasing protein concentration of crops by releasing the N for uptake later in the growing season (Grant and Wu, 2008).

Late applications of N can be effective at increasing protein content, particularly where environmental conditions promote losses of N prior to crop uptake. Extra N applied during the post-anthesis phase can increase the rate of grain protein synthesis, if the pre-anthesis N supply is low and the crop is able to absorb the late-applied N (Peltonen, 1993). Uptake of N fertilizer applied at anthesis, as indicated by increase in grain protein per unit of N applied, was more efficient than uptake from pre-plant additions in irrigated hard red spring wheat in California (Wuest and Cassman, 1992). In this study, level of pre-plant N addition had little influence on post-anthesis N uptake, while N applied at anthesis increased N uptake markedly. Soil analysis showed little difference in soil N content among the pre-plant N applications, indicating that much of the pre-plant N fertilizer was lost by leaching, denitrification or immobilization. Application of irrigation water after the late season N application likely contributed to the efficient uptake of the late N application.

In rainfed cropping systems, uptake of late-applied N from the soil may be impaired when conditions are dry. Foliar applications of N fertilizer have been investigated for decades as a method of providing N during grain formation to enhance grain protein concentration in cereal crops (Bly and Woodard, 2003; Gooding and Davies, 1992; Gooding et al., 2007; Souza et al., 1999). Uptake of N from late-season foliar applications would be less dependent on soil conditions and could be effective for protein enhancement. In studies with winter wheat, foliar applications of urea after anthesis were more rapidly translocated to the grain than soil applications of ammonium nitrate (Gooding et al., 2007).

Foliar N applications are more likely to increase protein content when N supply is low relative to the crop yield potential (Bly and Woodard, 2003; Finney et al., 1957; Gooding and Davies, 1992; Gooding et al., 2007). Gooding and Davies (1992) reported that yield responses to foliar applications of urea decrease as application is delayed beyond flag leaf emergence. While soil applications of N fertilizer are more effective at improving grain protein concentration when applied before anthesis, foliar applications seem to be more effective when applied after anthesis. Reduced root activity after anthesis may limit uptake of soil applied N. The optimum timing for foliar urea application to increase protein concentration is usually from anthesis through the following two weeks. This may be in part because at these later timings there is less likelihood of a yield increase and so less dilution from increased carbohydrate. Applications later than two weeks after anthesis tend to be less effective in increasing grain protein concentration, possibly because of the limited green tissue to intercept the spray and restricted incorporation and translocation of the N in the plant (Gooding and Davies 1992).

Phosphorus does not normally appear to have a direct influence on protein concentration in cereals under field conditions, but may indirectly decrease protein concentration through dilution when P fertilization causes a significant increase in grain yield (Halvorson and Havlin, 1992; Holford et al., 1992; May et al., 2008; Porter and Paulsen, 1983). Phosphorus may also influence protein accumulation through effects on N uptake and metabolism. In solution culture, grain protein concentration increased with increased P supply (Porter and Paulsen, 1983). Phosphorus fertilization increased N uptake of wheat from emergence to tillering, indicating that the beneficial effect of P on N absorption occurred early in plant development. Under field conditions, adequate P in combination with N may stimulate better root development so that more N can be absorbed by the plants (Boatwright and Haas, 1961). Since the effects of P on protein content tend to be indirect, P fertilization often has no significant effect on protein content (Brennan and Bolland, 2009b; McKercher, 1964).

Potassium is important in N relations within the plant. In nutrient solution culture, K increased the rate of amino acid translocation into wheat grain as well as the conversion of amino acids into grain proteins (Blevins et al., 1978). Higher K appeared to increase the transport rate of amino acids from the vegetative plant parts into the grain (Mengel et al., 1981). Protein synthesis in the grain was also promoted by improved K nutrition, likely indirectly through the improved accumulation rate of amino acids into the grain. Under highly K-deficient conditions, K fertilization can increase both protein yield and protein concentration (Bakhsh et al., 1986). However, under field conditions of moderate to low deficiency, K normally has little effect on protein concentration of wheat (Brennan and Bolland, 2009a; Campbell et al., 1996; May et al., 2008).

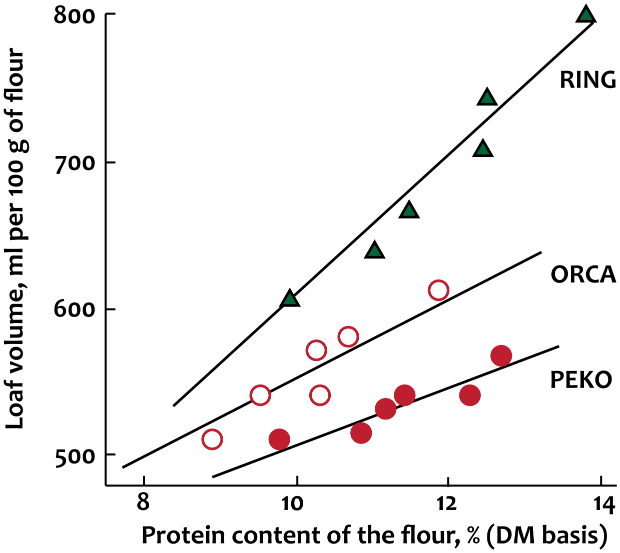

Figure 4. The relation between loaf volume and protein content of the flour for three wheat varieties of good (▲), intermediate (○) and poor (●) baking quality (Belderok, 2000).

Baking quality of wheat flour generally increases with increasing grain protein content, with loaf volume increasing as protein concentration increases (**Figure 4**).

Albumins and globulins are small, physiologically active proteins, comprising about 25% of the total grain protein and concentrated in the seed coat, aleurone cells and the germ, with a lower concentration in the endosperm. The remaining 75% of grain protein consists of storage proteins in the endosperm, gliadins and glutenins that have no enzymatic activity but are important in the functional quality of the wheat for bread-making (Belderok, 2000). These components absorb water during dough mixing and form gluten.

Gluten creates an elastic structure during mixing that holds the CO_2 formed by the yeast during fermentation in the bread-making process, making the bread rise. The more elastic and extensible the gluten structure, the more the dough rises. When the dough is baked, the protein structure sets, forming the crumb texture and loaf volume. The quality and quantity of gluten is the major factor affecting bread-making quality and is largely determined by crop genetics and N supply. Hard, high protein "strong" wheat generally has high gluten strength and is suitable for raised breads. Soft wheat flour has lower protein and forms softer gluten as compared to hard wheat. Soft wheat is not suitable for bread-making, but gives good results for biscuits and cookies. In pasta, both protein content and composition are important in determining cooking quality, with a high glutenin content encouraging good cooking quality (Porceddu, 1995).

While N fertilization increases all protein fractions in bread wheat, it has a proportionally greater effect on the gluten fractions, specifically low-molecular-weight glutenins (LMWG) and gliadins as compared to albumins and globulins and the high-molecular-weight glutenins (HMWG) (Kindred et al., 2008; Tea et al., 2004). Similarly, in durum wheat, N application increased the amount of LMWG at the expense of the HMWG (Lerner et al., 2006). Increases in protein content due to N fertilization may not improve nutritional value to the same extent because of the proportionally higher increase in the lysine-poor gluten proteins (Shewry, 2009).

The N:S balance is important in ensuring protein quality. Sulphur is an important component of protein in wheat, being a constituent of the SH-containing amino acids that contribute the enhanced quality of protein for baking (Zhao et al., 1999a; Zhao et al., 1999b; Zhao et al., 1999c). Sub-units of the gliadins and glutenins which comprise gluten are rich in S. The S-containing sub-units are important in determining the elasticity of dough because the inter-chain disulphide bands stabilize the polymer network formed by the gluten molecules (Shewry et al., 2002). Therefore, adequate amounts of S must be present for protein quantity and quality (Flæte et al., 2005; Thomason et al., 2007; Zhao et al., 1999b; Zhao et al., 1999c). Where soil S levels are deficient, both grain yield and grain quality can increase with S fertilization. Even where S availability is adequate for optimum yield, protein quality may be affected by S application (Flæte et al., 2005; Thomason et al., 2007).

Sulphur deficiency leads to a change in the composition of protein in wheat. Amounts of S-poor proteins such as ω-gliadins increase and S-rich proteins such as γ-gliadins and low molecular weight subunits of glutenin reduce with S deficiency (Tea et al., 2007; Tea et al., 2004; Wieser et al., 2004). The reduced proportion of S-rich compounds leads to tough, less extensible dough and lower bread volume even with a comparable protein content (Reinbold et al., 2008; Thomason et al., 2007; Zörb et al., 2009; **Figure 5**). Similarly, in durum wheat, on a site that was not S-deficient, S application increased the amount of LMWG at the expense of the HMWG (Lerner et al., 2006).

Sulphur does not remobilize from vegetative tissue to the grain as much as N does, therefore an adequate supply of S must be obtained through grain-fill to optimize crop quality. Under highly S-restricted greenhouse conditions, late S fertilization resulted in a higher loaf volume than early S fertilization (**Figure 5**; Flæte et al., 2005; Zörb et al., 2009. Application of high levels of N fertilizer to S-deficient areas may widen the N:S ratio, aggravating S deficiencies. Gooding et al. (1991) reported that a lack of improved loaf quality after urea application could sometimes be related to insufficient grain S concentrations, high N:S ratios and associated changes in the proportions of protein fractions. They suggest that more consistent effects of urea on bread-making quality might be achieved if S nutrition could be improved. In studies with wheat in England, application of N in the absence of S produced lower N concentration than applications of N with

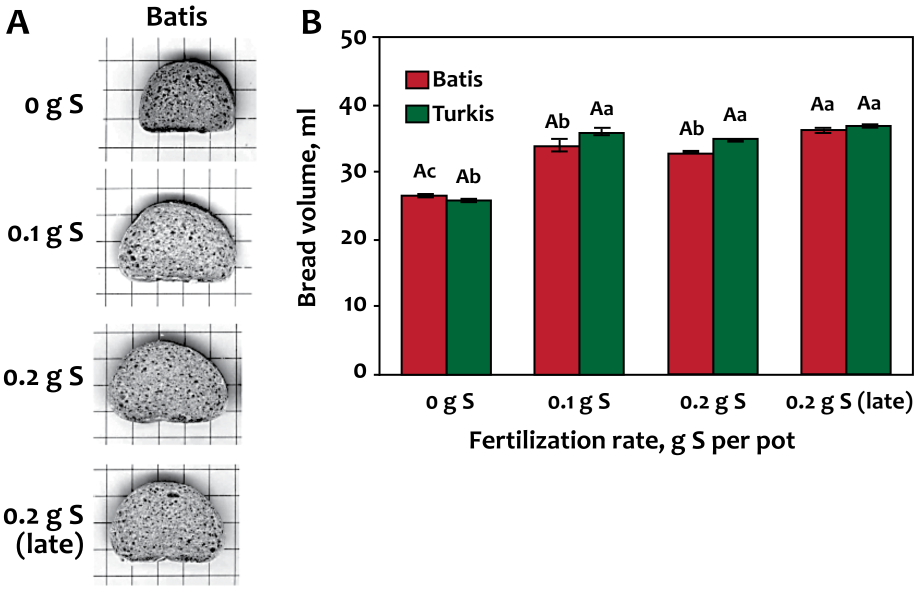

Figure 5. Effect of different S fertilization rates on the baking quality of two wheat cultivars as measured by a microscale baking test using 10 g of wholemeal flour. (A) Images of micro bread slices of the cultivar Batis at a comparable scale. (B) Histograms of bread volumes; different letters represent significant differences of the mean values. Error bars represent (standard errors of five independent pot replicates. Statistical significance ($p<0.05$) is indicated by small letters for the S rates and capitals for the cultivars (Zörb et al., 2009).

S, consistent with a need for a balance between N and S to support protein synthesis (Godfrey et al., 2010). Dough from grain grown with high N, but without applied S showed lower dough strength than samples grown with S (Godfrey et al., 2010; Wooding et al., 2000). Sulphur may also interact with foliar N applications, with a greater increase in protein occurring with foliar N fertilizer when applied to wheat that had previously received S applications (Thomason et al., 2007).

Carbohydrate

Starch is the major carbohydrate present in wheat and is used as a source of energy in the human diet. Starch is composed of amylose and amylopectin, both polymers of glucose, amylose being fairly linear and amylopectin being highly branched (Cornell, 2003). A notable difference between amylose and amylopectin is in gel formation. Amylopectin does not form gels, while amylose forms gels in mixtures with water. Starch forms paste upon heating in water. Viscosity increases as gelatinization begins and continues to increase until gelatinization is complete, then decreases as the structure breaks down. This property is important where starch is used as a thickener. Excess α-amylase in the grain increases breakdown of starch and causes a reduction in the viscosity of starch paste. This leads to sticky doughs that are difficult to process and to poorly structured and discoloured loaves (Kindred et al., 2005). Low Hagberg falling number (HFN) indicates high α-amylase in the dough and hence a weaker dough.

The amount of water absorbed by flour to give the best mixing also depends on the starch properties. Small granules give a higher water absorption than large granules and mixing time seems to be longer for large than for small granules (Eliasson, 2003). Amylose appears to be responsible for setting of the crumb structure. Bread goes stale largely because of recrystallisation of amylopectin and amylose appears to increase this recrystallisation.

Starch content also influences the quality of pasta produced from durum wheat. Normally starch content is negatively related with cooking firmness of pasta, although this may be a reflection of the negative relation between starch and protein content of grain (Porceddu, 1995).

Fiber in diets can reduce the risk of developing a range of diseases including coronary heart disease, stroke, hypertension, diabetes, obesity and some gastrointestinal problems (Anderson et al., 2009). The cellulose content of wheat can be an important source of dietary fiber, especially in whole wheat products. White wheat flour has about 0.6% cellulose while whole wheat flour contains over 2.4% cellulose due to the inclusion of the bran (Anderson and Bridges, 1988). Bran contains about 9% cellulose and about 30% other non-starch polysaccharides, such as the pentosans. Pentosans are hemicelloses that yield pentones upon hydrolysis. Arabinoxylans are pentosans in wheat grain that are composed primarily of D-xylose and L-arabinose, with the xylose unit backbone linked together in a β-1,4 linkage, and arabinose units linked to the main backbone as single unit sidechains (D'Applonia, 1980). As well as being important fibre components, pentosans such as arabinoxylan contribute to water absorption of flour and viscosity of doughs and batters and can increase loaf volume and improve crumb and crust characteristics (Buksa et al., 2010; D'Appolonia 1980).

Increasing protein content by application of N fertilization can decrease starch concentration in the kernel (Erbs et al., 2010; Kindred et al., 2008). Low N supply also reduced soluble β-amylase. Nitrogen fertilizer increased both protein content and Hagberg falling number (Kindred et al., 2005), related to a higher α-amylase activity in the absence of N fertilizer application.

Rice

Rice (*Oryza sativa* L.) is the cereal crop produced in the largest quantity worldwide and the most important crop as a human food. Before consumption, rice is dehulled to remove the lemma and palea and expose the brown rice, which consists of the embryo, endosperm and bran layers. The majority of rice is also polished before use, removing the seed coat and aleurone layer to varying degrees, leaving white rice. Polished rice grain is composed of up to 95% starch, 5 to 7% protein and 0.5 to 1% lipid (Fitzgerald et al., 2009), as well as a wide range of trace elements and components present in low concentrations. Quality traits for rice include physical appearance (i.e. shape, uniformity, and translucence of grains), cooking and sensory properties (gelatinization temperature, gel consistency, fragrance, taste) and nutritional value (Fitzgerald et al., 2009; Yang et al., 2007).

Protein

Although its protein concentration is low, rice contributes approximately 29% of the protein for human consumption in developing countries. This nutritional benefit could be improved by increasing the protein concentration and/or reducing the anti-nutritional components such as phytic acid (Ning et al., 2009).

Protein also affects the functional quality of rice such as texture, pasting capacity and sensory characteristics (Ning et al., 2010). Surface hardness of cooked rice is related to protein content. Rice grain breakage during milling can be a problem, because breakage reduces the amount of grain recovered during milling. In rice, proteins occupy the space between starch granules and may act as a binder. Therefore, increasing protein content may increase the resistance of the grain to breakage (Borrell et al., 1999). Percentage of unbroken rice is positively related to the storage protein content of the lateral region of the endosperm, so in cultivars susceptible to breakage, application of N to increase protein content can reduce breakage (Borrell et al., 1999; Leesawatwong et al., 2005). The increased protein increases hardness in the rice grains and increases the resistance to breakage during milling.

Protein content of rice increases most when N is applied at heading (Borrell et al., 1999; Perez et al., 1996). In a study with 31 cultivars of rice in Nanjing China, both the average total protein content, and the protein quality in terms of glutelin:protein ratio, increased with increasing N level (Ning et al., 2009). Nitrogen fertilizer also decreased the concentrations of phytic acid, an anti-nutritional factor that decreases the bioavailability of both protein and trace elements (Ning et al., 2009). Rice that received 340-55-375 kg/ha of N-P-K before transplanting had substantially higher protein content than rice that received no fertilizer (Champagne et al., 2009).

Timing of N application also affects protein content in rice. In studies conducted in Louisiana, protein content was increased by 90 and 130 kg N/ha fertilizer applications, with the greatest protein content found where all the N was applied subsurface at seeding or where the N was split into equal applications at seeding and at the 2-mm panicle stage (Patrick et al., 1974). Protein content was lower when all N fertilizer was broadcast prior to first flood, or with half broadcast at first flood and half at first joint.

In the studies with 31 rice cultivars in China, applying moderate amounts of N (185 kg/ha) equally as basal before transplanting and top-dressed at panicle initiation produced a higher yield than applying at the same times in a 80:20 ratio, and a yield equal to application of a higher N rate (300 kg/ha) (Ning et al., 2009). With the right timing, moderate N levels were sufficient to optimize grain yield and nutritional quality.

Nitrogen fertilizer applied at anthesis significantly increased protein concentration of rice and increased the percentage of unbroken kernels in cultivars susceptible to broken kernels (Leesawatwong et al., 2005). Nitrogen fertilizer application at flowering consistently increased rice protein and translucency, under both wet and dry conditions, at the International Rice Research Institute farm in the Philippines (Perez et al., 1996), while foliar application 10 and 20 days after anthesis increased rice protein concentration without decreasing grain yield (Souza et al., 1999).

Nitrogen management can also influence the protein distribution in rice. Nitrogen fertilization can increase the protein located in the outer cell layers of the endosperm, which can lead to extra water absorption, affecting the texture of the cooked rice (Champagne et al., 2009). Nitrogen fertilization had a greater effect than genotype on the prolamin and glutelin fraction of the rice protein while albumin and globulin were affected more by genotype than by N treatments (Leesawatwong et al., 2005; Ning et al., 2010). Prolamin is concentrated in the outer layers of rice while glutelin is proportionally higher towards the centre of the grain, therefore glutelin is more likely be retained in the finished product during the milling process than prolamin (Leesawatwong et al., 2005; Ning et al., 2010). In addition, glutelin has a higher proportion of lysine than does prolamin (Souza et al., 1999).

The degree of polishing may affect the amino acid balance of rice protein. Polishing removes the outer layers and the protein that they contain, therefore the protein in the finished product is primarily derived from the endosperm (mainly glutelin and prolamin). In studies conducted by Leesawatwong et al. (2005), polishing decreased the albumin-globulin concentration but increased the glutelin concentration in some cultivars, with the change being affected by the distribution of storage protein in the different layers of the grain. Like other cereal crops, rice tends to be low in the essential amino acid lysine, but is relatively rich in cysteine and methionine (Juliano, 1999; Ning et al., 2010).

Ning et al. (2010) reported that N application increased the concentration of most amino acids in both brown and milled rice, but that methionine in brown rice and lysine and methionine in milled rice were not significantly affected by N. However, lysine was increased by N application in brown rice and tended to increase in milled rice, reflecting the increase in the high-lysine glutelin proteins (Ning et al., 2010). Therefore, N fertilizer may increase both total protein content and protein quality, and may have an important effect on dietary protein contribution from rice in the diet (Leesawatwong et al., 2005).

Carbohydrate

Nutritionally, rice is mainly a supplier of energy in the form of starch. Starch characteristics have a dominating effect on rice quality (Champagne et al., 2009). Sensory quality of rice is largely determined by the amylose and amylopectin content and structure, with the firmness of cooked rice increasing with the amylose content and the number of long chains in the amylopectin (Fitzgerald et al., 2009). Generally, a higher ratio of amylose relative to amylopectin leads to harder cooked rice grains. Rice with low or intermediate amylose content is relatively dry and fluffy and retains a soft texture even after cooling (Yang et al., 2007). However, Bhattacharya (2009) noted that more recent data since the mid-1980s firmly attribute end-use quality mostly to amylopectin chain structure. Higher abundance of extra long chains of amylopectin correlates positively to quality. The long chains lead to strong and resilient starch granules that resist swelling and breakdown during cooking.

Amylose content showed a nonsignificant decreasing trend with N applications in studies conducted by Yang et al. (2007), while in studies by Champagne et al. (2009) amylose content decreased with N-P-K application. Stickiness and cohesiveness of the rice decreased with increases in amylose, while hardness increased. Fertilizer application affected rice flavour and texture, with initial starchy coating, slickness and stickiness between grains reduced by fertilization, and roughness, hardness and moisture absorption increasing with fertilization.

Gelatinization consistency was greatest at moderate N applications, decreasing with high or low N applications, while gelatinization temperature (related to cooking time requirement) was not affected by N application (Yang et al., 2007). Overall, grain quality was less responsive to N than was crop yield, showing trends of higher chalkiness and worse eating/cooking quality at high compared to moderate or low N levels. Quality may be improved by increasing the proportion of N applied after panicle initiation, to improve milling quality, appearance and protein content (Yang et al., 2007).

Maize

Maize or corn (*Zea mays* L.) is grown primarily for its high starch production and is a major energy source for animal feed (McKevith, 2004), and more recently for fuel ethanol production. The majority of maize produced in 2008 in the United States, the world's largest producer of maize, was used to feed livestock (45%), with the next largest proportion going for ethanol production (30%) (Iowa Corn, 2010). About 10% of maize production in the United States enters the human diet, as starch, corn oil, sweeteners, flour, meal and grist, or to produce beverage alcohol, with an additional 15% being exported outside of the country. While the majority of maize grown in the United States is used for non-food purposes, it is an important food in Asia, Africa, Latin America and parts of the former Soviet Union. For use in feed and food, high protein and hard endosperm are the most desirable. The standard maize cultivars commonly grown in the United States contain about 7 to 10% protein, 68 to 74% starch and 3 to 5% oil (Dado, 1999).

Protein

Maize is relatively low in protein compared to wheat, and maize protein is low in the essential amino acids lysine and tryptophan. Consumption of maize with legume crops such as soybean improves the nutritional quality of the diet, as the amino acid profiles of maize and soybean are complementary. Physical quality of maize is also affected by protein content because kernel hardness (vitreousness or translucence) increases and kernel breakage susceptibility decreases with increasing protein content (Mason and D'Croz-Mason, 2002). Increases in crude protein in maize would increase the value of maize in the diet considerably (Johnson et al., 1999). Protein quality can be improved though the production of opaque-2 maize cultivars that have a higher level of lysine and tryptophan than conventional cultivars (Mason and D'Croz-Mason, 2002). However, some of the opaque-2 cultivars have lower zein, leading to softer, flourier endosperms (Misra, 2009). Quality Protein Maize (QPM) cultivars have also been bred to have higher contents of lysine and tryptophan and reduced concentrations of zein (Sullivan et al., 1989), thus increasing the biological value of the protein. However, newer QPM lines have been developed with harder endosperm (Sullivan et al., 1989).

In maize, as in most crops, there is a general inverse relationship between protein content and starch content (Mason and D'Croz-Mason, 2002). Therefore, factors that increase grain yield tend to increase starch concentration and decrease protein concentration. However, grain protein concentration and crop yield both tend to increase with increasing N availability, if N levels are initially deficient (Genter, 1956; Miao et al., 2006; Miao et al., 2007; Riedell et al., 2009; Singh et al., 2005). Differences in factors affecting N supply relative to yield potential, such as field variability, hybrid, and growing season, affect the response of protein to N application (Mason and D'Croz-Mason, 2002). Miao et al. (2007) evaluated variable-rate N management for effects on the protein content and test weight of two maize hybrids, in one field in 2001 and two fields in 2003, in Illinois. They found that the N rate to maximize grain protein concentration was 45 to 50 kg/ha higher than the optimal rate for yield. Protein content was more sensitive than yield to N supply and required more available N to attain a maximum (Miao et al., 2007; Singh et al., 2005). Attempting to address the variability in soil N supply using variable-rate N application did not, however, reduce variability in protein content.

As protein concentration of maize increases with higher rates of N application, the proportion of the protein composed of zein in the endosperm increases as well (Sauberlich et al., 1953; Tsai et al., 1992). Isoelectric focusing analysis showed that increases in zein were primarily due to a quantitative increase in alpha- and gamma-zein polypeptides (Tsai et al., 1992). As zein is important for kernel hardness but is low in lysine and tryptophan (Wang et al., 2008), increases in protein concentration in conventional maize cultivars due to N applications may lead to harder, more vitreous kernels, but decrease the proportions of lysine and tryptophan. Therefore, increases in the protein content of maize with N application may not improve nutritional performance as much as raw protein content because of the low content of the limiting amino acids. In contrast, application of N fertilizer to opaque-2 cultivars maintained or increased the lysine and tryptophan concentration of the kernel (Tsai, 1983). The reduced biological value of maize protein in conventional but not opaque-2 cultivars caused by higher rates of N has been demonstrated through rat feeding experiments (Blumenthal et al., 2008).

As in wheat, a significant amount of seed-filling in maize is due to remobilization of N accumulated in the stalks, leaves and roots during the pre-anthesis period (Bennett et al., 1989; Weiland and Ta, 1992). Newer maize hybrids, however, tend to take up a greater proportion of their N after anthesis, but do not translocate more N to the grain (Ma and Dwyer, 1998). Nitrogen taken up by the crop after canopy formation is less likely to be immobilized in structural components than N taken up earlier in growth and therefore may be more incorporated into grain protein (Gooding and Davies, 1992; Gooding et al., 2007; Powlson et al., 1987).

Phosphate and K fertilization had no significant effect on maize protein content in studies conducted in Virginia, even when significant yield increase occurred with fertilization (Genter, 1956).

Carbohydrate

Maize grain is mainly used to provide energy in the form of fermentable carbohydrate. Increases in grain yield normally increase starch concentration and decrease protein concentration (Mason and D'Croz-Mason, 2002). However, N fertilization under conditions of N deficiency can increase yield, protein content and test weight, and decrease oil, starch content and extractable starch content (Miao et al., 2007; Singh et al., 2005; Riedell et al., 2009). Protein and oil content tend to be inversely related to starch content, so increases in these components would tend to decrease the fermentable energy from carbohydrates in maize (Dado, 1999). With the exception of protein, maize quality parameters tend to respond less to N applications than does yield, with starch content and test weight responding least and oil content responding moderately (Miao et al. 2007).

Oil

The oil in maize is an important energy source in feed and is highly unsaturated, hence a good oil for human consumption (Mason and D'Croz-Mason, 2002). More than 90% of the kernel oil is located in the germ in maize and oil concentration increases as the germ to endosperm ratio increases. Therefore, oil concentration is normally negatively related to starch concentration (Riedell et al., 2009). Agronomic practices have only a minor effect on the oil concentration of maize grain (Mason and D'Croz-Mason, 2002). In studies conducted in Illinois, oil content increased with N application, but to a lesser extent than protein content (Lang et al., 1956). Similarly, in studies conducted in Virginia, N, P and K fertilization had only minor and inconsistent effects on oil content (Genter, 1956). Percentage oil was shown to increase slightly with N, P and K fertilization, possibly because fertilization increased the ratio of oil-rich germ relative to endosperm (Riedell et al., 2009; Welch, 1969). Fertilization frequently increased grain yield as well, so total oil yield was increased to a greater extent by fertilization.

Potatoes

Eppendorfer and Eggum (1994) reported a comprehensive analysis of potato protein and starch qualities in response to application of mineral fertilizers. While their results were based on outdoor pot studies, making comparison to rates used in field production difficult, they found that nutrient addition levels producing maximum yields generally resulted in high protein and starch levels as well (**Table 3**). They reported that N applied to the soil strongly increased crude protein content of potato, but reduced its biological value. As crude protein increased, the proportion of asparagine increased while that of essential amino acids declined. Nevertheless, the reduction in biological value was smaller than the increase in crude protein, and thus the total production of bioavailable essential amino acids increased with N application, even beyond the rate of application required for maximum yield. Increasing levels of P and K reduced crude protein but increased its biological value. Sulphur deficiency strongly reduced biological value of protein as well, owing to reductions in methionine and cysteine.

Table 3. Effects of level of mineral nutrients applied to soil on yield, starch and protein characteristics of potato (Eppendorfer and Eggum, 1994).

Nutrient Level				Potato yield, g/pot	Starch content, %	Crude protein, %	
N	P	K	S			Content	Biological value
2	3	3	3	124	70	8.3	89
4	3	3	3	317	72	12.9	80
6	3	3	3	266	69	15.9	75
4	1	3	3	134	68	14.9	74
4	4	3	3	454	74	10.3	81
4	3	1	3	50	59	22.9	65
4	3	4	3	332	68	11.5	82
4	3	3	0	173	65	14.7	45

Starch content of potatoes can be reduced by either deficiency or excess of nutrients. Deficiencies of P, K and S reduced the starch content in boiled potatoes, but levels of N, P, and S that maximized yield also provided the highest starch levels (**Table 3**). When K input was increased to very high levels, small increases in yield were offset by small decreases in starch content. Westermann et al. (1994) reported reductions in potato tuber starch content with increasing levels of N and K in field-grown irrigated potatoes in Utah, USA.

Henderson (1965) found that sulphate of potash *"gave a drier potato with higher content of dry matter and higher specific gravity than muriate, and also a mealier product when cooked"* but also noted that these differences were not fully consistent, and were smaller than the variation among the 11 site-years of the study, conducted in Scotland. In India, Kumar et al. (2007a) found that the sulphate form of K was more suited for crisping potatoes than either KNO_3^- or KCl, since it increased tuber dry matter percentage and crisp yield and also decreased crisp oil percentage.

Working with high-yielding irrigated potatoes in Washington, USA, Davenport and Bentley (2001) reported no difference in specific gravity between sulphate and chloride forms of K fertilizer, applied at high rates, in either granular or liquid form, whether applied pre-plant or partially in-season.

Kyriacou et al. (2009) found no effect of increasing N rates over a range of 0 to 300 kg/ha on quality of chipping potatoes grown in Cyprus, concluding "*Completion of physiological crop senescence of the spring potato crop under Mediterranean climatic conditions seems to mitigate the potential interference of preplanting N fertilization with tuber maturation and subsequently cold storage performance, reconditioning potential and processing quality.*" For processing-grade potatoes in India, Kumar et al. (2007b) found that specific gravity and tuber dry matter percentage increased with increasing N rates from 0 to 360 kg/ha, while crisp color and reducing sugars were unaffected.

Acrylamide is a compound formed when high-carbohydrate foods are cooked and has been associated with adverse health effects. Gerendas et al. (2007) reported that the highest acrylamide contents were observed in French fries processed from potatoes grown with high N and low K supply. Ensuring adequate K may thus help reduce health risks associated with acrylamide.

Soybean

In soybean, both the oil content and the protein content of the seed are of value. Soybean contains high concentrations of protein (**Table 4**), with methionine and cysteine being the limiting amino acids. Generally, as the protein concentration of the seed increases, the oil concentration decreases.

As a legume crop, soybean normally fixes its own N in a symbiosis with *Bradyrhizobium* bacteria. However, application of P, K, and S fertilizers can influence seed yield, and protein content and composition. Soybeans show greater response in nodule activity and N-fixation capacity than in root or shoot growth when P is applied to P-deficient soils (Cassman et al., 1980; Israel, 1987; Brown et al., 1988). Thus P application could potentially increase protein levels in soybeans, and has been reported to do so in P-deficient soils of India (Majumdar et al, 2001; Tanwar and Shaktawat, 2003).

Effects of P and K fertilization on oil and protein content in soybean have been variable. Greenhouse studies using a P-deficient soil showed that P fertilization increased protein concentration under a range of water regimes (Jin et al., 2006). Potassium fertilization increased oil concentration but reduced protein concentration in soybean seed produced on soils with low to medium soil test levels (Gaydou and Arrivets, 1983), while fertilization with P or dolomite (a Mg-containing lime) increased both oil and protein along with yield.

In Ontario, Canada, Yin and Vyn (2003) reported that protein concentration declined from 43% to 42%, and that oil concentration increased from 21.5% to 21.8%, in response to band (but not broadcast) application of K fertilizer. In these studies, conducted on soils with low to medium K fertility, Yin and Vyn (2004) showed that oil concentration increased with increasing leaf K concentration, reaching a maximum when leaf K reached 2.2 to 2.5%.

Older research in Virginia also showed that K could decrease soybean protein in situations where it increased yield (**Table 4**). Since yield was increased more than protein was decreased, K still substantially increased protein production (Jones, 1976).

Table 4. Applied P and K fertilizer increased yield and protein production, even though the concentration of protein was reduced (two-year average, Virginia; soil test P and K were approximately 17 and 39 ppm, respectively, following five years of zero application; Jones, 1976).

P_2O_5, kg/ha	K_2O, kg/ha	Yield, kg/ha	Protein concentration, %	Protein production, kg/ha
0	0	1,710	41.8	716
135	0	1,770	41.8	741
0	135	3,130	39.2	1,227
135	135	3,680	39.2	1,443

Summarizing results from 112 field trials on soil and foliar P and K fertilization conducted across Iowa from 1994 to 2001, Haq and Mallarino (2005) concluded, "*Fertilization that increases soybean yield has infrequent, inconsistent, and small effects on oil and protein concentrations but often increases total oil and protein production.*" In other Canadian research, applied K only slightly reduced protein, and slightly increased oil and sugars (Zhang, 2003; **Table 5**). Similarly, effects of P and K fertilization on protein content of soybean were minimal in trials in Quebec, Canada, on soils with moderate to high initial fertility (Seguin and Zheng, 2006). In soils testing high or very high in P and K, applied P and K had little if any impact on qualities such as specific weight, visual quality, 100-seed weight, seed protein and oil content (Tremblay and Beausoleil, 2000).

Table 5. Potassium increased oil and sugar but decreased protein slightly in soybeans. Means of five cultivars over four years, 1999-2002 (Zhang, 2003).

Applied K_2O, kg/ha	Protein, %	Oil, %	Sugar, %
95	41.9	21.6	11.0
0	42.3	21.4	10.9

Sulphur may also influence the nutritional quality of soybean protein. Glycinin (11S) and β-conglycinin (7S) account for about 70% of the storage protein in soybean (Sexton et al., 1998). Glycinin contains about 3.0 to 4.5% S-amino acids while β-conglycinin contains less than 1%, so a higher ratio of 11S to 7S proteins indicates higher cysteine and methionine content and hence higher protein quality. Synthesis of low-S β-conglycinin is stimulated by an abundance of N and inhibited by an abundance of methionine, so the nutritional quality of soybean protein can be influenced by the relative N and S status of the plant (Imsande and Schmidt, 1998; Paek et al., 1997; Paek et al., 2000). Nitrogen appears to be remobilized more efficiently than S from vegetative material to the seed during pod filling; therefore increased tissue N may increase the N:S ratio in the seed (Imsande and Schmidt, 1998). Under hydroponic conditions, the 11S/7S ratio, and hence protein quality, increased substantially with provision of S during seed-filling, although protein concentration was not greatly affected (Sexton et al., 1998). Provision of S during vegetative growth had a much lower effect on the 11S/7S ratio than provision of S during seed filling. Therefore, an adequate supply of S through seed filling is important to ensure both optimum protein concentration and quality.

Canola (Rapeseed)

Canola (rapeseed) oil is viewed as healthy because it contains a relatively low proportion of saturated fatty acids compared to other vegetable oils. Canola is one of the richest sources of mono-unsaturated fatty acids and is a good source of ALA (Harland, 2009). Reducing saturated fatty acid intake with mono-unsaturated fatty acids can reduce total cholesterol and low-density lipoprotein cholesterol, potentially improving human health.

As with other crops, there is generally a negative relationship in canola between protein and oil concentration. Therefore, increased protein content due to application of N fertilization commonly results in a reduced oil concentration (Asare and Scarisbrick, 1995; Brennan and Bolland, 2009b; Gao et al., 2010; Malhi and Gill, 2007; Rathke et al., 2005; Rathke et al., 2006). Increasing N fertilization has also been reported to affect the oil composition, although results were variable: increasing the proportion of oleic acid and decreasing the concentration of linolenic, linoleic and erucic acids in some studies (Behrens, 2002 as cited by Rathke et al., 2006) and decreasing the concentration of oleic acid and increasing the concentration of linoleic acids in others (Gao et al., 2010).

Phosphorus and K appear to have little to no effect on oil content of canola (Brennan and Bolland, 2007; Brennan and Bolland, 2009a). However, concentration of oil in canola seed has been reported to increase with S application when S was deficient (Brennan and Bolland, 2008; Grant et al., 2003; Malhi and Gill, 2002; Malhi and Leach, 2002; Malhi and Gill, 2006; Malhi and Gill, 2007; Malhi et al., 2007; Nuttall et al., 1987) but no effects occurred in other studies (Asare and Scarisbrick, 1995; Malhi and Gill, 2007). Sulphur fertilization did not have a consistent effect on fatty acid composition or oil content in studies in Turkey (Egesel et al., 2009).

Summary

Proper nutrient management in crop production is important not only for improving crop yield and profitability, but also in optimizing the quality of crop-based food products. Protein, carbohydrate, and oil content composition and bioavailability can all be influenced by nutrient management. Adequate and balanced applications of N, P, K and S, managed in an efficient manner are critical to optimize the functional and nutritional quality of the staple crops wheat, rice, maize and potatoes and the major oilseed crops soybean and canola. In general, fertilizing for optimum yields does not differ greatly from fertilizing for optimum quality for most of the world's major food crops. In the long term, ensuring that soil fertility is maintained is important to avoid the major declines in both crop yield and nutritional quality that can be seen when crops are grown on highly depleted soils. FCHH

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Chapter 7

Fertilizer Application and Nutraceutical Content in Health-Functional Foods

By Moustapha Oke and Gopinadhan Paliyath[1]

Abstract

Fruits and vegetables are rich sources of nutrients and phytochemicals. Phytochemicals—such as polyphenols (flavonoids, anthocyanins), isoprenoids, S-containing compounds, soluble and insoluble fibre, etc.—are some of the agents that help to combat certain diseases, such as cancer and cardiovascular disease in humans. A growing body of research shows that fruits and vegetables are critical to disease prevention and promotion of good health. With an increasing consumer interest in healthy food, there is a need to improve the content of phytochemical and nutraceutical components in crop products. Fertilizers have been generally used to increase crop yields and improve crop quality. In the last decade, increased attention has been given to the use of fertilizers for enhancing nutraceutical content. The objective of this review is to summarize the effects of plant mineral nutrition on functional food components and nutraceuticals in crop products. Overall, fertilizers have diverse effects on the biosynthesis of health-promoting phytochemicals. Some have positive effects, but others have negative or inconclusive outcomes. Nonetheless fertilizers could play an important role in increasing the levels of nutraceuticals and functional food ingredients in crop plants, although more studies are required to optimize some of the reported successes.

Introduction

In this chapter, the term fertilizers relates to chemical fertilizers that are manufactured products used in agriculture for the supply of plant nutrients. These include N, P, K, S, and combinations of them. Before the introduction of mineral fertilizers in the 19th century, soil fertility was maintained mostly by the recycling of organic materials and crop rotations that included N-fixing leguminous crops. The results

Abbreviations specific to this chapter: APX = ascorbate peroxidase; CAT = catalase; DNA = deoxyribonucleic acid; FOSHU = Foods for Specified Health Use; GSH = glutathione; LDL = low-density lipoprotein; POX = guaiacol peroxidase; SOD = superoxide dismutase.
For abbreviations and symbols used commonly throughout this book see page v.

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were insufficient food production for the increasing world population. There was a particular concern at the beginning of the 20th century about the availability of adequate quantities of N fertilizers. This issue was resolved through the industrial fixation of atmospheric N. Improved agricultural productivity through the use of fertilizers and novel productive varieties of food crops resulted in increased food production, that to date satisfies the food requirements in several countries. World fertilizer use has increased almost five-fold since 1960. Smil (2002) estimated that fertilizer N has contributed an estimated 40% to the increases in per-capita food production in the past 50 years, and Erisman et al. (2008) noted that this contribution continues to increase, reaching 48% by 2008.

Consumers are increasingly interested in the health benefits of foods and have begun to look beyond the basic nutritional benefits of food to disease prevention and health-enhancing compounds contained in many foods. This, combined with a more widespread understanding of how diet and lifestyle affect disease development and health-care costs, has created a market for functional foods and natural health products. Functional foods and nutraceuticals provide an opportunity to improve health, reduce health care costs and support economic development. They also offer a way for some producers to diversify their agriculture and marine-based resources. The global functional food and nutraceutical market is growing at a rate that is outpacing the traditional processed food market.

While fertilizers are mainly used for their primary purpose of increasing yield and improved quality, more emphasis is being given to increase nutraceuticals in foods. An increasing amount of research is being conducted to investigate the effects of various fertilizers on nutraceuticals such as lycopene, isoflavone, flavonoids, and organosulphur compounds. The focus of this chapter is to critically review the effects of fertilizers on nutraceuticals and functional foods.

Crop Quality

Factors Influencing Crop Quality

Quality is defined in various terms depending on the crop, ranging from how seed storage proteins of wheat define bread-making quality of wheat flour, to the long list of native antinutrients or phytotoxins found in many crops, particularly beans and other legumes. Nonetheless, quality is made up of many attributes, both intrinsic and extrinsic (Jongen, 2000). These attributes will vary depending on the expectations and memory of the consumer. Intrinsic features of the product include external attributes such as color, shape, size, and freedom from visible defects. In addition, internal attributes include texture, sweetness, acidity, aroma, flavor, shelf-life, and nutritional value. These are important components of the subjective approach used by the consumer in deciding what to purchase. Extrinsic factors refer to the production and distribution systems. These factors include chemicals used during production, package types and their recycling capability, sustainability of production and distribution in relation to energy utilization. These extrinsic factors are increasingly influencing consumer decisions to purchase.

Nutraceuticals and Health-Functional Foods

Nutraceuticals

The term nutraceutical was originally defined in 1989 by Dr. Stephen L. DeFelice, founder and chairman of the Foundation for Innovation in Medicine, Crawford, New Jersey. The word "nutraceutical" is formed by connecting the words "nutrition" and "pharmaceutical" and is a nutritional product—a single entity or combination which includes special diets—that reasonable clinical evidence has shown to have a medical benefit. However, the manufacturer or a physician cannot proclaim its benefits unless approved by the regulatory agencies [e.g. United States Food and Drug Administration, Health Canada] (Kalra, 2003; Cohen 2008). Since the term was coined by Dr. DeFelice, its meaning has been modified by Health Canada, which defines nutraceuticals as a product isolated or purified from foods, and generally sold in medicinal forms not usually associated with food and demonstrated to have a physiological benefit or provide protection against chronic disease development.

Functional Foods

A functional food, on the other hand, is similar in appearance to, or may be, a conventional food, but is consumed as part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions. The general category of functional foods includes processed food or foods fortified with health-promoting additives, like "vitamin-enriched" products. Fermented foods with live cultures are considered as functional foods with probiotic benefits. The study of functional foods is an emerging field in food science owing to increasing popularity of these foods among health-conscious consumers because of their health benefits. The term functional food was first used in Japan in the 1980s where there is a government approval process for functional foods called Foods for Specified Health Use (FOSHU) as the need arose for different age groups.

Health Benefits of Food

Centuries ago our ancestors proclaimed and believed in the medicinal properties of foods. The use of plants and foods for healing was used in the traditional Ayurvedic practices in India appearing in the Vedic texts, and as traditional medicine in China over 3,500 years ago. In Europe, the Greek physician and philosopher Hippocrates has been linked to the understanding of the healing properties of foods, and with the proclamation "Let food be thy medicine and medicine be thy food." He is considered to be the father of modern medicine (Kochhar, 2003). The Sumerians (4,000 BC) realized the medicinal use of licorice, opium, thyme, and mustard. Later, the Babylonians further developed the plant formulary to include saffron, cinnamon, coriander, and garlic. The modern concept of functional foods is attributed to the Japanese government's move to consider the limitations and cost-effectiveness of curative medicine's contribution to a healthier society. The term functional food as used in Japan refers to processed foods containing ingredients that aid specific bodily functions, in addition to being nutritious. To date Japan is the only country that has a legal definition of functional foods (Kochhar, 2003).

The Japanese FOSHU concept is based on knowledge concerning the relationship between particular foods or food components and certain expected health beneficial properties. A widely accepted definition of functional foods can be found in the consensus document produced by the European concerted action on functional food science in 1999. According to this definition, a particular food is functional if its beneficial effects on one or more targets in the body, beyond adequate nutritional effects, can be demonstrated (e.g. specific functional foods for infants and other functional foods for elderly adults). This must lead to an improved state of health and well-being and to a reduction in disease risk. Functional foods must remain as foods and achieve their effects in amounts consumed normally in the diet. Functional foods are not pills or supplements (Kochhar, 2003).

Biochemistry of Functional Foods

According to Ferrari (2004) aging is associated with mitochondrial dysfunctions, which trigger leakage across membranes, release of reactive species from oxygen and N, and subsequent induction of peroxidative reactions. Peroxidative reactions result in damage to the biological macromolecules. Free radicals induce neuronal cell death, which could be associated with a loss of memory. These pathological events are involved in cardiovascular, neurodegenerative and carcinogenic processes. Dietary bioactive compounds from different functional foods, herbs, and nutraceuticals (ginseng, ginkgo, nuts, grains, tomato, soy phytoestrogens, curcumin, melatonin, polyphenols, antioxidant vitamins, carnitine, carnosine, ubiquinone, etc.) can ameliorate or even prevent diseases. Protection from chronic diseases by nutraceuticals involves antioxidant activities, mitochondrial stabilizing functions, metal chelating activities, inhibition of apoptosis of vital cells, and induction of cancer cell apoptosis. Functional foods and nutraceuticals constitute a great promise to improve health and prevent aging-related chronic diseases (Ferrari, 2004; Bates et al. 2002).

Protecting Pathways

Flavonoids such as quercetin, kaempferol, and luteolin as well as polyphenols (from grapes and wine), vitamin E, chlorophyllin (water-soluble chlorophyll analogue) and other phenols can protect membrane polyunsaturated fatty acids from oxidation, avoiding mitochondrial and other biomembrane disruptions (Brown et al. 1998; Frankel 1999; Terao and Piskula 1999; Boloor et al. 2000; Ferrari, 2004). Dietary ω-3 fatty acids improved mitochondrial membrane lipids, decreasing calcium release (apoptosis trigger), and pyruvate dehydrogenase activity (Pepe et al. 1999). Recently, it was observed that the antioxidant N-acetylcysteine prevented Bcl-2 down-regulation increasing cell survival and life span (Kumazaki et al. 2002). Ebselen, ($C_{13}H_9NOSe$) an organoselenium compound, can significantly abrogate apoptosis of myocardial cells exposed to ischemic injury (Maulik et al. 1998). Namura et al. (2001) observed that ebselen decreased cytochrome-c release from mitochondria and increased survival of stroke-induced brain cells.

Tocopherol, glutathione (GSH) and idebenone abrogated the oxidative decay of complex III, but only GSH blocked damage to complexes II and V (Ferrari, 2004). Aged rats have decreased brain and plasmatic levels of dopamine, serotonin and some of their metabolites (Lee et al. 2001). Melatonin reversed alcohol-induced hepatic mitochondrial DNA strand-breaks and massive DNA degradation possibly by its antioxidant actions (Mansouri et al. 1999). Melatonin administration to Adriamycin patients improved cognitive functions, decreased nocturnal activity and prolonged sleep period (Asayama et al. 2003). Ubiquinone also improves mitochondrial respiration and enhances post-ischemic myocardial contractile function and decreases myocardial damage (Rosenfeldt et al. 2002). L-Carnitine is a mitochondrial membrane fatty acid transporter and stabilizer in aging cells and neurons (Hagen et al. 1998; Binienda 2003; Virmani et al. 2003), improving heart-related conditions and encephalomyopathy (Mahoney et al. 2002). Lipoic acid supplementation decreased heart mitochondrial DNA oxidation (Suh et al. 2001), hence it has many free radical scavenging activities (Pioro 2000). Carnosine stabilizes mitochondrial structure of stressed cells (Zakharchenko et al. 2003), blocking membrane permeability transition, cytochrome c leakage and subsequent events that lead to cell apoptosis (Kang et al. 2002, Ferrari, 2004).

Anti-aging Mechanisms

By modulating many biological mechanisms in mammalian body and cells, functional foods can exert general health benefits and specific anti-aging benefits. Based on extensive literature review regarding normal aging and chronic diseases of aging (Ames et al. 1993; Mahoney et al. 2002; Reiter et al. 2002; Driver, 2003; Ferrari, 2004), the following anti-aging mechanisms of functional foods could be proposed: (1) Stabilizers of mitochondrial membranes and enhancers of mitochondrial function, agents that avoid cell death by apoptosis (programmed cell death) or necrosis (accidental cell death); (2) Metal chelating activities of functional foods; (3) Antioxidants that decrease cell injury, including those that stimulate antioxidant cell defense systems, protect DNA from oxidation or even inhibit apoptosis of target cells in vital organs; and (4) Inducers of apoptosis of preneoplastic and neoplastic cells.

Antioxidant Activities

Consumption of fruit and vegetable is beneficial to human health, since numerous studies have shown that they reduce the risk of developing cancer and cardiovascular disease (Hertog et al., 1993, 1995; Steinmetz and Potter, 1996; Shi et al., 2002; Bao and Fenwick, 2004; Dorais, 2007). Phytochemicals that possess antioxidant characteristics are believed to contribute to the overall health-protective effects of fruits and vegetables. They are thought to be protective against oxidative stress resulting from mitochondrial respiration and lifestyle factors such as smoking, exposure to environmental pollutants and solar radiation, all of which lead to disease development and aging process. The antioxidant properties of the most abundant types of phytochemicals found in fruits and vegetables such as vitamin C, carotenoids and phenolics result from their electron-rich structure in the form of oxidizable double bonds and hydroxyl groups. Antioxidant vitamins can counteract the oxidizing effects of lipids by scavenging oxygen free radicals that have been found to be major agents of cardiovascular disease, certain cancers, neurodegenerative disease, diabetes, rheumatoid arthritis, cataract, and others (Shi et al., 2002). The antioxidant activity of fruits and vegetables varies according to species.

Many authors reported that aging impairs mitochondrial function resulting in oxidative imbalance and increased peroxidation biomarkers (lipid, protein, DNA), inducing heatshock proteins, and depleting antioxidant defense enzymes [catalase (CAT), SOD, GSH, glutathione-S-transferase] (Lucas and Szweda, 1998; Yang et al., 1998; Brack et al., 2000; Hall et al., 2001; Sandhu and Kaur, 2002; Rattan, 2003, Ferrari, 2004). This deleterious phenotype can be reversed by overexpression of SOD and CAT extending life span of *Drosophila melanogaster* and *Caenorhabditis elegans* (Larsen 1993; Sohal et al. 1995). Higher levels of vitamin A and E were found in healthy human centenarians (Mecocci et al. 2000), reinforcing the theory of an antioxidant–life span relationship. Rather than directly increasing life span, antioxidants' benefits are related to the control of free radicals that negatively influence healthy aging (Le Bourg 2003), saving antioxidant enzymes and performing the following protective mechanisms:

- Antioxidant gene expression – ginsenoside Rb2 found in panaxadiol (*Panax ginseng* fraction) induced expression of SOD-1 gene, but total saponins and panaxatriol did not affect SOD-1 expression (Kim et al. 1996). Propolis was also able to induce SOD production in rats (Sforcin et al. 1995).
- Protection of LDL cholesterol from oxidation (Frankel 1999).
- Antiapoptotic protection of liver, brain and heart, preserving tissues (Green and Kroemer 1998; Ferrari 2000).

Fertilizer Impacts on Nutraceuticals and Functional Foods

Flavonoids in Apple

Plant flavonoids constitute one of the largest groups of naturally occurring phenolics, possessing chemical structures that can act as antioxidants, free radical scavengers and metal chelating agents (Rice-Evans et al. 1997). Apple fruits are rich in quercetin glycosides, catechin, epicathechin, procyanidins, dihydrochalcones such as phloretin and phloridzin, as well as anthocyanins in blushed cultivars, all of which are generally concentrated in the skin (Awad et al., 2001).

Awad and Jager (2002) investigated the relationship between fruit nutrients (N, P, K, Mg, and Ca) and concentrations of flavonoids and chlorogenic acid in "Elstar" apple skin and concluded that the most important variable in predictive models for the anthocyanin and total flavonoids concentration was N concentration in the fruit. The result suggested that the concentration of flavonoids in the fruit skin could be increased by optimizing fertilization, especially that of N. Paliyath et al. (2002) studied the effect of soil and foliar P supplementation on the post harvest quality of apples (*Malus domestica* Borkh. cv. 'McIntosh' and cv. 'Red Delicious') and found that P fertilization increased the percentage of red skin on both varieties at harvest. They have also found that fruit from sprayed sides of the trees subjected to foliar treatments with P and Mg or P and Ca from blossom until a week before commercial harvest had increased red color compared to those from the non-sprayed side.

Lycopene in Tomatoes

Tomato fruit pigment is derived largely from the carotenoids lycopene and beta-carotene. Unlike anthocyanin pigments, which can be stimulated by increasing carbohydrate reserves, increasing carotenoid content seems to depend more on up regulating the general plant health, and by increasing phytoene, the carotenoid substrate needed to form lycopene. Abiotic and biotic factors often mask these effects, especially when studies are moved from greenhouse to field conditions. For instance, high air temperatures can shift carotenoid biosynthesis by oxidizing lycopene to beta carotene.

Potassium fertilization has been reported to stimulate lycopene production in tomato (Trudel and Ozbun, 1970, 1971; **Table 1**). Ramírez et al. (2009) found that increasing K increased the level of lycopene with a concomitant decrease in the level of β-carotene in greenhouse grown tomato fruits (**Table 2**). Carotenes change very rapidly as the tomato fruits mature, and thus nutrient effects on maturation rate can interact with their effect on carotene content. Hartz (1991) demonstrated that a higher concentration of K in field soil directly increased carotenoid biosynthesis enzyme activities and subsequently increased lycopene content. Taber et al. (2008) found that tomato cultivars with higher lycopene responded more to high rates of KCl applied in field conditions than low-lycopene cultivars. In field conditions, lycopene content increased by as much as 22% and β-carotene fell by as much as 53% in response to added K.

Table 1. Carotenoid content (mg/kg fresh mass) of fresh market tomato fruit in response to various levels of K in the nutrient solution (adapted from Trudel and Ozbun, 1970, 1971).

K levels, mmol/L	Total carotenes	Phytoene	Phytofluene	Beta-carotene	Lycopene
0	72	11.8	4.1	3.5	36.8
1	75	12.7	4.1	3.6	41.9
2	91	16.2	5.4	3.1	53.6
4	92	15.2	4.9	2.8	52.7
6	110	14.7	5.0	2.8	59.3
8	111	15.1	4.8	2.6	61.5
10	104	16.3	5.3	2.4	52.4

Table 2. Tomato fruit concentrations of carotenoids (mg/kg fresh mass) as affected by cultivar and K nutrient solution level in the greenhouse (fruit was harvested at 7 days after breaker-stage of development (n=111) (adapted from Taber et al., 2008).

Cultivars	Total carotenes	Phytoene	Phytofluene	Beta-carotene	Lycopene
Mountain Spring	2,056	9.8	5.8	5.6	50.5
Florida 91	2,067	11.2	6.7	6.0	51.7
Fla.8153	2,088	14.4	8.8	2.7	70.5
SED	NS	0.7	0.38	0.34	2.78
K levels, mmol/L	**Total carotenes**	**Phytoene**	**Phytofluene**	**Beta-carotene**	**Lycopene**
0	-	9.5	5.9	5.0	51.3
2.5	-	11.5	7.0	4.8	55.9
5.0	-	13.5	8.0	4.7	60.0
10.0	-	12.8	7.6	4.6	63.0
Significance	-	Q**	Q**	NS	L**

SED-standard error of difference for comparison among cultivars.

Regression analysis in which NS-not significant; L-linear; Q-quadratic.

**, **¬significance at $p < 0.05$ and $p < 0.01$ respectively.*

Other researchers have reported contradictory results on the effects of fertilizers on the levels of lycopene and vitamin C. Lycopene production in tomato fruits depends not only upon K ion concentration in cytoplasm and vacuoles (Taber et al., 2008), but also upon other limiting factors, such as temperature and watering regime (Oded and Uzi, 2003; Dumas et al. 2002, 2003).

Addition of P has been reported to increase vitamin C and with variable effect on lycopene in tomato (Zdravković et al., 2007). Ahn et al. (2005) investigated the effects of P fertilizer supplementation on antioxidant enzyme activities in tomato fruits such as superoxide dismutase (SOD), guaiacol peroxidase (POX), and ascorbate peroxidase (APX). The results suggested that antioxidant enzyme activities may be influenced by the availability of P, but are subject to considerable variation depending on the developmental stage and the season. Oke et al. (2005) studied the effects of P fertilizer supplementation on processing quality and functional food ingredients in tomato and did not find any significant increase in lycopene, vitamin C, and flavor volatiles. Also, Dumas et al. (2002, 2003) did not find a positive influence of mineral nutrition on the levels of health-beneficial components. In other studies, the level of P, S, Mg, vitamins, total minerals, lycopene, and β-carotene did not show a dependency on soil K levels, but increased tomato fruit yield (both size and number of fruit) (Fontes et al., 2000, Zdravković et al., 2007). Toor et al. (2006) reported that a NO_3^--dominant fertilizer produced tomato fruit with lower acidity than NH_4^+-dominant or organic fertilizers. They also found higher phenolic and ascorbic acid content in tomatoes grown using chicken manure and grass-clover mulch as compared to mineral sources of N, but the lycopene content was 40% lower in tomatoes grown with either high Cl^- fertilizers or grass-clover mulch.

Isoflavones in Soybeans

Soybean [*Glycine max* (L.) Merr.] seeds contain isoflavones that have several positive impacts on human health. Soybean-based foods have been implicated in the prevention of chronic diseases including cancer, heart disease and osteoporosis, as well as menopausal symptoms (Caragay, 1992, Hasler, 1998 and Messina, 1995) due to the regulation of estrogen-related functions (Kitts et al. 1980, Naim et al. 1976). Isoflavones are also antioxidants (Akiyama et al. 1987) and tyrosine protein kinase inhibitors (Vyn et al. 2002). Total isoflavone concentration in soybean seeds has been reported to range between 276 and 3,309 μg/g, across a range of studies with different cultivars and environmental conditions (Carrao-Panizzi et al., 1999; Hoeck et al., 2000; Wang et al., 2000; Lee et al., 2003; Seguin et al., 2004). Total and individual isoflavone concentrations in soybean seeds are both genetically and environmentally determined. Cultivars differ widely in their isoflavone concentrations, with variations of up to 220% having been reported between cultivars grown in the same environment (Seguin et al., 2004).

Several abiotic and biotic factors have been found to affect soybean isoflavone concentrations, including air temperature, soil moisture level, and soil fertility (Tsukamoto et al., 1995; Wilson, 2001; Nelson et al., 2002; Vyn et al., 2002). Vyn et al. (2002) reported that in soils containing low to medium levels of K, isoflavone concentration in seeds may be increased up to 20% by K fertilization compared with an unfertilized control. Wilson (2001) reported that N fertilization negatively affected soybean isoflavone concentration, with a 90 kg/ha rate causing an almost ten-fold reduction in total isoflavone concentration compared with a 10 kg/ha rate. Seguin and Zheng (2006) investigated the effect of P, K, S, and B fertilization on soybean isoflavone content and other seed characteristics for two years and found that across years and cultivars, no fertilizer treatment effects were observed for most variables. This overall lack of response to fertilizers was attributed to the relatively high initial fertility of the sandy loam and sandy clay loam soils used.

Organosulphur Compounds in Brassicaceae

Brassicaceae members are major vegetables in the diets worldwide. *Brassica oleracea*, for example, includes the following staple food cultivars: cabbage, broccoli, cauliflower, kale, kohlrabi, and Brussels sprouts. Brassicaceous plants are also known for their production of the S-containing secondary plant metabolites, glucosinolates. Moderate intake of glucosinolate-containing plants is associated with a decreased risk of cancer (Gross et al., 2000; Hecht, 2000; Zhang and Talalay, 1994). When consumed, glucosinolates are hydrolyzed to isothiocyanates, which in turn stimulate the activities of the anticarcinogenic phase II human enzymes. The production of glucosinolates in brassicaceous plants is influenced by a number of factors, including plant nutrition. A plant nutrient of specific interest is Se. Selenium is similar to S in both size and chemistry, and therefore often substitutes for S in physiological and metabolic processes. Charron et al. (2001) found that total glucosinolate production in rapid-cycling *Brassica oleracea* decreased when grown in the presence of sodium selenate.

Toler et al. (2007) studied the extent of Se impact on S uptake and glucosinolate production in *Brassica oleracea* L. and found that Se increases S uptake and regulates glucosinolate metabolism. They also demonstrated that it may be possible to produce a crop of *B. oleracea* vegetables that not only have preexisting anticarcinogen-inducing health benefits from glucosinolates, but also have the added health benefit of appropriate amounts of Se.

Concluding Remarks

Fertilizers are important for human nutrition through their direct effect on producing healthy plants for food and through indirect effects of altering the nutraceuticals and other anti-aging and disease preventing compounds in plants. Fertilizer effects on nutraceuticals in plants include anthocyanin content of apples, carotenoid content of tomato, and grapefruit, glucosinolates in *Brassica*, and isoflavones in soybean. The plasticity of plant responses has made conclusive demonstrations of fertilizer effects difficult, as soil pH, season, moisture level, temperature, cultivars, and type of fertilizer can strongly influence outcomes. The increasing and aging global population underscores the need for fertilizer and a more tailored approach to optimize plant nutrition effects on key nutraceuticals and functional foods to prevent chronic disease and maintain good health. FCHH

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Chapter 8

Fertilizer Use and Functional Quality of Fruits and Vegetables

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Abstract

The crucial role that fertilizers can play in addressing the global food security problem is quite obvious since fertilizer input and crop productivity are directly related. Many initiatives aimed at securing an adequate food supply have focused primarily on improving crop productivity and market quality, thereby missing the opportunity to capture the nutritional and health benefits of foods. Consumers are increasingly aware of the health benefits of diets rich in fruits and vegetables or "Functional Foods," since these are excellent sources of essential nutrients and plant secondary compounds (phytonutrients) that have been linked with disease prevention and promotion of good health and well being. Consumption of functional foods often falls short of recommended guidelines, especially in developing economies where inadequate food supply and low phytonutrient densities of staple foods are prevalent. Besides genetics, pre-harvest farming practices, particularly fertilizer management, have a strong influence on the functional properties of foods and thus provide a sustainable and inexpensive approach for improving these attributes. Scientific evidence from numerous sources has demonstrated that judicious fertilizer management can increase productivity and market value as well as the health-promoting properties of foods. Low input or organic production systems could benefit the most from these findings since

For abbreviations and symbols used commonly throughout this book see page v.

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they often lack access to expensive, high-yielding, nutrient-fortified hybrid varieties. This chapter summarizes key findings from studies where fertilizers have impacted phytonutrient content of fruits and vegetable crops, with examples drawn from studies highlighting viable fertilization practices that can be useful in guiding policy interventions for addressing the food security and nutrition problem.

Introduction

Plant-derived foods are major components of staple diets around the world, and play a crucial role in the well being of human beings. They provide the essential nutrients (water, carbohydrates, protein, fats, minerals, and vitamins) that serve as substrates for energy, growth, and development (Lester, 1997; Kushad et al., 2003; Liu et al., 2003). Plants also synthesize and/or accumulate a diverse collection of minerals and complex secondary metabolites collectively known as phytonutrients, which have been associated with good health, disease prevention, and well being (Croteau et al., 2000; Kim et al., 2010; Prior and Cao, 1999). Phytonutrients include mineral elements, organic and inorganic compounds with known health benefits such as the essential nutrients (K, Fe, Ca, Mg, Zn) and vitamins (ascorbic acid or vitamin C, vitamin E, pro-vitamin A carotenoids), as well as trace elements (e.g. Se and Si) and secondary metabolites such as phenolic compounds, alkaloids, terpenes, and glycosides (Bramley et al., 2000; Cassidy et al., 2000; Milner, 2000; Mithen et al., 2000; van den Berg et al., 2000; Tsao and Akhtar, 2005; Winkels et al., 2007). Many of these plant secondary metabolites are synthesized in small amounts and used to attract pollinators (Croteau et al., 2000), or for protection against pathogens, herbivores, and environmental stresses (Heldt, 2005; Bray et al., 2000; Croteau et al., 2000). The vast diversity of defense-related phytonutrients that plants synthesize in response to environmental cues is a reflection of the high level of phenotypic plasticity which is essential for stress tolerance. This high degree of plasticity indicates that management practices can be manipulated to maximize the synthesis and accumulation of specific phytonutrients in target crops.

The role of nutrition in disease prevention and healthy living is receiving considerable attention, in part because of the need for alternatives to conventional strategies of disease management, and the potential for reducing health care costs through nutrition-based disease prevention programs (Milner, 2000; Bidlack, 1996; Tucker and Miguel, 1996). The current state of knowledge indicates that fruits, vegetables, and minimally-processed whole foods are excellent sources of phytonutrients and also constitute a superior delivery mechanism for dietary intake of these compounds compared to intake of supplements (Wahlqvist and Wattanapenpaiboon, 2002; USDA, 2005; WHO-FAO, 2003; Winkels et al., 2007). The awareness that plant food components have health benefits is one possible explanation for the expanding use of supplements by apparently healthy people (Eliason et al., 1997; Greger, 2001; Milner, 2000). However, purified phytonutrient compounds contained in dietary supplements often lack the synergism among multiple compounds found in whole foods (Salucci et al., 1999;

Pignatelli et al., 2000; Freedman et al., 2001; Liu, 2003; Winkels et al., 2007). These problems with supplements clearly demonstrate that the greatest benefits may be obtained only through intake of a diversified diet with fruits and vegetables as major components.

In many societies, the current per capita consumption of fruits and vegetables is very low (Johnston et al., 2000), in part due to the fact that only 20 plant species, of the approximately 7,000 described edible species, make up 90% of plant-derived human foods (FAO, 2009). Such dependence on a narrow genetic base makes the world's food supply vulnerable to disease or sudden climatic change and puts food sustainability at risk. The food security problems in many parts of the world are also compounded by limited arable land, and the fact that subsistence agriculture is still the easiest and dominant method of sustaining many families (Sanchez, 2010). Soils in these regions of the world are also highly weathered and deficient in important macro- and micronutrients that are essential for plant growth and productivity as well as for basic human nutrition (Wild, 1993). Continuous intensive cropping and inadequate replacement of nutrients removed with harvested crops have resulted in reduced productivity and nutritional quality of foods (Sanchez, 2010; Lal, 2006). This trend has also contributed to persistent chronic malnutrition in many developing countries (Sanchez and Swaminathan, 2005; Stein, 2010). Nutrient mining and the associated reductions in productivity and quality are predicted to worsen with continued population growth and climate change (Sanchez, 2010; St. Clair and Lynch, 2010; Lal, 2004; Bohle et al., 1994). Various interventions to alleviate global malnutrition such as crop fertilization and/or breeding for increased nutrient use efficiency or micronutrient contents continue to focus heavily on crop yields (Sanchez, 2010; Stein, 2010; Sanchez and Swaminathan, 2005), with little regard for consumer preferences or functional properties. It has also been suggested that some of the yield gains brought about by the Green Revolution through the use of improved cultivars, fertilization, irrigation, and mechanization might have been achieved at the expense of essential nutrient and phytonutrient contents (Davis, 2009). Such potential tradeoffs are unfortunate since billions of people globally are malnourished in mineral nutrients and vitamins (Welch, 1997). Besides genetics (cultivar or variety), farming practices (including production location, planting date, planting density, irrigation, fertilization, maturity stage at harvest) and environmental factors can have a strong influence on the composition and diversity of phytonutrients in foods (Mozafar, 1993; Dixon and Paiva, 1995; Lester and Eischen, 1996; Lester and Crosby, 2002; Crosby et al., 2003, 2008). These factors also influence consumer preference characteristics such as taste (sweetness), texture, size, color, aroma, year-round availability, ease of processing, and absence of defects. Advances in farming techniques, including fertilizer management, and the diversity of underutilized edible fruits and vegetable crops hold promise for achieving food security as well as improving health and well being (Crosby et al., 2006, 2008, 2009; Welch and Graham, 2004).

The remainder of this review will focus on the important role that fertilizer management can play on the phytonutrient content of foods. Examples are drawn from studies highlighting key fertilization practices that have so far shown promise as viable strategies for improving the phytonutrient content of fruits and vegetables. Where appropriate, a discussion of how fertilizer management can guide policy interventions for addressing the nutrition security problem (i.e. intake of essential nutrients, including proteins, minerals, vitamins, and phytonutrients) is included.

Nutrient Management

The impacts of fertilizer management on crop productivity and basic nutritional quality parameters (proteins, minerals, vitamins, and essential oils) are well documented (FAO, 1981; Marschner, 1995; Havlin et al., 2005; Stewart et al., 2005). However, information on the effects of mineral nutrients on phytonutrient compounds is limited. The potential for improving the bioactive properties of foods through fertilizer management strategies is very attractive since many mineral nutrient elements are either structural constituents of phytonutrients or participate in processes involved in phytonutrient synthesis and accumulation.

The available data suggests that fertilizers can have varying effects on phytonutrient profiles depending on the modulating effects of other environmental conditions on plant growth and development. For instance, Lester and Crosby (2002) found that vitamin C (ascorbic acid) and folic acid contents of green-flesh honeydew muskmelons were higher when grown on a clay loam compared to a sandy loam soil. Differences in the nutrient supply capacities of the different soil types probably accounted for the observed results. These modulating effects will be discussed in the succeeding sections and should be taken into account when developing fertilization strategies to improve phytonutrient contents.

Nitrogen Fertilization

Nitrogen is an essential component of nucleic acids (DNA, RNA), amino acids, proteins, and enzymes that are needed to support growth and development. Close correlations between N fertilization, leaf N concentration, leaf soluble proteins, photosynthesis and productivity have been documented for many crops (FAO, 1981; Millard, 1988; Marschner, 1995; Havlin et al., 2005; Stewart et al., 2005). Increased leaf area and photosynthetic carbon dioxide assimilation associated with adequate N supply ultimately represents a source of carbon skeletons for phytonutrient synthesis.

Depending on crop cultivar and the harvested portion, N fertilization can have varying effects on the phytonutrient composition and nutritional quality of foods. In a detailed review of the literature on the impact of N fertilization on vitamin contents in plants, Mozafar (1993) noted that the concentrations of carotenes and vitamin B_1 tend to increase with N fertilization whereas the concentration of vitamin C decreases. Recent studies have also confirmed these trends. For instance, Barickman et al. (2009) reported positive correlations between N supply amount and concentrations of antioxidant carotenoids (beta-carotene, lutein, neoxanthin and zeaxanthin) in watercress (*Nasturtium officinal* R. Br.). Similarly, Kopsell et al. (2007a) found linear increases in carotenoid concentrations (lutein, beta-carotene, and chlorophyll pigments on a dry mass basis) in leaf tissues of kale in response to N fertilization. Lutein and beta-carotene are potent antioxidant pigments and play an important role in eye health. Together with vitamins A, C, and E, they can help lower the risk of developing, or slow down the progression of age-related macular degeneration (AMD), which is one of the leading causes of blindness (AREDS, 2007).

Fertilizer N source and form can also influence phytonutrient composition (Chance et al., 1999; Errebhi et al., 1990; Xu et al., 2001). For instance, Kopsell et al. (2007a) found that increasing the $NO_3^-:NH_4^+$ ratio in fertilizer solutions resulted in significant increases in both the dry and fresh mass concentrations of lutein and beta-carotene in kale leaves. Similarly, Toor et al. (2006) found that total phenolic and ascorbic acid concentrations were highest in tomato fruits grown on grass-clover mulch (29%) compared to fruits grown on soils fertilized with chicken manure (17%) or mineral nutrient solutions containing NH_4^+ and NO_3^-. This result may form the basis for some of the quality differences often reported between organic and conventional production systems (Rosen and Allan, 2007; Lester and Saftner, 2011). Species and cultivar sensitivities to variations in N form should, therefore, be considered when designing fertilizer management strategies for phytonutrient enhancement.

In contrast to the enhancement effects of N fertilization on carotenoids, some studies have reported negative correlations between elevated N fertilization and vitamin C (ascorbic acid) concentrations in fruits and vegetables including some of the most widely consumed foods such as citrus, potatoes, spinach, cauliflower, tomato, and lettuce (Nagy, 1980; Lee and Kader, 2000; Lisiewska and Kmiecik, 1996; Sørensen, et al., 1995; Mozafar, 1993; Abd El-Migeed et al., 2007). Excessive N fertilization has also been associated with reductions in the concentrations of naringin and rutinoside in grapefruits (Patil and Alva, 1999; 2002), anthocyanin in apples (Awad and Jager, 2002), and polyphenolic compounds and antioxidant activity in basil (Nguyen and Niemeyer, 2008).

Nitrate and ammonium are the two major N forms available for crop uptake from the soil. Nitrate-N is highly soluble and is subject to leaching and groundwater contamination under certain conditions (Havlin et al., 2005). High NO_3^- levels in harvested plant products, especially fresh vegetables, have been the subject of health concerns, since consumption of NO_3^--enriched foods is sometimes linked to illnesses such as methemoglobinemia (or the blue baby syndrome) (Correia et al., 2010; Greer and Shannon, 2005), and since nearly 80% of dietary nitrates are derived from vegetables. In a survey of 34 vegetables (different varieties of cabbage, lettuce, spinaches, parsley, and turnips) collected at several locations of intensive crop production in Europe, Correia et al. (2010) found that NO_3^- and NO_2^- levels ranged from 54 to 2,440 mg NO_3^-/kg and 1.1 to 57 mg NO_2^-/kg, respectively. Since the maximum acceptable daily intake (ADI) limits for these compounds were not exceeded, the authors concluded that consumption of these vegetables would still be beneficial for human health.

Phosphorus Fertilization

Phosphorus is one of the primary essential macronutrients required for crop growth, productivity, and quality. It is involved in numerous biochemical reactions as a substrate and/or catalyst, and is a key component of many structural compounds such as phospholipids, DNA, and RNA (Marschner, 1995). Oxidative phosphorylation, the process that produces adenosine triphosphate (ATP), depends on adequate supply of P. Phosphorus is also a key substrate in reversible phosphorylation of proteins which regulates many metabolic processes in plants and animals (Marschner, 1995; Cohen, 2001). Phosphorus is a well-known yield-limiting macronutrient, especially in highly-weathered soils (Cramer, 2010; Sanchez, 2010). In such soils, most of the available P is sorbed by various soil constituents and taken out of the soil solution. Hence, P must be supplied from external sources, including commercial fertilizers, plant and animal manures, wastes, and P-containing parent materials in soils (Oberson et al., 2006; Havlin et al., 2005; Brady and Weil, 2001).

Few studies have investigated the role of P fertilization on phytonutrient content of foods. The available data are often inconsistent or sometimes contradictory. Paliyath et al. (2002) reported that supplemental soil and foliar P fertilization (with superphosphate, Hydrophos®, and Seniphos®) increased the intensity of red color in peels of 'Red Delicious' apple fruits. This suggests that P fertilization can increase the concentration of anthocyanin and other flavonoids such as proanthocyanidins and flavonols which determine peel coloration in red-skinned apples. The authors speculated that the increase in color intensity may be related to activation of the pentose phosphate pathway, from which the precursor for flavonoid synthesis (erythrose-4-P) is derived (Bruulsema et al., 2004). Anthocyanins give fruits and vegetables the characteristic purple and red color appearances and have been shown to protect tissues from oxidative damage (Kushad et al., 2003; Croteau et al., 2000; Close and Beadle, 2003; Chalker-Scott, 1999). Foliar application of P-containing substances namely, ethephon (2-chloroethyl phosphonic acid) and a fertilizer that contained P, Ca, and N, has also been shown to enhance red peel color, and an increase in the concentration of flavonoids in 'Fuji' apples (Li et al., 2002). In an investigation of the effects of N, P, and K supply on the growth and chemical properties of celery, Gurgul et al. (1994) reported an increase in the activities of key antioxidant enzymes (peroxidase, catalase, and acid phosphatase) in response to P fertilization. In contrast, Ahn et al. (2005) found no consistent effects of soil and foliar P supplementation on the activities and levels of superoxide dismutase, guaiacol peroxidase, and ascorbate peroxidase in tomato fruits. Effects of P fertilization on functional quality seem to be highly variable and dependent on production location, season, crop maturity stage, weather conditions, and other environmental factors during growth (Oke et al., 2005; Ahn et al., 2005). Bruulsema et al. (2004) also noted that weather conditions modulated flavonoid responses to P fertilization. Warm sunny days and cool nights are known to stimulate anthocyanin production (Close and Beadle, 2003).

Numerous reports have linked P deficiency with increased anthocyanin content in plant tissues (Jiang et al., 2007; Close and Beadle, 2003; Stewart et al., 2001). Reddish-purple discoloration of leaves is a common symptom of such P-deficient plants. Stewart et al. (2001) found that P deficiency elicited an increase in flavonol content in early (mature green) stages of tomato fruit ripening, but not in the later (breaker and red) stages. They speculated that induction of flavonols in the skins of young tomato fruits may be important to protect fruit tissues and developing seeds from potentially damaging UV-B radiation. Anthocyanin accumulation during P deficiency has been linked to reduced gibberellins (GA) activity, or an increase in tissue concentrations of GA antagonists such as ethylene and abscisic acid (ABA) (Jiang et al., 2007; Saure, 1990). Besides P starvation, other biotic and abiotic stresses such as herbivory, temperature, and radiation stress have also been shown to elicit anthocyanin accumulation and purple coloration of leaves (Close and Beadle, 2003; Saure, 1990; Chalker-Scott, 1999). Due to their high antioxidant capacity, flavonoids, including anthocyanins, are believed to help plants cope with environmental stresses (Close and Beadle, 2003; Chalker-Scott, 1999). Increased dietary intake of flavonoids is also associated with potential health benefits (Pietta, 2000). These established relationships between P availability and flavonoid accumulation indicate that the functional quality of fruits and vegetables can be enhanced by altering P fertilizer management strategies. Further research is needed to characterize the magnitude of any potential tradeoffs between yield and enhanced functional quality.

In addition to its role in crop growth and yield, P fertilization has been associated with the synthesis and accumulation of phytic acid (or phytate, its salt form) (Marschner, 1995; Kumar et al., 2010). Phytic acid is the principal storage form of P in many plant tissues, especially high-fiber foods such as nuts, seeds, grains, and other foods including soy products, oatmeal, corn, peanuts, kidney beans, whole wheat, and rye (Kumar et al., 2010; Sotelo et al., 2010). Because of its tremendous affinity for dietary mineral elements, especially Fe, Zn, Ca, and Mg, phytic acid, and phytates have been the subject of intense nutritional scrutiny. They interfere with the bioavailability of proteins, lipids, and essential vitamins and minerals (Kumar et al., 2010; Sotelo et al., 2010). This effect is worsened if the Zn, Fe, or Ca content of the diet is low, as is the case in many developing countries, where unrefined cereals and/or pulses constitute the staple foods (Bouis and Welch, 2010). Phytate concentrations in raw and cooked potatoes were found to range from 1.1 g/kg to 2.6 g/kg dry weight among eight potato varieties, and were 1.74, 0.95, and 2.05 g/kg in french fries, potato chips, and dehydrated potato flakes, respectively (Phillippy et al., 2004). In a survey of representative staple foods from Sidama, Southern Ethiopia, for phytate, Zn, Fe, and Ca contents, Abebe et al. (2007) found that local oilseeds (nyjer, *Guizotia abyssinica*, and sesame, *Sesamum indicum*) had the highest phytate contents (approximately 1,600 mg/100 g), whereas fermented foods prepared from enset (*Ensete ventricosum*, a root crop) and tef [*Eragrostis tef* (Zucc.) Trotter; a grain crop] had low phytate, phytate:Zn, and phytate:Fe molar ratios, whereas unleavened corn bread, kidney beans, sesame, and nyjer seeds had higher molar ratios. They concluded that the phytate content of staple foods such as enset and tef is unlikely to limit the availability of essential mineral elements unless such foods are consumed together with other high-phytate foods such as corn bread, legumes, and oil seeds.

Contrary to its status as an anti-nutrient, there is increasing evidence that dietary phytates have beneficial health effects, such as protection against a variety of cancers, heart-related diseases, diabetes, and renal stones. They can also act as antioxidants in preventing the formation of free radicals, thereby reducing oxidative stress and preventing related diseases such as cardiovascular disease, kidney and other cancers (Graf and Eaton, 1990; Hanson et al., 2006; Kumar et al., 2010; Prieto et al., 2010). More research is needed to characterize the phytate profiles of staple and underutilized crops, their responses to P fertilization, production system (conventional versus organic) and potential anti-nutritional interactions prior to implementing fertility programs as a strategy to alleviate malnutrition and food insecurity.

Potassium Fertilization

Together with N and P, K is one of the primary essential macronutrients involved in numerous physiological processes that control plant growth, yield, and quality (Marschner, 1995; Lester et al., 2005). Even though K is not an integral part of any plant structures, it plays a key regulatory role in many physiological processes. Other documented K-mediated processes include enzyme activation, osmoregulation, regulation of stomatal opening/closing, photosynthesis and transpiration, phloem transport, and fruit sugar accumulation (Usherwood, 1985; Geraldson, 1985; Kafkafi et al., 2001; Pettigrew, 2008; Marschner, 1995; Mengel and Kirkby, 1987). Many market quality and consumer preference traits such as taste, texture, and appearance are positively correlated with K availability (Usherwood, 1985; Lester, 2006). The positive effects of K nutrition on quality development are largely related to the promoting effects on enzyme activation, photosynthesis, and assimilate transport to storage sink organs such as fruits (Jifon and Lester, 2009; Pettigrew, 2008; Lester et al., 2006; Marschner, 1995).

As in plants, K is important for animal physiology in maintaining a constancy of the internal environment (homeostasis), thus allowing normal functioning of vital processes such as enzyme activation, nerve impulses, heartbeat, and muscle activity. Inverse associations between K intake and the incidence of cardiovascular diseases such as stroke and coronary heart disease have been reported (He and MacGregor, 2008). Most fruits and vegetables including muskmelons (cantaloupes and honeydew melons), watermelons, squash, pumpkins, tomatoes, broccoli, orange juice, potatoes, bananas, avocados, peaches, pears, apples, soybeans, and apricots are good sources of dietary K (USDA, 2010; Lester, 1997). Insufficient consumption of fruits and vegetables has been implicated as the reason for low dietary K intake, currently at ~2 g/day, compared to the recommended 3-5 g/day (He and MacGregor, 2008; US Institute of Medicine, 2005).

Insufficient consumption of fruits and vegetables and hence K intake is partly linked to poor market and consumer preference quality characteristics such as taste, flavor, and texture, which are directly related to K availability during plant growth. In many species, K uptake occurs mainly during the vegetative stages when root growth is not inhibited by carbohydrate availability. Competition for photoassimilates between developing fruits and vegetative organs during reproductive growth stages can limit root growth/activity and K uptake (Ho, 1988). This creates an apparent K deficiency that can limit photoassimilate translocation to developing seeds and fruits, potentially reducing yield and quality.

Muskmelons (*Cucumis melo L.*), tomato (*Solanum lycopersicum*), citrus (Citrus spp), and banana (*Musa sapientum*) are commercially important horticultural fruit crops that are widely consumed for their nutritional benefits, and more recently for their functional properties. They are also excellent examples of the positive influence of K fertilization on yield and functional quality parameters, as briefly highlighted in the following examples.

Muskmelons

Muskmelons (*Cucumis melo L.;* including the Reticulatus and Inodorus or honeydew Groups) are rich sources of K and other phytonutrients such as ascorbic acid, beta-carotene, and folic acid. In the U.S., muskmelons are one of the few fruits that have experienced a significant increase (>2-fold) in consumer demand within the last three decades (Lester, 2006) thanks to preference traits such as sweetness and year-long availability.

Compared to nine other highly consumed fresh fruits, muskmelons ranked among the top five foods in Dietary Reference Intake (DRI) levels for beta-carotene (pro-vitamin A), ascorbic acid, K, and folic acid (Vitamin B9) (Lester, 2006). This high diversity of phytonutrients in muskmelon makes it an excellent dietary component for healthy living. However, there is considerable variability in the levels of these phytonutrients in muskmelon fruits due to cultivar differences as well as environmental factors (Jifon and Lester, 2009; Lester and Crosby, 2002; Lester and Eischen, 1996). One of the critical environmental factors regulating muskmelon phytonutrient contents is K availability (Lester, 2006). As discussed in preceding sections, however, soil-derived K is not always optimal during the critical fruit development period, and this is partly responsible for poor fruit quality, including phytonutrient contents.

Controlled-environment and field investigations have shown that supplementing soil-derived K with foliar applications can alleviate this deficiency and enhance quality traits such as sweetness, texture, color, vitamin C, beta-carotene, and folic acid contents (Jifon and Lester, 2009; Lester et al., 2005, 2006). These quality improvements were generally greater with S-containing K sources [e.g. potassium thiosulphate ($K_2S_2O_3$) and potassium sulphate (K_2SO_4)] and amino acid-K chelates (e.g. Metalosate® Potassium) compared to standard mineral K sources (KCl and KNO_3). Plausible mechanisms for the promoting effects of K fertilization include a combination of improved photosynthesis, assimilate translocation from leaves to fruits, improved leaf and fruit water relations, increased enzyme activation, and substrate availability for biosynthetic pathways (Marschner, 1995). Among several K salts studied, late-season foliar KNO_3 application consistently resulted in non-significant effects on fruit quality, perhaps due to its tendency to stimulate vegetative growth (mainly stems and leaves) at the expense of roots, fruit yield and quality development. Foliar fertilization with KNO_3 would be more beneficial during the vegetative growth stages when N is most needed for canopy development to establish a high photosynthetic capacity. Foliar fertilization is generally not meant to replace soil fertilization. In environments where soil K uptake is limited, carefully-timed, soil and late-season foliar K fertilization can improve the market, and functional quality of fruits. Late-season foliar K fertilization is a readily-applicable practice that growers can easily adopt to improve the functional properties of foods.

Tomato

Tomato (*Solanum lycopersicum*) is one of the most important horticultural fruit crops globally, and is an important source of phytonutrients such as lycopene, beta-carotene, ascorbic acid, phenolics, flavonoids, and vitamin E (Clinton, 1998;

Kaur et al., 2004; Dorais et al., 2008). Differences in shape, size, color, and maturity stages have been shown to influence tomato phytonutrient contents; for instance, the small-fruited (cherry) types generally have higher lycopene and antioxidant activities (Cox et al., 2003; Kaur et al., 2004; Wold et al., 2004; Passam et al., 2007).

Tomatoes require large amounts of K for optimum yield and quality; K deficiency results in slow and stunted growth, blotchy ripening, puffiness, hollow fruits, misshapen fruits, poor color development and a reduction in yield and the proportion of marketable fruits (Usherwood, 1995; IFA, 1992; Geraldson, 1985). Various effects of K fertilization on fruit lycopene and other carotenoids have been reported (Saito and Kano, 1970; Trudel and Ozbun, 1971; Dumas et al., 2003; Passam et al., 2007). Tomato yield improvements in response to K fertigation (Hartz et al., 2005) as well as improvements in fruit soluble solids, titrable acid, and ascorbic acid contents (Peyvast et al., 2009), in response to foliar K applications have also been reported. As with K fertilization of muskmelons (Jifon and Lester, 2009), additional factors such as timing and source of K fertilization can intensify or mask the beneficial effects of K fertilization on fruit quality (Hartz et al., 2001). For instance, late-season foliar fertilization with N-containing K sources such as KNO_3 can have the undesired effect of stimulating vegetative development and delay fruit development and maturation (Neuweiler, 1997; Jifon and Lester, 2009). Such factors should, therefore, be taken into consideration in fertilization programs for phytonutrient improvement.

Citrus

Citrus fruits (including oranges, grapefruit, tangerines limes, lemons, and others) are produced in many countries, and are among the richest sources of a variety of phytonutrients including ascorbic acid, lycopene, beta carotene, limonoids, and flavonone glycosides such as naringin, narirutin, and hesperidin (Somasundaram et al., 2009; Murthy et al., 2009; Park et al., 2009; Harris et al., 2007). Global consumption of citrus products continues to increase, thanks, in part to increasing consumer awareness of its health benefits, as well as improved quality, year-round availability and affordability.

Potassium fertilization is a critical factor for citrus fruit quality development (Koo, 1985). It is estimated that approximately 2 kg of K is removed per ton of harvested citrus fruit, which exceeds the amount of any other nutrient element removed and reflects the high K content of citrus juice (IFA, 1992; Koo, 1985). Citrus phytonutrients, particularly ascorbic acid, and other quality factors such as fruit juice content, soluble solids and acid concentrations, soluble solids/acid ratio, fruit size, and color, fruit size, shape, and rind thickness are all impacted by K nutrition (Koo, 1985). In pink grapefruit, supplemental foliar K resulted in increased lycopene, beta-carotene, and vitamin C concentrations (Patil and Alva, 2002). However, higher levels of soil-applied K resulted in lower fruit total ascorbic acid levels (Patil and Alva, 2002) perhaps highlighting the effects of timing as well as application method on K uptake and metabolism. The positive effects of K fertilization are probably related to improved enzyme activity, carbohydrate assimilation, transport, and sugar metabolism (Marschner, 1995).

Banana

Bananas (*Musa spp*) are cultivated in over 130 countries, and are one of nature's best known sources of K and one of the most convenient and nutritionally dense food items. They are also good and inexpensive sources of vitamins A, C, B6, and minerals (Robinson, 1996). In addition to the well-known effects of K in lowering the risk of developing diseases such as heart attack and strokes, functional compounds in banana are reported to relieve constipation, heartburn, ulcers, and have been linked to prevention of anaemia by stimulating the production of haemoglobin in the blood (Robinson, 1996).

Banana productivity and quality are strongly influenced by K nutrition. According to IFA (1992), bananas are among the highest accumulators of fertilizer K, with uptake/removal amounts ranging from ~20 kg K/t whole bunch (for Cavendish type varieties) to 50 kg K/t in other cultivars. Von Uexküll (1985) estimated that a banana plantation yielding 50 t/ha requires approximately 1,625 kg K/ha with most of the K being absorbed during bunch growth. Several studies have reported positive correlations between K nutrition and banana fruit quality parameters such as total soluble solids, reducing sugars, non-reducing sugars, total sugars and ascorbic acid, and negative correlations with fruit acidity (Al-Harthi and Al-Yahyai, 2009; Kumar and Kumar, 2008; Hongwei et al., 2004). Investigations by Kumar and Kumar (2008), comparing the impact of fertilizer K source (KCl versus K_2SO_4) on banana quality have also confirmed the positive effects of K supply on fruit quality parameters. This study also demonstrated that the beneficial effects of K fertilization were greater with K_2SO_4 than with KCl, which is similar to results obtained for muskmelon fruit by Jifon and Lester (2009).

Many current fertilizer recommendations are designed to optimize crop yields, while quality attributes are assumed to depend on other cultural practices such as variety selection or timing of produce harvest. It is apparent from the preceding discussion that carefully-designed fertilizer management strategies can play a key role in quality enhancement. With respect to K management, the current evidence indicates that supplementing soil K with foliar K fertilization during

the fruit development and maturation period can improve consumer preference attributes and functional quality of fruit and vegetable crops. However, in order to develop K fertilizer recommendations for improving the functional quality of foods, information regarding crop nutrient removal amounts, production season, fertilizer source, and soil properties is required. This information is useful in determining nutrient amounts that must be applied to sustain yields and quality while maintaining soil fertility. This information can also be useful in selecting cultivars for specific sites based on their nutrient accumulation/removal capacities.

Sulphur and Selenium Fertilization of *Allium* and *Brassica* Crops

Allium crops including onion, garlic, leeks, and chives, have been cultivated and used in human diets for centuries for their unique flavors. More recently, their benefits to human health including antiplatelet activity, anticarcinogenic properties, antithrombotic activity, antiasthmatic and antibiotic effects have been reported (Turner et al., 2009; Havey, 1999). The functional flavor components of *Allium* crops are organo-S compounds that are synthesized from a common precursor, the S-alk(en)yl cysteine sulfoxides (ACSOs) (Yoo and Pike, 1998). Sulphur is directly involved in the synthesis of the ACSOs, and is a major constituent of the flavor compounds; higher available S in the soil generally results in greater flavor intensity and also alters the composition of ACSOs (Randle et al., 2002; Randle et al., 1995; Coolong and Randle, 2003; Randle and Brussard, 1993; Bloem et al., 2005; McCallum et al., 2005).

Brassica crops, including broccoli, brussels sprouts, kale, and radish, are also excellent sources of S-containing phytonutrients such as glucoraphanin, which have been associated with numerous health benefits (Cartea and Velasco, 2008; Johnson, 2002; Osmont et al., 2003). Positive correlations between S fertilization and the concentrations of these phytonutrients have been reported (Barickman et al., 2009; Kopsell et al., 2007b; Aires et al., 2007; Finley, 2007; Bloem et al., 2007).

Selenium fertilization of crops, especially *Brassicas*, has recently gained considerable attention, in part because Se is an essential trace element involved in protein synthesis, and has shown antioxidant, anti-inflammatory, and anti-carcinogenic properties (Ip et al., 1992). Although trace amounts of Se are necessary for cellular function, adverse health effects associated with Se deficiency or excess supply have been observed, in part due to the relatively narrow range for optimal Se requirements (Jackson-Rosario and Self. 2010). Selenium is also closely related to S, and may be substituted for S in metabolic pathways (Young 1981; Mäkelä et al., 1993; Goldman et al., 1999; Arthur, 2003; Finley, 2007). Selenium concentration in foods is directly related to the soil content where the crops were grown (Arthur, 2003). Several studies have demonstrated that Se fertilization increases crop uptake (Kopsell et al., 2009; Kopsell et al., 2007b; Finley, 2007; Toler et al., 2007; Charron et al., 2001; Barak and Goldman, 1997), and may alter the relative levels of individual S-containing phytonutrients (Charron et al., 2001). Many countries have now established Se-fortification programs aimed at increasing intake rates to recommended sufficiency levels (Lintschinger et al., 2000; Mäkelä et al., 1993).

Concluding Remarks and Future Perspectives

The central role that fertilizers play in addressing the global food security problem is irrefutable. While fertilizer use in crop production has been instrumental in increasing food production in many societies, lack of access (availability and affordability) to foods with health benefits (functional foods) is still a global problem. Previous policies to eliminate hunger by increasing access to food have focused heavily on improving crop productivity. The compelling evidence linking diet and health presents a unique opportunity for redefining global agricultural food policies to promote the production of foods rich in a wide variety of phytonutrients. This has the potential to improve food supplies and reduce disease incidences and health care costs globally.

The link between fertilizer management and phytonutrient concentrations in fruits and vegetables is becoming much stronger. Fertilizer management represents a sustainable and inexpensive complement to conventional breeding and biotechnology for improving the human-health properties of foods. However, major gaps still exist in the knowledge regarding interactive effects among fertilizers, and among phytonutrients in foods. For instance, the relationships between P fertilization, phytate accumulation, and micronutrient bioavailability discussed earlier, clearly demonstrate that alternative P management guidelines are needed to maintain an optimal balance between crop growth, micronutrient and phytonutrient contents, and bioavailability. Fertilizer management strategies should take into account the potential for significant tradeoffs among biosynthetic pathways and functionality of target phytonutrients. Human nutrition guidelines that emphasize consumption of balanced/diversified diets, containing a wide variety of fruits, vegetables, and whole-grain products, can ensure optimal bioavailability of essential nutrients and phytonutrients, and minimize some of the observed negative interactions. Given the rapid global increase in consumer demand for organically-grown foods, research documenting the relative effects of fertilizers on the functional properties of foods derived from conventional or organic production systems is warranted. Research is also needed to characterize the caloric and phytonutrient contents of the vast majority of edible, non-staple food crops, and farmers should be encouraged to focus not only on yield, but also on nutritional and health benefits of produce. Nevertheless, enhancing the human-health quality of foods through carefully-planned fertilizer management practices can be an effective dietary approach for enhancing the health, well being, and productivity of human beings. FCHH

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