

BETA-CRYPTOXANTHIN: AN OVERVIEW ON DIETARY SOURCES, METABOLISM,
BENEFITS IN HUMAN HEALTH, AND BIOFORTIFICATION

by

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CHAPTER 1

Literature Review

Carotenoids are a group of naturally occurring phytochemicals in plants, many of which are important in human nutrition and health. Among more than 700 carotenoids, β -carotene, α -carotene, β -cryptoxanthin, lutein, and zeaxanthin, are the most abundant, especially in yellow and orange fruits, dark green leafy vegetables, and yellow and orange maize. Many carotenoids have demonstrated important roles in human health. Some of which include potent antioxidant properties to protect cells and tissues from oxidative stress and free radicals, enhancement of immune function, and suppression of certain types of cancer (Bendich 1989; Nishino 1998; Rao and Rao 2007). The provitamin A activity is the most well-studied nutritional role for some carotenoids, in particular, β -carotene (Figure 1). Promotion of foods rich in provitamin A carotenoids could help prevent vitamin A deficiency, a major global health concern and one of the leading micronutrient deficiencies prevalent in impoverished areas.

β -Cryptoxanthin is a provitamin A carotenoid that has recently received attention in its role in human biological functions. Provitamin A activity of β -cryptoxanthin comes from its structure, which produces one retinol molecule upon central cleavage. β -Cryptoxanthin is also classified as a xanthophyll because of the hydroxyl group on one side of the molecule. This bipolar property may differentiate β -cryptoxanthin from the hydrocarbons α - and β -carotene, which are more hydrophobic, or the dihydroxy-xanthophylls with no vitamin A value, such as lutein and zeaxanthin (Figure 1).

Because of the free radical quenching ability and effects on cell differentiation and proliferation, multiple recent studies have suggested that β -cryptoxanthin protects against

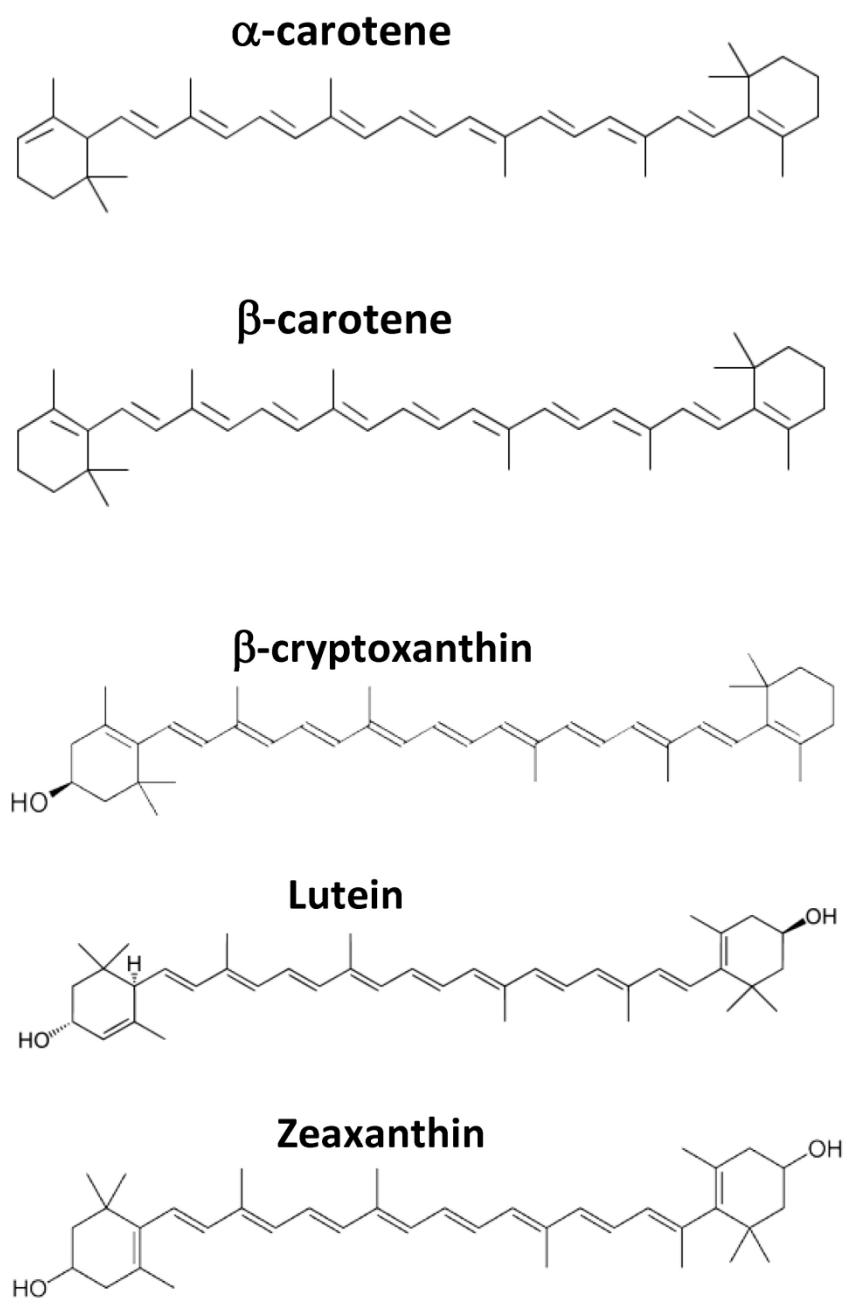


Figure 1. Major carotenoids found in the human diet. Hydrocarbon carotenoids include α - and β -carotene; and β -cryptoxanthin, lutein, and zeaxanthin are classified as xanthophylls. Provitamin A carotenoids are cleaved to yield retinol. Theoretically, two molecules of retinol are produced by cleaving β -carotene, while the cleavage of β -cryptoxanthin and α -carotene yield one.

certain diseases such as cardiovascular disease, osteoporosis, and cancer (Uchiyama and Yamaguchi 2005; Lian and others 2006; Voutilainen and others 2006; Lorenzo and others 2009).

Dietary Sources, Bioavailability and Conversion of β -Cryptoxanthin to Vitamin A

Food Sources

Fruits and vegetables are the major dietary sources of 80 – 90% of total carotenoid intake (Maiani and others 2009). β -Cryptoxanthin is primarily found in citrus fruits such as tangerines, oranges, mandarins, and other tropical fruits such as papaya, mango, guava, apricots, and nectarines (Irwig and others 2002; Chandrika and others 2003; Schlatterer and Breighaupt 2005; Maiani and others 2009; Arscott and others 2010). Other good dietary sources include red chilies and pumpkins (Maiani and others 2009; Burri and others 2011). Some varieties of local and indigenous foods specific to certain areas of the world have also identified β -cryptoxanthin as a dietary component. The camu-camu, acerola (Barbados cherry), pitanga, and pequi, which are indigenously grown and locally consumed in Brazil, are a few examples (Azevedo-Meleiro and Rodriguez-Amaya 2004). In addition, some varieties of typical maize contain modest concentrations of β -cryptoxanthin (Kurilich and Juvik 1999); although in general, provitamin A carotenoids are not naturally abundant in staple foods (e.g., rice and maize). Recently, increased efforts to biofortify maize through various methods have produced many varieties of maize with enhanced provitamin A carotenoids containing substantially elevated β -cryptoxanthin concentrations (Davis and others 2008a; Harjes and others 2008). The continuous promotion of maize biofortified with

provitamin A carotenoids could help alleviate the prevalence of vitamin A deficiency in impoverished regions of Sub-Saharan Africa, Central America, and South-East Asia where people mainly rely on staple foods (Tanumihardjo 2008; Tanumihardjo and others 2008; Liu and others 2012).

Bioavailability and Bioconversion of β -Cryptoxanthin

Understanding how the human body can obtain, absorb, and utilize β -cryptoxanthin efficiently from food is important. Bioavailability refers to the fraction of ingested carotenoid that is absorbed and available for physiological function. The esterified form as laurate, myristate, or palmitate is the primary storage form of β -cryptoxanthin in fruits and vegetables (Breithaupt and Bamedi 2001). Prior to absorption, β -cryptoxanthin esters are hydrolyzed by intestinal enzymes. The non-esterified β -cryptoxanthin has a much higher bioavailability than esterified β -cryptoxanthin, as the increased polarity may make β -cryptoxanthin more easily incorporated into micelles, ready for a more efficient transport from the lumen into enterocytes (Dhuique-Mayer and others 2007; During and Harrison 2007). β -Cryptoxanthin is suspended in oil droplets in some citrus fruits and maize, which is the most bioavailable form (Howe and Tanumihardjo 2006; O'Connell and others 2007).

Other nutrients, such as fat and fiber, when consumed with β -cryptoxanthin, have an effect on bioavailability (Deming and others 2000; Ribaya-Mercado and others 2007). Accumulated evidence has confirmed the role of dietary fat intake in the absorption of provitamin A carotenoids and their bioconversion into vitamin A (Prince and Frisoli 1993; Khachik and others 1997; Ribaya-Mercado and Blumberg 2004; Unlu and others 2005).

Relatively low amounts of dietary fat, suggested by previous studies, are necessary for promoting optimal absorption and maximizing bioavailability (Nestel and Nalubola 2003; Brown and others 2004). Dietary fiber, on the other hand, could interact with bile acids by binding with carotenoids, increasing fecal excretion, and leading to decreased bioavailability (Rock and Swendseid 1992; Hoffmann and others 1999).

The amount of vitamin A produced from bioavailable carotenoids defines the bioconversion of provitamin A carotenoids, which predominantly occurs in enterocytes or liver cytosol (Leuenberger and others 2001). β -Cryptoxanthin is centrally cleaved by 15, 15' carotenoid monooxygenase (CMO1). From the chemical structure, central cleavage of β -cryptoxanthin theoretically produces one molecule of retinol compared to two molecules of retinol from β -carotene (Figure 1). The process of converting provitamin A carotenoids to biologically active retinol is dependent on many physiological factors such as the vitamin A status of the host (Ribaya-Mercado and others 2000; Tang and others 2000; Haskell and others 2004, Tanumihardjo and others 2010). The conversion rate of dietary β -cryptoxanthin to vitamin A, proposed by the Institute of Medicine (IOM), is the same as α -carotene, *i.e.*, 24 μg to 1 μg retinol, and is twice the amount of β -carotene, *i.e.*, 12 μg to 1 μg retinol (Institute of Medicine 2001). Although studies directly investigating the vitamin A value of β -cryptoxanthin from food sources are relatively few, previous evidence using Mongolian gerbils fed maize diets with varying quantities of β -cryptoxanthin or supplements suggests that β -cryptoxanthin is as efficacious as β -carotene in maintaining vitamin A status (Davis and others 2008a and 2008b). The conversion factor achieved was 2.74 μg β -cryptoxanthin to 1 μg retinol (Davis and others 2008a). Other studies with humans have investigated the

vitamin A value of β -cryptoxanthin. Indonesian schoolchildren with a marginal vitamin A status who consumed orange fruit, containing a substantially high concentration of β -cryptoxanthin, had a greater increase in serum retinol than those who obtained more provitamin A carotenoids from vegetables (De Pee and others 1998). Based on these findings, future investigations may challenge and improve the current knowledge of the human body's ability to convert dietary β -cryptoxanthin to vitamin A.

Purification and Synthesis of β -Cryptoxanthin

Extraction and Purification from Foods

A process that employs simultaneous extraction and saponification procedures primarily for the purification of lutein and zeaxanthin as well as several dietary carotenoids including β -cryptoxanthin from Marigold flowers *Tagete erecta* and *Lycium Chinese Mill* (LCM berries) was previously described by Khachik (Khachik 2001). The plants were mixed and homogenized with tetrahydrofuran (THF), ethanol, and 10% potassium hydroxide (KOH) at room temperature for 2 hours. The procedure extracted carotenoids and simultaneously hydrolyzed the esters of major dihydroxy-xanthophylls such as lutein and zeaxanthin as well as monohydroxy-xanthophylls such as β -cryptoxanthin. The mixture was filtered off, the solids washed with THF, and the solvents evaporated. The residue was repeatedly washed with water and ethanol until the aqueous wash returned to pH=7, removing all the base and salts of fatty acid esters. The solids were centrifuged with ethanol and dried to give crystals that were 70% pure lutein or zeaxanthin, which were re-dissolved in THF and passed through a glass column packed with silica gel. The column was washed using THF and water to

collect fractions; the first fraction obtained was enriched in α -cryptoxanthin and β -cryptoxanthin in high purity (Khachik 2001).

An alternative method was documented for isolating β -cryptoxanthin from papayas (700 g) (Breithaupt and Bamedi 2001). After repeated extraction of the pulp with diethyl ether/light petroleum ether (1:1 v/v), the extracts were combined, dried, filtered, and the solvent evaporated. The residue was re-dissolved in diethyl ether and 30% potassium hydroxide (KOH) and methanol were added for saponification at room temperature overnight. The solution was repeatedly washed with water for the removal of base and dried before the resulting residue was re-dissolved in hexane and passed through a glass column on silica gel (open column chromatography). While the first band contained mainly β -carotene, the second band, obtained by elution with light petroleum ether/acetone as the mobile phase, consisted primarily of β -cryptoxanthin. A yield of 2.0 mg β -cryptoxanthin was reported by the authors (Breithaupt and Bamedi 2001).

The extraction and isolation from foods and large-scale production of purified dietary carotenoids such as β -cryptoxanthin are of great importance. Individually or combined with other carotenoids, β -cryptoxanthin may be safely used as a supplement and food color enhancer in animal feeding studies and human interventions where its potential health benefits could be evaluated.

Synthesis of β -Cryptoxanthin

β -Cryptoxanthin is considered a rare carotenoid in nature, and its synthesis for industrial production is not a very efficient or economically viable process. Although total

synthesis of optically inactive β -cryptoxanthin (Isler and others 1957; Loeber and others 1971) and optically active (3R)- β -cryptoxanthin- β -D-glucopyranoside (Yamano and others 2000) have previously been reported, these elaborate synthetic methods are expensive and difficult to implement, involving numerous steps.

A two-step method involving acid-catalyzed dehydration of commercially available lutein followed by ionic hydrogenation was developed and reported by Khachik *et al.*, transforming lutein to a mixture of optically active α -cryptoxanthin and β -cryptoxanthin where β -cryptoxanthin was the major product (Khachik and others 2007). The first step of the reaction was achieved with acid-catalyzed dehydration of (3R,3'R,6'R)-lutein at elevated temperatures (90–97°C) in a reflux solution of propanol, water, and acid. This resulted in a mixture of anhydrolutein I; 2',3'-anhydrolutein II; and 3',4'-anhydrolutein III in which the latter was the major product due to the efficient isomerization of anhydrolutein I and II to anhydrolutein III at high temperature. In the second step of the reaction, the mixtures of anhydroluteins underwent ionic dehydrogenation with trifluoroacetic acid and trimethylamine borane (TFA/Me₃N·BH₃) to yield a mixture of (3R)- β -cryptoxanthin (76%), (3R,6'R)- α -cryptoxanthin (23%), and only 1% of (3R,5'RS,6'R)-3',4'-didehydro-5',6'-dihydro- β , β -caroten-3-ol (a regioisomer of α -cryptoxanthin) in nearly 80% isolated yield (Khachik and others 2007). Although not confirmed, the findings also suggested that (3R,6'R)- α -cryptoxanthin was not isomerized to give (3R)- β -cryptoxanthin under the reactions, and β -cryptoxanthin was exclusively formed from 3',4'-anhydrolutein III (Khachik and others 2007).

Potential Benefits of β -Cryptoxanthin in Human Health

Lung Cancer and Cardiovascular Diseases

In addition to providing vitamin A, epidemiological studies have suggested a link between consumption of fruits and vegetables containing β -cryptoxanthin and reduced risk of developing many chronic diseases (Ziegler 1991; Tanumihardjo 2012). β -Cryptoxanthin may be associated with improved pulmonary health. Increased levels of β -cryptoxanthin, both dietary and serum, was associated with a reduced risk for lung cancer in Chinese populations (Yuan and others 2001 and 2003). In fact, results from a pooled analysis of seven cohort studies evaluating the correlation between dietary carotenoids and lung cancer risk determined that β -cryptoxanthin was the only major dietary carotenoid with a 24% reduction in relative risk (Männistö and others 2004). Additional findings suggest that high concentrations of β -cryptoxanthin from mandarin juice reduced lung tumor development in mice with chemically induced carcinogenesis compared with a control group (Kohno and others 2001). Prior intervention studies reported that β -carotene supplementation failed to provide such protection (Gallicchio and others 2008).

In addition to β -cryptoxanthin antioxidant protection from oxidative stress (Lorenzo and others 2009), a connection between the suppression of tumor cell growth in the lung and up-regulated expression of retinoic acid receptor- β was recently observed, suggesting a different mechanism for improved pulmonary health (Lian and others 2006). Retinoic acid receptor (RAR) and retinoid X receptor (RXR) bind to retinoic acid and retinoid X response elements, respectively, and act as transcription factors to regulate gene expression. They are important in regulating cellular growth and differentiation, and in particular, inhibiting the

development of non-small cell lung cancer (Brabender and others 2005). Based on previous results, all RAR and RXR subclasses exhibit down-regulated expression and reduced function in tumor development and prognosis of non-small cell lung cancer (Brabender and others 2005). According to the findings by Lian *et al.*, β -cryptoxanthin effectively inhibited the growth of BEAS-2B cells, a pre-malignant immortalized human bronchial epithelial cell line and A549 cells, a non-small cell lung cancer cell line (Lian and others 2006). β -Cryptoxanthin also escalated the mRNA levels of RAR- β in both BEAS-2B cells and A549 cells, which was consistent with suppression of lung tumor cell growth, although this gene expression up-regulation effect was less profound in the A549 cells (Lian and others 2006). More investigations are needed to understand the biological activity and molecular mechanisms by which β -cryptoxanthin may affect pulmonary tumorigenesis.

Although previous observational data suggested that dietary consumption of carotenoids from fruits and vegetables in general may decrease the morbidity and mortality associated with cardiovascular diseases (Gaziano and others 1995), evidence linking individual carotenoids to cardiovascular health and information on β -cryptoxanthin specifically is lacking (Sesso 2006). However, a recent review discussing a previous observational study, the Atherosclerosis Risks in Communities Study, noted an inverse association between serum β -cryptoxanthin and lutein plus zeaxanthin concentrations and the extent of atherosclerosis (Voutilainen and others 2006). Other evidence has demonstrated that high dietary intake of β -cryptoxanthin reduced the risk of angina pectoris (Ford and Giles 2000).

Inflammatory Disorders

Free oxygen radicals and their oxidation products play an important role in the development of inflammatory disorders such as rheumatoid arthritis, a chronic disease characterized by persistent inflammation of the joints, leading to damage and ultimately destruction of the joints. Recent epidemiological findings from the Iowa Women's Health Study reported that high dietary intake of β -cryptoxanthin, and not any other major carotenoid was strongly associated with reduced risk of rheumatoid arthritis, suggesting that β -cryptoxanthin may be protective against the oxidative damage and the development of this inflammatory disease (Cerhan and others 2003). In a separate study, dietary β -cryptoxanthin had an independent and strong association with reduced risk of developing inflammatory polyarthritis (Pattison and others 2005). Although these prior results suggest that the antioxidant property of β -cryptoxanthin may explain the association, specific mechanisms outlining this relation are still unclear and additional studies are needed to confirm the potential protective benefit of β -cryptoxanthin for such serious chronic diseases.

Osteoporosis, Bone Health, and Other Potential Benefits

β -Cryptoxanthin has potential protective effects against osteoporosis, a disease characterized by loss of bone mass and deterioration of bone tissue leading to brittle, fragile bones and fractures. Although calcium and vitamin D deficiencies lead to an imbalance of bone remodeling and adequate intakes may prevent and treat osteoporosis (Nieves JW 2003 and 2005), other factors, particularly oxidative stress, could also play a role (Garrett and others 1990; Basu and others 2001; Mody and others 2001). A combination of *in vitro* studies

show that β -cryptoxanthin is uniquely effective in stimulating osteoblastic bone formation and suppressing osteoclastic bone resorption (Yamaguchi and Uchiyama 2003 and 2004 and 2008; Uchiyama and Yamaguchi 2006a). Previous *in vivo* studies in rats have demonstrated similar anabolic effects on bone components and preventive effects against bone loss (Yamaguchi and others 2006; Uchiyama and Yamaguchi 2006b). Additionally, recent investigations found that consumption of β -cryptoxanthin-reinforced mandarin juice had a stimulatory effect on bone formation and an inhibitory effect on bone resorption in healthy individuals, suggesting that dietary intake of β -cryptoxanthin may have preventive effects on bone loss during aging (Yamaguchi and others 2004 and 2005). In a previous study among postmenopausal women, decreased serum concentrations of β -cryptoxanthin were observed in women with osteoporosis despite a higher dietary β -cryptoxanthin intake than women without the disease (Yang and others 2008). However, definitive evidence of β -cryptoxanthin to lower osteoporosis risk has not been observed in epidemiological studies (Maggio and others 2006).

Other evidence has demonstrated that high dietary intake of β -cryptoxanthin reduced the risks of hyperglycemia (Suzuki and others 2002) and certain types of cancer (Zeegers and others 2001; Chen and others 2002). These results from previous studies suggest that further investigations are imperative to better understand the mechanisms of health benefits provided by β -cryptoxanthin, and the association between the consumption of β -cryptoxanthin and prevention of chronic diseases.

Format of Dissertation

Chapter one is a short literature review that emphasized chemical structure, dietary sources, bioavailability and bioconversion, purification and synthesis, and potential roles of β -cryptoxanthin in human health. The remainder contains a review article, an original research paper and a concluding chapter. Chapter two is a comprehensive review of citrus fruits, the most common and concentrated food source of β -cryptoxanthin, with primary focus on their constituents, nutritional importance, and protective benefits in improving human health. The chapter is in submission to *Comprehensive Reviews in Food Science and Food Safety*. Chapter three investigated the influence of feeding β -cryptoxanthin biofortified maize to laying hens on concentrations of carotenoids and provitamin A equivalents in chicken eggs. The study also evaluated the change in yolk color in response to feeding. This chapter was published in *Poultry Science* (Liu and others 2012). Chapter four summarizes the findings presented in the dissertation with brief conclusions and suggestions for future research.

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CHAPTER 2

History, Global Distribution, and Nutritional Importance of Citrus Fruits

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YuQiu Liu conducted the literature search and drafted the majority of the following manuscript. Emily Heying prepared the sections related to β -cryptoxanthin (included in Fat-soluble vitamins, Micronutrients and Phytochemicals) and Flavonoids (included in Micronutrients and Phytochemicals) of the manuscript. Dr. Sherry Tanumihardjo, the academic advisor, provided support and revised the manuscript.

History, Global Distribution, and Nutritional Importance of Citrus Fruits

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Abstract: Although the mysteries of its history and origin remain unsolved, worldwide cultivation and high-demand production for citrus fruit (genus *Citrus* in family *Rutaceae*) make it stand high among fruit crops. Growth of the citrus industry, including rapid development of the processing technology of frozen concentrated orange juice after World War II, has greatly expanded with international trade and steadily increased consumption of citrus fruits and their products during the past several decades. Characterized by the distinct aroma and delicious taste, citrus fruits have been recognized as an important food and integrated as part of our daily diet, playing key roles in supplying energy and nutrients and in health promotion. With low protein and very little fat contents, citrus fruits supply mainly carbohydrates, such as sucrose, glucose, and fructose. Fresh citrus fruits are also a good source of dietary fiber, which is associated with gastrointestinal disease prevention and lowered circulating cholesterol. In addition to vitamin C, which is the most abundant nutrient, the fruits are a source of B vitamins (thiamin, pyridoxine, niacin, riboflavin, pantothenic acid, and folate), and contribute phytochemicals such as carotenoids, flavonoids, and limonoids. These biological constituents are of vital importance in human health improvement due to their antioxidant properties, ability to be converted to vitamin A (for example, β -cryptoxanthin), and purported protection from various chronic diseases.

Keywords: beta-cryptoxanthin, carotenoids, citrus fruit, citrus oils, flavonoids, hesperidin, naringin,

Introduction

Citrus, also known as *agrumes* (sour fruits) by the Romance loanword, is one of the world's major fruit crops with global availability and popularity contributing to human diets. Due to unclear numbers of natural species and wide areas for cultivation, the most well-known examples of citrus fruits with commercial importance are oranges, lemons, limes, grapefruit, and tangerines. Although citrus fruits are grown all over the world in more than 140 countries, most of the crop grows on either side of a belt around the equator covering tropical and subtropical areas of the world 35°N and 35°S latitudes with cultivation and production concentrated in major regions in the Northern Hemisphere (Ramana and others 1981; UNCTAD 2004) (Figure 1). Annual global production of citrus fruit has witnessed strong and rapid growth in the last several decades, from approximately 30 million metric tons in the late 1960s (FAO 1967) to a total estimate of over 105 million metric tons between 2000 – 2004, with oranges contributing more than half of the worldwide citrus production (UNCTAD 2004). According to 2009 data from the Food and Agriculture Organization of the United Nations (FAO), China, Brazil, the U.S.A., India, Mexico, and Spain are the world's leading citrus fruit-producing countries, representing close to two-thirds of global production (FAO 2009) (Table 1). In the U.S.A., a total of 10.9 million metric tons of citrus production was reported for 2009 – 2010, with Florida constituting 65% as the leading state, California 31%, followed by Texas and Arizona (USDA 2010) (Table 2).

While many citrus fruits, such as oranges, tangerines, and grapefruits can be eaten fresh, about a third of citrus fruit worldwide is utilized after processing, and orange juice production accounts for nearly 85% of total processed consumption (USDA 2006). Since the introduction of frozen concentrated orange juice after World War II, which preserves fresh flavor and full color,

reduces transportation costs, and minimizes losses due to storage diseases, the U.S.A. has seen a significant increase in the use of citrus fruit (Florida Citrus Processors' Association 1978).

California is the main producer for consumption as fresh citrus fruit, while citrus processing and orange juice production primarily occur in Florida (USDA 2010). Because of the preferred flavor, delightful taste, affordable economic reach, and consumer awareness of the increasingly recognized potential health properties, citrus fruits and products are very prevalent with widespread nutritional and economic impact in both developed and developing countries (Ting 1980).

The origin of citrus fruit is identified with a history full of controversy and interesting legends. Some researchers believe that citrus is native to the subtropical and tropical areas of Asia, originating in certain parts of Southeast Asia including China, India, and the Malay Archipelago (Bartholomew and Sinclair 1952; Sinclair 1961; Scora 1975; Ramana and others 1981; Gmitter and Hu 1990). According to old manuscripts found among ancient Chinese documents, the earliest reference to citrus was documented during the reign of Ta Yu (around 2205 – 2197 BC) when citrus fruits, particularly mandarins and pummelos, were considered highly prized tributes and were only available for the imperial court (Webber 1967; Nagy and Attaway 1980). Lemon was originally grown in India and sweet oranges and mandarins are indigenous to China. Recent research suggests that, while some commercial species such as oranges, mandarins, and lemons originally came from Southeast Asia, the true origins of citrus fruit are Australia, New Caledonia (off eastern Australia), and New Guinea (Anitei 2007). The spread of citrus to other parts of the world was slow, including northern Africa and southern Europe. The first introduction of citrus to America was achieved by Spanish and Portuguese explorers, and orchards first appeared in Florida and California around 1655 and 1769,

respectively. The commercial production, processing, and global trade of citrus have significantly increased since then, placing citrus as the most important fruit in the world (UNCTAD 2004; Ramana and others 1981).

Previous work reviewed the botanical classification and horticultural varieties of citrus in different ways (Tanaka 1954; Hodgson 1967; Swingle and Reece 1967). In addition to the traditional morphological identification, chemical characteristics of citrus fruits, such as enzymes, fatty acids, hydrocarbon profiles, flavonoid patterns, and carotenoid composition, were used to develop systems for studying citrus species (Yokoyama and White 1966; Iglesias and others 1974; Nordby and Nagy 1974, 1975; Dass and others 1977; Esen and Scora 1977). Because many named species of citrus are hybrids and the numerous varieties were derived from very few ancestral species based upon genetic evidence, the number of natural species remains unknown due to difficult systematics and complex taxonomy (Nicolosi and others 2000; De Araújo and others 2003). Some researchers believe that there may be only 25 true-breeding species of citrus (Anitei 2007). Citrus plants generally are evergreen shrubs or small trees, bearing flowers which yield a strong scent. The fruits can have different forms (for example, round, oblong, or elongated) and various sizes from 3.8–14.5 cm in diameter (UNCTAD 2004; Ranganna and others 1983). Citrus fruits generally consist of an outer skin or rind made up of an epidermis (a leathery and waxy layer), the flavedo (a subepidermal layer that contains color and oil sacks producing aromatic oils), the albedo (a spongy layer below the flavedo, a source of flavanones), and vascular bundles (a network of thin threads along the flesh) (Figure 2). The inner flesh has segments, usually aligned and situated around the soft central core of the fruit and wrapped by a thin segment membrane called the septum. Small and densely packed sacs containing juice and seeds in most varieties fill the segments, and the citric acid contained in the

juice together with a complex mix of other acids, oils, and sugars, give the characteristic flavor (Albrigo and Carter 1977; Ranganna and others 1983). An increase in total sugar, decreases in acidity and ascorbic acid content, change of peel color, and increase in fruit size indicate advancement through maturation and ripening. Citrus fruits are ready to be consumed and processed upon harvesting with no further significant change in composition (Harding 1947; Bain 1958; Sinha and others 1962; Ramana and others 1981).

The established nutrient values of citrus fruits are beyond providing vitamin C (Nagy 1980). The fruits are abundant in macronutrients, such as simple sugars and dietary fiber, and are a source of many micronutrients including folate, thiamin, niacin, vitamin B₆, riboflavin, pantothenic acid, potassium, calcium, phosphorus, magnesium, and copper, which are essential for maintaining health and normal growth (Rouseff and Nagy 1994; Economos and Clay 1999). Citrus fruits are also low in energy density and free of sodium and cholesterol (Guthrie and others 1995; Whitney and others 2009; USDA Natl. Nutrient Database 2011a) (Table 3). In addition, understanding the variety of naturally occurring phytochemicals, including limonoids, flavonoids, and carotenoids, is actively being researched. Recent epidemiological studies and other investigations have demonstrated that these bioactive compounds have a broad range of physiological effects and may contribute to the associations between citrus fruit consumption and prevention of chronic diseases (Steinmetz and Potter 1991; Silalahi 2002; Liu 2003; Yao and others 2004), such as cardiovascular disease (Clinton 1998; Ford and Giles 2000), cancer (Steinmetz and Potter 1996; Nishino 1997), neurological deficits (Youdim and others 2002), cataracts (Taylor and others 2002), age-related macular degeneration (Gale and others 2003; Zhou and others 2011), and osteoporosis (Yang and others 2008). For example, a significant reduced risk for lung cancer was associated with an increase of one serving/day for grapefruit or

grapefruit juice, while no other associations were found for other fruits or vegetables (Feskanich and others 2000). In a pooled analysis of cohort studies, a significant inverse relationship between lung cancer risk and orange and tangerine fruit or orange and grapefruit juice consumption were found when comparing the lowest to the highest quartiles of intake (Smith-Warner and others 2003). Some epidemiological studies report a 40-50% reduced risk of certain cancers with increased citrus consumption (Baghurst 2003). This review focuses on the nutritional value and economic importance of citrus consumption, as well as the health benefits related to prevention of micronutrient deficiencies and suggested protection against chronic diseases.

Macronutrients

Citrus fruit composition varies significantly due to fluctuating effects from rootstock, fruit size, variety, maturity, storage, horticultural conditions, and climate, suggesting that nutrient and constituent analysis only provide within- and between-variety estimates, and general conclusions are difficult to form (Kefford and Chandler 1970). Different processing procedures with capabilities to adjust extraction pressure affect juice composition, which is particularly important for fruits that contain flavonoids and have thick peels (Danziger and Mannheim 1967). Because protein and fat are low in citrus fruits, carbohydrate is the essential macronutrient supplying nutritional and caloric values (Watt and Merrill 1963; USDA Natl. Nutrient Database 2011a) (Table 3).

Carbohydrates

Citrus fruits can be separated into those with soluble and others with insoluble constituents, based on 80% ethanol extraction (Sinclair and Jolliffe 1960). The soluble portion largely contains mono- and disaccharides, nonvolatile organic acids, amino acids, and other minor components, and the insoluble fraction primarily consists of cell structure polysaccharides, which establish the nature and profiling of citrus carbohydrates (Sinclair and Jolliffe 1960). Sucrose, glucose, and fructose, with a general ratio of 2:1:1, represent the major components of citrus fruit carbohydrates and hold the key to sweetness of the juice (Bartholomew and Sinclair 1943; Curl and Veldhuis 1948; McCready and others 1950; Ting and Attaway 1971). The ratios of sucrose to other reducing sugars tend to fluctuate with various stages of maturity and different varieties, and to decrease in the acidic environment with long-term storage (Ting and Attaway 1971; Chan and Kwok 1975; Kuraoka and others 1976; Daito and Sato 1985) (Table 4). Depending on the specific fruit, total sugar content in the juice could range from lower than 1% in some limes to as high as 15% in some oranges (Ranganna and others 1983). In addition to the 3 major sugars, trace amounts of mannose, maltose, heptuloses, and galactose have been reported, and rhamnose, xylose, and trehalose have been identified in some Israel oranges, grapefruits, and lemons (Stepak and Lifshitz 1971; Ladaniya and Mahalle 2011). Citrus peels also contain substantial amounts of sucrose, glucose, and fructose, as well as traces of other free sugars such as xylose and rhamnose. Although the sugars soluble in ethanol generally increase with maturity and contribute 30 to 50% of the peel's dry weight, the sucrose content is considerably lower than that of the total reducing sugars (Ting and Deszyck 1961).

The insoluble solid portion in 80% ethanol yields polysaccharides upon further extractions (Sinclair and Jolliffe 1960). In citrus peel, pulp, juice, and membrane, 45 to 75% of

the total solids are insoluble in ethanol and most solids are polysaccharides (Ting and Deszyck 1961; Ting 1970). The main fractions of citrus polysaccharides are pectic substances, hemicelluloses, cellulose, and lignin (Ting 1980) (Table 5). Individual hydrolysis of the pectin, hemicelluloses, and cellulose revealed that certain monosaccharides, such as arabinose and galactose, were discovered in all fractions, whereas free xylose primarily existed in the hemicellulose fraction, and galacturonic acid and glucose dominated the pectin and cellulose fractions (Ting and Deszyck 1961; Rouse and others 1962, 1964). Previously reported results, based on the assumption that each hydrolyzed product is derived from a homogeneous polysaccharide, suggested that orange and grapefruit peels consist of 7 to 10% araban, 5 to 6% galactan, 2.5% xylan, 15 to 28% cellulose glucosan, and 23% polygalacturonic acids, accounting for approximately 53 to 70% of the ethanol-insoluble solids in the peel (Ting and Deszyck 1961).

Synthesis of starch has been documented in lemon fruit tissue by Kordan (Kordan 1965, 1974). Starch is abundant in the albedo and can also be found in the flavedo when fruits are still green (Shomer and Erner 1989). Starch can be found in all fruit components throughout the course of early citrus development as storage carbohydrate, which supplies energy for growth and respiration in immature fruits, and is completely degraded and fully metabolized during maturation (Webber and Batchelor 1943; Ranganna and others 1983; Lelièvre and others 1997; Holland and others 1999; Cajuste and others 2011).

Fiber

For citrus fruits, dietary fiber generally refers to the alcohol-insoluble compounds listed above, which is conventionally composed of cellulose, lignin, and pectin. In addition to the ability of dietary fiber to decrease transit time of food through the gastrointestinal tract and hence

prevent digestive disorders (Truswell 1993; Hillemeier 1995), the methoxyl content of pectin is associated with a cholesterol-lowering benefit (Spiller and Amen 1974; McCready 1977; Ting 1980). Previous data on grapefruit showed changes in the composition and level of dietary fiber with maturity, suggesting that early harvest may result in a higher dietary fiber content (Larrauri and others 1997). In general, the polysaccharides of citrus fruits, particularly in the peel and pulp, are a source of dietary fiber (Church and Church 1970).

Organic acids

Citrus acidity not only impresses consumers as sourness, but also plays a key role in the criteria assessing the commercial acceptability of citrus fruits, and together with appropriate sugar levels, provides the delightful and typical taste. Carboxylic acids, particularly citric, malic, and succinic acids, comprise the content of organic acids (Vandercook 1977a) (Table 6). The organic acids, in addition to the free form, also exist in the form of salts such as citrates and malates (Clements 1964). While citric acid prevails in juices, malic, malonic, oxalic, and quinic acids are major organic acids in citrus peel (Sinclair and Eny 1947; Ting and Deszyck 1959; Clements 1964; Sasson and others 1976). During maturity, citrus acidity may demonstrate different fates depending on fruit species. As fruits ripen, the gradual decrease of citric acid leads to declined acidity while malic acid content remains relatively constant (Rasmussen 1963; Gepshtain and Lifshitz 1970). This trend has been documented in several orange and grapefruit varieties (Shaw and Wilson 1983). In lemons, however, acidity increases with maturity, resulting in a lower pH (Vandercook 1977a). Other acids, such as adipic, isocitric, lactic, aconitic, α -ketoglutaric, and benzoic acids, have also been recorded (Sasson and Monselise 1977). As acids enter the tricarboxylic acid cycle, mainly as malic and citric acids, these substrates are oxidized

to yield ATP and produce new compounds. Many flavor and aromatic compounds are synthesized as metabolites during the utilization of organic acids (Kealey and Kinsella 1978).

Protein

Total nitrogen content is between 0.08 to 0.11% in oranges, 0.08% in grapefruit, and 0.06% in lemons (Clements and Leland 1962; Sawyer 1963; Vandercook 1977b). Free amino acids contribute the most to citrus nitrogen values, which account for about 70% of the nitrogenous constituents in all varieties (Zamorani and others 1973; Zamorani and Russo 1974; Russo and others 1975; Ranganna and others 1983). Therefore, citrus fruits are not considered a major protein source (Table 3).

Using electrophoresis to isolate proteins on polyacrylamide gels, previous results by Clements indicated that bands produced from different portions of orange, grapefruit, and lemon shared similarity, suggesting that a number of proteins are common in citrus fruits (Clements 1966). These proteins, in spite of their relatively low content, are largely enzymes, which include transferases, hydrolases, lyases, ligases, and oxidoreductases in different parts of the fruits (Vandercook 1977b). Seeds of citrus fruits, however, have higher amounts of protein. Previous studies reported from 9.8% protein in lime seeds to 18.2% in whole seeds of citrus fruits on a dry weight basis (Ammerman and others 1963; Kunjukutty and others 1966).

Considerable attention has been given to qualitative and quantitative estimation of individual amino acids in different fruits and products. Based on previous data, the majority of citrus amino acids are considered nonessential (Block and Bolling 1944). With little seasonal variation observed in free amino acid concentration (Wallrauch 1980), nonessential amino acids such as alanine, arginine, asparagine, aspartic acid, glutamic acid, glycine, serine, and proline are

found in several orange, lemon, and mandarin varieties, while indispensable amino acids such as valine, phenylalanine, threonine, leucine, methionine, and lysine are reported in certain oranges and grapefruits (Zamorani and others 1973; Giacomo and others 1974; Zamorani and Russo 1974; Benk 1975; Russo and others 1975; Wallrauch 1980; USDA Natl. Nutrient Database 2011a) (Table 7). Interestingly, while proline reportedly dominates the free amino acids in juices of all citrus fruits, it has the lowest concentration in lime juice (Vandercook 1977b) (Table 7). In addition, among the many important amino acids in citrus juices, arginine is the only semiessential amino acid (Imura and Okada 1998) that exists in relatively detectable amounts (Attaway and others 1972). Overall, free amino acids in citrus fruits likely make a small impact on human nutrition.

Lipids

Although citrus flesh is not a good source of lipids, they are primarily found in the seeds and rinds. As seed moisture declines and lipid content increases during fruit maturation, about 30–45% lipids are present in dried seeds of oranges and 29–37% in grapefruit seeds (Hendrickson and Kesterson 1963a; Kesterson and Braddock 1976). However, the bitterness of these seed oils, owing to the presence of limonoids, often make them unpopular and rarely used, and need to be removed by treatment with alkali for refinement. As illustrated in Table 8, a mixture of unsaturated fatty acids, such as oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids, as well as saturated fatty acids such as palmitic (C16:0) and stearic (C18:0), characterize the citrus seed oils with different varieties sharing similar fatty acid composition (Braddock 1973; Nagy 1977) (Table 8). Measured by iodine values, an association between refractive index and degree of unsaturation has been observed (Hendrickson and Kesterson 1963b, 1964).

Linoleic acid is abundant in mandarin seeds, with the highest refractive indices and iodine numbers, but also in lemon and lime seed oils. Orange seed oils, on the other hand, demonstrate the lowest refractive indices and iodine numbers. In addition to the major unsaturated fatty acids, other acids previously reported in trace amounts are lauric (C12:0), myristic (C14:0), and palmitoleic (C16:1) acids (Sattar and others 1987).

Minor amounts of nonpolar and polar lipids exist in citrus juice and flesh (Swift and Veldhuis 1951). While the lipid content stays relatively constant in some varieties, fluctuation has been observed during fruit maturation (Kadota and others 1982). Previous data showed that total lipids in citrus juice generally range from 84 – 101 mg/100 mL in orange, 75 – 86 mg/100 mL in grapefruit, and 58 – 78 mg/100 mL in lemon (Nagy 1977). Free fatty acids form an essential part of nonpolar lipids, with palmitic, oleic, linoleic, and linolenic acids as the major components (Nordby and Nagy 1969; Nagy 1977). The major fatty acid profiles in juice are identical in orange and grapefruit, while lemon and lime juices have a decreased level of oleic acid and elevated concentrations of linolenic acid (Table 9). Because of this, previous data by Nagy and others suggested that citrus fruit fatty acid composition could be used to distinguish species (Nagy and Nordby 1974). Lemons, for example, can be differentiated from other varieties by the higher $C_{16:0}/C_{16:1}$ ratio, and together with lime, they contain higher concentrations of branched-chain fatty acids than other species. The polar lipids consist of a nonionic group of sugar-containing lipids, which include glycosyl glycerides and sterol glucosides (Nordby and Nagy 1971). The ionic polar lipids are generally characterized by phospho-, sulfo-, amino, or carboxyl groups, with phospholipids being 50% of the total juice lipid content (Nagy 1977). This number increases in commercially processed citrus juice due to extraction, heating, and evaporation leading to physical disruption of membranes and tissues

(Braddock 1972). According to previous findings by Vandercook and others, phospholipids can also distinguish a citrus juice from other beverages (Vandercook and others 1970).

Like most plants, the aerial surfaces of citrus fruits are covered with a multilayered cuticle, which is primarily composed of cutin, a lipid whose long-chain constituents are interlinked with ester bonds and cross-polymerized with compounds of high molecular weight and intermediate size, and also poorly soluble in most solvents (Baker and Procopiou 1975; Holloway 1982). In addition, a layer of waxy lipid, called epicuticular wax, exists on the outer surface of the cutin, and can easily be dissolved in organic solvents (Albrigo 1972; Baker and others 1975). The wax content of cuticle lipids in the fruit peel plays an important role in controlling moisture loss, protecting the fruits from insects and pests, and reducing physical damage such as chilling injury (Nordby and McDonald 1990, 1991).

Micronutrients and Phytochemicals

Fat-soluble vitamins

In citrus fruits, vitamin A is the only fat-soluble vitamin that exists in an adequate quantity in the form of provitamin A carotenoids, with the carotenes and β -cryptoxanthin as the major vitamin A precursors (Ting 1977). While α - and β -carotene are a minor portion of carotenoids in some oranges, β -cryptoxanthin is the main vitamin A precursor in tangerines, mandarins, and oranges (Curl and Bailey 1954, 1957; Ting 1961; Stewart 1977). Using high-performance liquid chromatography (HPLC), separation of cryptoxanthin from other carotenoids in citrus juice suggests that only the β -isomer has provitamin A activity and the α -isomer is inactive, as determined by its structure (Stewart 1977). Total provitamin A carotenoids vary widely among different citrus fruits; mandarins, tangerines, and red and pink grapefruits are the

major sources (USDA Carotenoid Database 1998; Holden and others 1999) (Table 10). The concentrations are dramatically lower in oranges and almost undetectable in white grapefruits (Lime and others 1954; Ting and Deszyck 1958; Ting 1961; Stewart 1977; Holden and others 1999).

Citrus fruits are the most concentrated dietary source of β -cryptoxanthin among foods. The best dietary sources are papaya, tangerine, and orange (Arscott and others 2010), but it is also found in red chilies, peaches, pumpkins (Burri and others 2011), and guava (Maiani and others 2009). β -Cryptoxanthin bioavailability from these food sources is affected by the food matrix, processing, and storage state. While conversion to retinol does not occur until digestion, the degradation of β -cryptoxanthin in its native form (before consumption) is caused by natural light and heat, which results in isomerization. Cooking and other thermal processing alters the food matrix, making carotenoids more bioavailable for digestion (Shardell and others 2011).

Information on dietary β -cryptoxanthin intake exists from various sources, but little in-depth analysis is available. Because the major source of β -cryptoxanthin is citrus fruits, intake correlates with the geographic regions where they are grown or available through shipment. In European countries, sources of dietary β -cryptoxanthin include orange juice, oranges, and tangerines (O'Neill and others 2001); whereas in certain other regions the dietary source is largely papaya because of availability. In a study of Costa Rican adolescents, plasma concentrations of β -cryptoxanthin increased significantly with increasing servings of fruit per day. This took into account that one of the most consumed fruits was papaya, a high source of β -cryptoxanthin (Irwig and others 2002). An observational study in Chinese women showed that fruit intake was significantly and positively associated with plasma concentrations of β -cryptoxanthin, as well as other provitamin A carotenoids (Frankenfeld and others 2011).

Compared with provitamin A carotenoids, citrus fruits house negligible amounts of vitamin E and vitamin K (Newhall and Ting 1965; USDA Natl. Nutrient Database 2011a, USDA and HHS 2011). Although previous findings of several sterols were documented, neither sterol compounds related to vitamin D nor plant-derived precursors of vitamin D have been found in citrus fruits (Swift 1952; Mazur and others 1958; Williams and others 1967). However, fortification of orange juice with vitamin D has lately received considerable attention as an effective approach to ensure adequate vitamin D intake and increase calcium absorption in children and adults (Tangpricha and others 2003; Biancuzzo and others 2010).

Water-soluble vitamins

The high concentration of vitamin C (ascorbic acid) is probably the most significant contribution of citrus fruits to human health and nutrition. Previous findings suggested that daily intake of as little as 5 mg ascorbic acid is adequate to prevent vitamin C deficiency and scurvy symptoms in adults (Mapson 1967). The most recent intake recommendations provided by the US Department of Agriculture and US Department of Health and Human Services set 75 to 90 mg as the Recommended Dietary Allowance (RDA) for adults and even higher values for individuals who are cigarette smokers and women during pregnancy and lactation (USDA and HHS 2011). Although citrus fruits are not the single supplier of vitamin C, they are particularly rich and a popular dietary source among vegetables and fruits, providing average vitamin C concentration ranging from 23 to 83 mg/100 g fresh weight (West and others 1966, Lee and Kader 2000) (Table 11). A medium-sized orange or grapefruit contains approximately 56 to 70 mg ascorbic acid, and an average 225-mL serving of orange juice typically contains 125 mg ascorbic acid (Whitney and others 2009). The edible portion contains about one-fourth of the

total vitamin C content in the whole fruit. The peels (flavedo and albedo), although generally recognized as nonedible parts, contain a higher concentration than other components (Nagy 1980). A recent study suggested that total vitamin C concentration was more than 1.5 times higher in pulp of orange (*Citrus sinensis* Osb.) than that in pulp of Satsuma mandarin (*Citrus unshiu* Marc.) during fruit development and ripening (Yang and others 2011).

The variability of vitamin C content in fresh citrus fruits and their commercial products is greatly influenced by variety, maturity, climate, handling, processing, and storage conditions. Vitamin C in freshly extracted juice is quite stable during short storage periods and processing into the various juice products results in no serious loss of vitamin C potency if kept at refrigerator temperature for reasonable times (Moore and others 1945; Lopez and others 1967; Horton and Dickman 1977). Even in open containers such as glass and cans, loss of vitamin C is still minimal as long as the juice products are kept cold. However, considerable degradation of vitamin C results from storage of finished citrus products with atmospheric oxygen at high temperatures (Mudambi and Rajagopal 1977; Smoot and Nagy 1980).

In addition to ascorbic acid, citrus fruits also provide vitamin B complex, in particular thiamin (vitamin B₁) and pyridoxal phosphate (vitamin B₆). According to a previous study comparing different parameters for nutrient density in nonfortified 100% fruit juices, citrus juices received higher rankings than other juices (Rampersaud 2007). Although the applied methods differed in approach as far as number or type of nutrients, the findings suggested that citrus juices, especially orange and pink grapefruit juices, were more nutrient dense than other commonly consumed juices (Rampersaud 2007). The current thiamin RDA for adults is 1.0 to 1.2 mg/day and 1.4 mg/day for women in pregnancy and lactation (USDA and HHS 2011).

Citrus fruits and products provide similar or higher thiamin amounts than some well-known foods such as milk supplying this nutrient (USDA Natl. Nutrient Database 2011a).

Vitamin B₆ plays an essential role as a coenzyme governing many reactions of amino acids and glycogen metabolism. The current RDA is set at 1.3 mg/day for adult males and females aged 19 to 50 years old, and 1.9-2.0 mg/day for pregnant and lactating women (USDA and HHS 2011). Apart from orange juice, other foods that are good sources of vitamin B₆ include meat, dairy, whole grains, vegetables, bananas, and nuts (Nelson and others 1977; McCormick 2006). Orange juice, for example, contains an average vitamin B₆ concentration of 40 µg/100 g fresh weight, suggesting that orange juice supplies this nutrient at a comparable level to that of milk, an average of 36 µg/100 g fresh weight (USDA Natl. Nutrient Database 2011a).

Folic acid, a pteroyl-glutamic acid, or folate as the naturally occurring form, is another water-soluble B vitamin which acts as an essential coenzyme involved in many important biological functions such as synthesis, repair, and methylation of DNA; cell division and growth; and metabolism of homocysteine (Kamen 1997; Fenech and others 1998). Deficiency of folate could result in megaloblastic anemia because humans need this vitamin to synthesize normal red blood cells (Zittoun 1993). Current dietary recommendations set 400 µg folate/day as the RDA for adults [1 µg food folate is also called a Dietary Folate Equivalent (DFE)], 600 µg DFE/day for pregnant women, and 500 µg DFE/day for lactating women (IOM 1998; USDA and HHS 2011). While certain foods are high in folate, such as leafy green vegetables (for example, asparagus, spinach, and turnip greens), egg yolk, and legumes (that is, dried beans and peas), its dietary level is usually low in most foods (USDA Natl. Nutrient Database 2011a), and the requirement to enrich processed grains by adding folic acid has made foods such as breads,

cereals, flours, and corn meal contributors to folic acid intake in the US diet (Oakley and others 1996; Daly and others 1997; Crandall and others 1998; Malinow and others 1998). Citrus fruits and juice are natural sources of folate, and orange juice contains higher concentrations of folate than other commonly consumed fruit juices (Hill and others 1971; USDA Natl. Nutrient Database 2011a) (Table 12).

Niacin, riboflavin, and pantothenic acid, three other water-soluble vitamins of the B complex, are all present in citrus fruits and juice products in minor amounts. The concentrations are low in the range of 2 to 4% of the current RDA per serving, with grapefruit juice having the lowest concentrations (Ting and others 1974; USDA and HHS 2011).

Flavonoids

In addition to being a source of carotenoids (for example, β -cryptoxanthin), citrus fruits are also a source of flavonoids (Holden and others 2005; USDA Flavonoid Database 2011b) (Table 13). Flavonoids have a polyphenol structure and are responsible for the flavor in many fruits and vegetables (Ross and Kasum 2002). Flavonoids are considered to be plant secondary metabolites and have many possible health-promoting effects when consumed (Wang and others 2011). One of the most common classes of flavonoids found in citrus fruits are flavonones, in particular naringin, which imparts the bitter flavor to grapefruit (Ross and Kasum 2002). Naringin is hydrolyzed to naringenin by gut bacteria before absorption (Shulman and others 2011), which is a common precursor to many other classes of flavonoids (Wang and others 2011). Naringenin is thought to have several health-related benefits and biological effects, which include acting as an antioxidant, inhibiting microsomal triglyceride transfer protein and acetyl-

coenzyme A acetyltransferase, and playing a role in regulating cytochrome P450 enzymes (Shulman and others 2011).

An emerging role of naringenin is its involvement in the prevention of bone loss and osteoporosis. A big contributor to bone loss is the activation of osteoclast cells, which occurs in response to the protein called receptor activator of nuclear factor- κ B ligand (RANK-L) located on osteoblasts and macrophage colony-stimulating factor (Ang and others 2011). Osteoclast formation is a crucial mechanism in bone loss, as it reduces and withdraws the calcium from bones, contributing to bone loss and osteoporosis. This pathway is thought to be the target of flavonoid interaction in the prevention of bone loss. Naringenin inhibited RANK-L and macrophage colony-stimulating factor induced differentiation of cultured primary human osteoclast precursors in a dose-dependent manner, whereas an absence of naringenin resulted in almost complete differentiation (La and others 2009). Naringenin treatment also reduced the total number of osteoclast cells formed. This was supported again in 2011, when naringin, which is metabolized to naringenin, was administered to osteoclastic cells and inhibited the RANK-L-induced activation of nuclear factor- κ B and phosphorylation of extracellular signal-regulated kinase, blocking osteoclast cell formation and bone resorption (Ang and others 2011). The inhibition of osteoclast cell formation is potentially an effective method for reducing the amount of bone loss and the delay and/or prevention of osteoporosis.

A less abundant citric flavonone known as hesperidin, may provide several health benefits. Low hesperidin has been linked with abnormal capillary leakiness, fatigue, and night leg cramps (Garg and others 2001). In addition to dietary sources, such as sweet oranges, lemons, and green fruits, supplementary hesperidin is often administered to relieve swelling and fluid accumulation in the legs (Garg and others 2001). Hesperidin possesses many of the same

properties as naringenin. It has anti-inflammatory properties through the inhibition of prostaglandins E_2 and F_2 and thromboxane A_2 (Manthey and others 2001). Hesperidin was reported to increase HDL cholesterol and lower LDL cholesterol, triglycerides, and plasma lipids when administered to rats (Garcia and Castillo, 2008). Hesperidin's aglycone form, hesperetin, has antioxidant potential in rats when administered as a supplement rather than from the diet (Shagiritha and Pari 2011). More research is needed on dietary hesperidin to gain a better understanding of the potential health benefits.

Minerals

Although sodium and potassium are the major cations of the cells, citrus fruits and products are low in sodium, with less than 2 mg/100 g fruit weight (USDA Natl. Nutrient Database 2011a). In contrast, citrus fruits are good sources of potassium, and according to previously documented data could constitute up to 40% of the total ash, with concentrations of 4 to 6 meq (156 to 235 mg) in 100 mL orange juice (Benk 1965). Calcium, magnesium, and phosphorus have relatively low amounts in citrus fruits (USDA Natl. Nutrient Database 2011a), contributing only 2 to 3% of the US RDA per serving (USDA and HHS 2011). In the U.S.A., inadequate calcium intake is a common problem among children and adolescents who often do not drink milk or consume dairy products (Black and others 2002; Nicklas 2003). Adult dietary calcium intakes are also well below recommended values especially for the elderly (Morgan and others 1985; Looker and others 1993). Calcium-fortified citrus juices, such as those commercially available, are considered an economically viable option to help adolescents and the elderly meet adequate calcium intake (Martini and others 2002; Gao and others 2006). While supplied as important plant nutrients during cultivation and growth, copper, zinc, iron, and

manganese, which are essential in numerous enzymatic reactions and human bodily functions, are trace minerals found in all citrus fruits.

Other Important Components of Citrus Fruit

Citrus oils and volatile flavoring constituents

Stored ripe citrus fruits have a distinctive odor. The aroma-active volatile flavoring compounds contained in the peel oils characterize the aroma emanated by citrus fruits and are associated with their flavors. The release of volatile compounds increases with rising temperature, maturity, and ruptured peel and juice components (Ladaniya 2008). There are over 300 citrus volatiles and oils, and the chemical constituents include terpene hydrocarbons (such as monoterpenes and sesquiterpenes), esters, aldehydes, ketones, alcohols, and volatile organic acids (Perez-Cacho and Rouseff 2008). They are primarily found in the ductless oil sacs in the flavedo, as cold-pressed peel oil, and can be incorporated into the juice during extraction (Ranganna and others 1983; Perez-Cacho and Rouseff 2008; Rouseff and others 2009).

The largest fraction by weight, representing approximately 90% of all citrus oil compounds, is total monoterpene hydrocarbons (including d-limonene) (Hunter and Brogden 1965a). Other hydrocarbons present in orange peel oils include the sesquiterpenes, such as valencene (Hunter and Brogden 1965a, b). Previous findings also suggested that the fruity aromas in citrus oils, which are specific to different varieties, contain oxygenated terpenes (Stanley 1962). Although representing a small portion of all citrus oils, esters, such as ethyl butyrate in orange essence, provide the characteristic aroma to citrus fruits (Wolford and others 1963; Ikeda and Spitler 1964; Shaw 1979). Terpene aldehydes and ketones are important flavoring constituents in lemons, oranges, and grapefruits (Stanley and others 1961; MacLeod Jr.

and Buigues 1964; Hunter and Brogden 1965b). Other volatile flavoring constituents and citrus oils include alcoholic compounds, such as linalool and octanol, which are in trace amounts in lemon, lime, grapefruit, and tangerine (Attaway and others 1962; Hunter and Moshonas 1965; Hunter and Moshonas 1966). Volatile organic acids are present in trace amounts in natural orange essence (Attaway and others 1964).

In industrial practice, cold-pressed peel oils are prepared by mechanically pressing and rupturing the oil sacs in the flavedo, and then centrifuging the extracted peel oils from its aqueous emulsion portion for separation. For most citrus fruits, such as orange, grapefruit, mandarin, and lemon, cold-pressed peel oils prepared using this method are well-received by consumers because natural aroma compounds are present, yielding the pleasant odor of freshly squeezed juice (Rouseff and others 2009). Distilled lime oil is sometimes commercially preferred due to its distinct terpene character rather than the natural aroma (Ranganna and others 1983). The peel oils of limes, lemons, mandarins, oranges, and grapefruits exhibit toxic insecticidal properties, with lime as the most effective (Abbassy and others 1979).

Essence oil is a byproduct recovered from the concentration process in the preparation of frozen fruit juice concentrate (Moshonas and Shaw 1979). During juice evaporation, water in the juice is vaporized, and the vapor contains aqueous components, oils, and aromas. After the vapor mixture of water and oil is removed, condensed, and separated by decantation, the oil recovered is called essence oil and contains most of the flavoring components present in juice (Ranganna and others 1983). The essence oil is characterized by a fresh-juice aroma and is used commercially as an important flavoring agent to add desirable bouquet to frozen concentrated juice (Shaw 1977; Moshonas and Shaw 1979).

Conclusions

With high demand and popular dietary preference, citrus fruit is widely consumed and has become an inseparable part of our diet. Recent developments in horticultural utilization and improved analytical technology have helped establish the analysis of citrus fruit chemical constituents. Characterized by their distinctive flavor, citrus fruits are a good source of carbohydrates, dietary fiber, many B vitamins, minerals, and biologically active phytochemicals such as carotenoids and flavonoids, which provide provitamin A activity and purported antioxidant benefits, respectively. Such nutrient density, the low-fat, low-sodium profiles, and associations between citrus fruit intake and prevention of chronic diseases make promotion of citrus consumption important in improved human health.

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Table 1-World's leading citrus fruit producers.^a

Country	Grapefruit	Lemons and limes	Oranges	Tangerines^b	Other	Total
	(tons)					
China	2,768,308	1,014,446	4,864,959	9,746,287	4,694,471	23,088,471
Brazil	66,895	972,437	17,618,500	1,094,430	NA ^c	19,752,262
U.S.A.	1,182,970	827,350	8,280,780	401,880	47,170	10,740,150
India	193,822	2,571,530	5,201,350	NA	161,691	8,128,393
Mexico	395,000	1,987,450	4,193,480	442,108	106,539	7,124,577
Spain	38,700	551,000	2,617,700	2,026,200	6,500	5,240,100

^aSource: FAO 2009. All values listed are tons.

^bIncludes tangerines, mandarins, and clementines.

^cNA = not available.

Table 2-Citrus production and utilization – individual states and the entire U.S.A..^a

State and season	Production		
	Total (1,000 tons)	Utilization	
		Fresh	Processed
		(1,000 tons)	(1,000 tons)
Arizona ^b			
2007-2008	90	62	28
2008-2009	133	54	79
2009-2010	97	51	46
California			
2007-2008	3,312	2,511	801
2008-2009	2,954	2,327	627
2009-2010	3,410	2,650	760
Florida			
2007-2008	9,119	891	8,228
2008-2009	8,470	867	7,603
2009-2010	7,127	824	6,303

Texas

2007-2008	317	191	126
2008-2009	282	181	101
2009-2010	294	195	99

U.S.A.

2007-2008	12,838	3,655	9,183
2008-2009	11,839	3,429	8,410
2009-2010	10,928	3,720	7,208

^aSource: USDA 2010. All values listed are 1,000 tons.

^bOranges not included in the 2009-2010 season.

Table 3-Nutritional characteristics for citrus fruits.^a

	Orange^b	Grapefruit^c	Tangerine^d	Lemon^e
	(per 100 g fruit)			
Energy (kcal)	47	42	53	29
Carbohydrates (g)	11.75	10.66	13.34	9.32
Protein (g)	0.94	0.77	0.81	1.10
Total fat (g)	0.12	0.14	0.31	0.30
Cholesterol (g)	0	0	0	0
Dietary fiber (g)	2.40	1.60	1.80	2.80
Folate, total (μg)	30	13	16	11
Niacin (mg)	0.282	0.204	0.376	0.100
Pantothenic acid (mg)	0.250	0.262	0.216	0.190
Pyridoxine (mg)	0.060	0.053	0.078	0.080
Riboflavin (mg)	0.040	0.031	0.036	0.020
Thiamin (mg)	0.087	0.043	0.058	0.040
Vitamin C (mg)	53.20	31.20	26.70	53
Vitamin A (IU)	225	1150	681	22

Vitamin E (mg)	0.18	0.13	0.20	0.15
Vitamin K (µg)	0	0	0	0
Sodium (mg)	0	0	2	2
Potassium (mg)	181	135	166	138
Calcium (mg)	40	22	37	26
Copper (µg)	45	32	42	37
Iron (mg)	0.10	0.08	0.15	0.60
Magnesium (mg)	10	9	12	8
Manganese (mg)	0.025	0.022	0.039	0.030
Zinc (mg)	0.07	0.07	0.07	0.06
β-Carotene (µg)	71	686	155	3
α-Carotene (µg)	11	3	101	1
β-Cryptoxanthin (µg)	116	6	407	20
Xanthophylls ^f (µg)	129	5	138	11
Lycopene (µg)	0	1419	0	0

^aSource: USDA National Nutrient Database for Standard Reference, Release 24, 2011a.

^bOranges, raw, all commercial varieties. Nutrient Databank no. 09200.

^cGrapefruit, raw, pink and red, all areas. Nutrient Databank no. 09112.

^dTangerines, raw (mandarin oranges). Nutrient Databank no. 09218.

^eLemons, raw, without peel. Nutrient Databank no. 09150.

^fXanthophylls represent combined lutein and zeaxanthin.

Table 4-Average sugar composition of citrus juices.^a

Fruit	Glucose	Fructose	Total reducing sugars^b	Sucrose	Total sugars
	(g/100 g)				
Orange	2.03	2.48	4.51	4.81	9.32
Grapefruit	1.66	1.75	3.41	2.56	5.97
Tangerine	1.13	1.54	2.67	6.53	9.20
Lemon	1.40	1.35	2.75	0.41	3.16
Lime	NA ^c	NA	3.48	0	3.48

^aSource: Ting and Attaway 1971. All values listed are g/100 g.

^bTotal reducing sugars represent combined glucose and fructose.

^cNA = not available.

Table 5-Percent distribution of polysaccharide fractions in citrus fruits.^a

Fruit	Pectic substance			Hemicellulose			Cellulose			Other		
	(%)											
	Peel ^b	Pulp ^c	Juice ^c	Peel ^b	Pulp ^c	Juice ^{c, d}	Peel ^b	Pulp ^c	Juice ^c	Peel ^b	Pulp ^c	Juice ^c
Oranges												
Hamlin	55.4	68.0	88.7	8.7	6.8	11.3	31.2	25.1	NA ^e	4.9	0.1	0
Pineapple	56.5	59.5	92.5	9.9	6.3	7.0	26.6	33.7	NA	7.0	0.6	0
Valencia	51.1	62.0	93.0	11.3	7.0	7.0	30.4	30.0	NA	7.2	1.0	0
Tangerine												
Dancy	NA	64.5	91.7	NA	8.6	8.3	NA	26.7	NA	NA	0.2	0
Grapefruit												
Duncan	NA	62.5	94.0	NA	6.6	6.0	NA	28.6	NA	NA	0.3	0
Marsh	52.4	63.0	92.6	11.7	6.7	7.4	30.8	29.0	NA	5.1	1.3	0

^aValues listed are percentage distribution of various fractions in the alcohol-insoluble solids of citrus peel, pulp, and juice.

^bSource: Ting and Deszyck 1961.

^cSource: Ting 1970.

^dCombined hemicelluloses and cellulose fractions in juice.

^eNA = not available.

Table 6-Concentrations of organic acids in some varieties of citrus fruit juices.^a

Variety	Citric	Malic	Succinic
	(g/100 mL)		
Orange			
Valencia	0.22 – 0.98	0.06 – 0.26	Trace – 0.54 ^b
Navel	0.14 – 0.72	0.11 – 0.15	0.06 – 0.90
Pineapple	0.30 – 0.36	0.15 – 0.26	0.26 – 0.85
Hamlin	0.17 – 0.70	0.15 – 0.31	0.02 – 0.24
Parson Brown	0.40 – 0.50	0.21 – 0.27	0.27 – 1.48
Shamouti	0.88 – 2.37	0.075 – 0.182	NA ^d
Mandarin and tangerine	0.86 – 1.22	0.08 – 0.21	NA
Grapefruit			
Marsh	0.42 – 0.95 ^c	0.03 – 0.23	0.06 – 0.86
Lemon			
Eureka	4.00 – 4.38	0.07 – 0.26	NA

^aSource: Vandercook 1977a. All values listed are g/100 mL citrus juice.

^bValues as high as 1.27 and 1.59 have been reported in Valencia oranges grown in Texas and Arizona, respectively.

^cValues as high as 1.41, 1.79, and 2.1 have been reported in Marsh grapefruit grown in Florida, California, and Arizona, respectively.

^dNA = not available.

Table 7-Amino acid concentrations in some varieties of citrus fruits and juice products.^a

Amino acid	Grapefruit, white^b	Grapefruit, pink and red^c	Lime juice^d	Oranges^e	Orange juice^f	Tangerines^g
	(mg/100 g fresh weight)					
Tryptophan	7	8	2	9	2	2
Threonine	12	13	2	15	8	16
Isoleucine	7	8	2	25	8	17
Leucine	13	15	16	23	13	28
Lysine	17	19	16	47	9	32
Methionine	7	7	2	20	3	2
Cystine	7	8	2	10	5	2
Phenylalanine	41	13	11	31	9	18
Tyrosine	7	8	2	16	4	15
Valine	14	15	11	40	11	21
Arginine	78	87	15	65	47	68
Histidine	7	8	2	18	3	11
Alanine	22	24	24	50	15	28
Aspartic acid	123	138	114	114	75	129
Glutamic acid	176	197	67	94	33	61
Glycine	13	15	11	94	9	19
Proline	56	63	30	46	44	74
Serine	25	28	35	32	13	33
Hydroxyproline	NA ^h	NA	NA	NA	NA	NA

^aAll values listed are mg/100 g fresh weight, edible portion. Source: USDA National Nutrient Database for Standard Reference, Release 24, 2011a.

^bGrapefruit, raw, white, all areas.

^cGrapefruit, raw, pink and red, all areas.

^dLime juice, raw.

^eOranges, raw, all commercial varieties.

^fOrange juice, raw.

^gTangerines, (mandarin oranges), raw.

^hNA = data not available.

Table 8-Composition ranges of major fatty acids in citrus seed oils.^a

Variety	Refractive index^b	Iodine value^b	Major fatty acids (%)^c				
			Palmitic	Stearic	Oleic	Linoleic	Linolenic
Orange	1.4649–1.4712	86.1–101.7	26–31	3–5	24–28	35–37	2–4
Grapefruit	1.4698–1.4700	100.9–106.3	26–36	1–4	18–25	32–41	3–6
Mandarin	1.4693–1.4702	94.1–107.3	22–30	2–5	20–25	37–45	3–5
Lemon	1.4707–1.4742	107.3–109.2	18–24	2–4	26–34	31–38	6–12
Lime	1.4699–1.4701	101.6–106.2	24–29	3–5	20–22	37–40	6–11

^aValues listed are percentage in citrus seed oils.

^bSource: Braddock and Kesterson 1973

^cSource: Nagy 1977.

Table 9-Major fatty acids in citrus juices.^a

Fatty acid	Orange^b	Grapefruit^c	Lemon^d	Lime^e
	%			
Palmitic C _{16:0}	21.2–23.3	21.7–23.7	23.0–23.4	21.7–22.3
Palmitoleic C _{16:1}	4.0–4.6	3.1–4.3	0.7–0.9	5.4–5.6
Oleic C _{18:1}	24.1–26.7	23.4–24.4	9.3–9.5	14.8–15.0
Linoleic C _{18:2}	27.8–35.2	33.5–35.5	34.8–36.0	26.9–27.5
Linolenic C _{18:3}	7.9–13.6	8.2–9.4	18.8–19.0	13.8–14.4
Others	5.9–7.1	5.8–7.2	12.0–12.4	16.0–16.6

^aSource: Nagy 1977. All values listed are percentage.

^bRange of values for early (Hamlin), mid (Pineapple), and late (mainly Valencia) oranges.

^cJuice from Florida factories.

^dJuice from California factories.

^eFourfold concentrate diluted to juice from Florida factories.

Table 10-Major dietary carotenoid contents of selected citrus fruits and juice products.^a

Fruit description	Carotenoid concentration, µg/100 g fresh weight, edible portion			
	<i>α</i> -Carotene	<i>β</i> -Carotene	<i>β</i> -Cryptoxanthin	Lutein + Zeaxanthin
Grapefruit, raw, white	8	14	NA ^b	NA
Grapefruit, raw, pink and red	5	603	12	13
Orange, blood, raw	ND ^b	120	69	NA
Orange, raw, commercial varieties	16	51	122	187
Orange juice, raw	2	4	15	36
Orange juice, raw, hybrid varieties	8	39	324	105
Orange juice, frozen concentrate, unsweetened, diluted	2	24	99	138
Tangerines, raw (mandarin oranges)	14	71	485	243
Tangerine juice, raw	9	21	115	166
Tangerine juice, frozen concentrate, sweetened, undiluted	NA	227	2767	NA

^aAll values listed are μg of carotenoid/100 g fresh weight (edible portion), presented in weighted means. Source: Holden and others 1999.

^bNA: data not available. ND: values not detected or below the detection limit.

Table 11-Vitamin C contents of some citrus fruits.^a

Fruit variety	Vitamin C content, mg/100 g fresh weight		
	l-Ascorbic acid^b	Dehydroascorbic acid^b	Total^b
Grapefruit (fresh) ^c	21.3	2.3	23.6
Lemon (fresh) ^d	50.4	23.9	74.3
Mandarins (Ellendale) ^d	34.0	3.7	37.7
Orange (California Navel) ^c	75.0	8.2	83.2
Orange (Florida) ^c	54.7	8.3	63.0
Orange (China) ^e	57.8	5.0	62.8
Satsuma mandarins (China) ^e	30.5	1.2	31.7

^aAll values listed are mg of vitamin C/100 g fresh weight. Source: Lee and Kader 2000.

^bl-Ascorbic acid, primary bioactive form of vitamin C; dehydroascorbic acid, the oxidized form also with biological activity of vitamin C; total vitamin C concentration.

^cSource: Vanderslice and others 1990.

^dSource: Mitchell and others 1992.

^eSource: Yang and others 2011. Values were based on molecular weights of 176.12 g/mol for ascorbic acid and 174.11 g/mol for dehydroascorbic acid, respectively.

Table 12-Folate content of some commonly consumed citrus fruits and juice products.^a

Fruit description	Weight (g)	Common measure	Dietary folate equivalent (µg) per measure	Amount (µg)^b per 100 g FW
Grapefruit, raw, white	118	½ grapefruit	12	10
Grapefruit juice, white, raw	247	1 cup	25	10
Grapefruit, raw, pink and red	123	½ grapefruit	16	13
Grapefruit juice, pink, raw	247	1 cup	25	10
Lemons, raw, without peel	58	1 lemon	6	11
Lemon juice, raw	47	juice of 1 lemon	9	20
Limes, raw	67	1 lime	5	8
Lime juice, raw	38	juice of 1 lime	4	10
Oranges, raw, all commercial varieties	131	1 orange	39	30
Orange juice, raw	248	1 cup	74	30
Tangerines, raw (mandarin oranges)	84	1 tangerine	13	16
Tangerine juice, raw	247	1 cup	11	5

^aSource: USDA National Nutrient Database for Standard Reference, Release 24, 2011a.

^bValues listed are µg of folate/100 g fresh weight, edible portion.

Table 13-Flavonoid contents of selected citrus fruits and juice products.^a

Fruit description	Subclass	Flavonoid	Mean (mg/100 g fresh weight)
Grapefruit, raw, white	Flavanones	Hesperetin	0.64
		Naringenin	21.34
Grapefruit juice, white, raw	Flavanones	Eriodictyol	0.65
		Hesperetin	2.35
		Naringenin	18.23
	Flavonols	Kaempferol	ND ^b
		Myricetin	0.05
Grapefruit juice, white, frozen concentrate, unsweetened, diluted	Flavanones	Quercetin	0.40
		Naringenin	31.18
	Flavanones	Hesperetin	0.35
		Naringenin	32.64
Grapefruit, raw, pink and red	Flavones	Apigenin	ND
		Luteolin	0.60
	Flavonols	Kaempferol	0.01
		Myricetin	0.01
		Quercetin	0.33
Grapefruit juice, pink, raw	Flavanones	Eriodictyol	ND
		Hesperetin	0.78

		Naringenin	17.19
		Eriodictyol	21.36
	Flavanones	Hesperetin	27.90
		Naringenin	0.55
Lemons, raw, without peel	Flavones	Apigenin	ND
		Luteolin	1.90
		Kaempferol	0.03
	Flavonols	Myricetin	0.50
		Quercetin	1.14
		Eriodictyol	4.88
Lemon juice, raw	Flavanones	Hesperetin	14.47
		Naringenin	1.38
		Hesperetin	43.00
Limes, raw	Flavanones	Naringenin	3.40
	Flavonols	Quercetin	0.40
		Eriodictyol	2.19
Lime juice, raw	Flavanones	Hesperetin	8.97
		Naringenin	0.38
		Hesperetin	27.25
	Flavanones	Naringenin	15.32
Oranges, raw, commercial varieties		Kaempferol	0.13
	Flavonols	Myricetin	0.15
		Quercetin	0.45

		Eriodictyol	0.17
Orange juice, raw	Flavanones	Hesperetin	20.39
		Naringenin	3.27
Orange juice, frozen concentrate, unsweetened, diluted	Flavanones	Hesperetin	26.21
		Naringenin	3.27
Tangerines, raw (mandarin oranges)	Flavanones	Hesperetin	7.94
		Naringenin	10.02
Tangerine juice, raw	Flavanones	Hesperetin	9.56
		Naringenin	1.20
Tangerine juice, frozen concentrate, sweetened, diluted	Flavanones	Hesperetin	22.01
		Naringenin	3.61

^aAll values listed are mg flavanoid/100 g fresh weight (edible portion), presented in weighted means. Source: USDA Database for the Flavonoid Content of Selected Foods, Release 3.0, 2011b

^bND: values not detected or below the detection limit.

(Adapted according to FAO data from UNCTAD 2004).

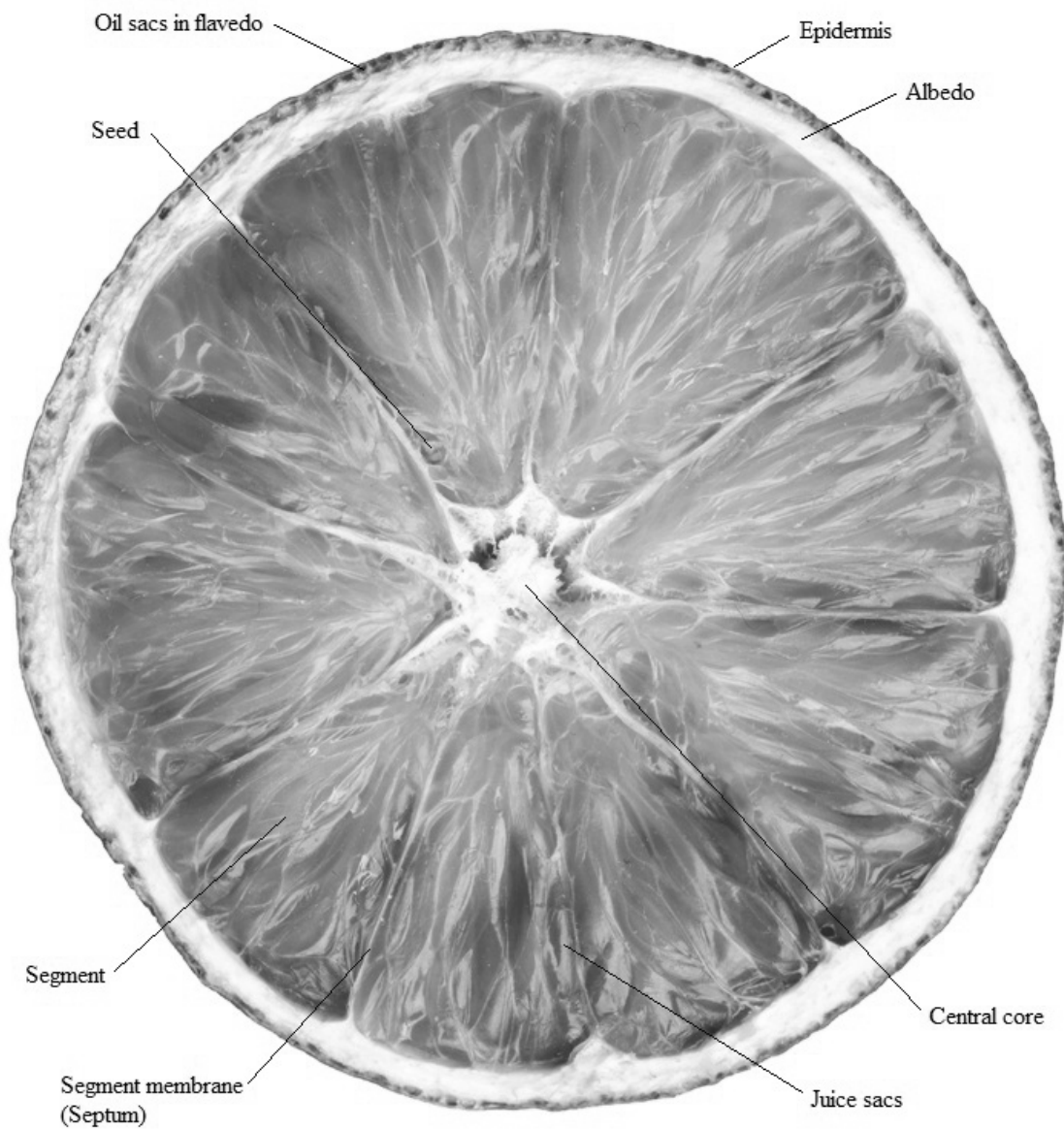


Figure 2—A schematic section of a typical citrus fruit illustrating different structures.

CHAPTER 3

**β -Cryptoxanthin biofortified maize (*Zea mays*)
increases β -cryptoxanthin concentration and
enhances the color of chicken egg yolk**

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Author Contributions

YuQiu Liu conducted the chicken feeding study, collected and analyzed the samples, processed the data, and drafted the following manuscript. Chris Davis provided technical support on carotenoid analysis with HPLC. Samantha Schmaelzle assisted with analyzing egg yolk samples for carotenoid profiles. Dr. Torbert Rocheford, Professor in the Department of Agronomy of Purdue University, provided β -carotene and β -cryptoxanthin biofortified maize varieties. Dr. Mark Cook, Professor in the Department of Animal Science at University of Wisconsin-Madison, provided assistance in the Poultry Science Laboratory and helped with diet preparation. Dr. Sherry Tanumihardjo, Professor in the Department of Nutritional Sciences at University of Wisconsin-Madison and the academic advisor, provided experimental design, and supervised overall manuscript preparation.

β -Cryptoxanthin biofortified maize (*Zea mays*) increases β -cryptoxanthin concentration and enhances the color of chicken egg yolk^{1,2}

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ABSTRACT The laying hen has a natural ability to deposit carotenoids into its egg yolks, especially the xanthophyll carotenoid lutein that is used commercially as an egg colorant. Can this ability to deposit carotenoids be used to enrich egg yolk provitamin A value? After a 10-d carotenoid depletion period in hens ($n = 24$), the effects of a 20-d intervention with high- β -cryptoxanthin, high- β -carotene, or typical yellow maize on color and carotenoid profile were compared with the effects of a white maize diet ($n = 6/\text{treatment}$). Eggs were collected every other day and yolks were analyzed by using a portable colorimeter to define the color space and by using an HPLC to determine the carotenoid profile. The high- β -cryptoxanthin and yellow maize increased β -cryptoxanthin in the yolk (0.55 ± 0.08 to 4.20 ± 0.56 nmol/g and 0.55 ± 0.08 to 1.06 ± 0.12 nmol/g, respec-

tively; $P < 0.001$). Provitamin A equivalents increased in eggs from hens fed high- β -cryptoxanthin maize ($P < 0.001$) but not the high- β -carotene maize. The color (L^* , a^* , and b^*) assessment of the yolks showed an increase in the high- β -cryptoxanthin treatment for the red-green a^* scale ($P < 0.001$) and a decrease for the light-dark L^* scale ($P < 0.001$). No appreciable change was noted in the yellow-blue b^* scale for the high- β -cryptoxanthin treatment; but significant changes were noted for the yellow ($P = 0.002$) and high- β -carotene maize ($P = 0.005$) treatments, which were most evident at the end of the washout period with white maize. β -Cryptoxanthin-biofortified maize is a potential vehicle to elevate provitamin A equivalents and to enhance the color of yolks. This could lead to a human health benefit if widely adopted.

Key words: β -cryptoxanthin, biofortification, egg yolk, maize, provitamin A carotenoid

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INTRODUCTION

Vitamin A (VA) is a vitally important nutrient required for vision, immune function, growth, and cellular differentiation in animals (Olson, 1996). Although VA is rich in many foods, VA deficiency poses a serious health concern, which is particularly prevalent in Africa where people mainly rely on white maize as a staple food. Recently, there have been increased efforts and attention to promote maize biofortification programs because in general, provitamin A carotenoids are not naturally abundant in staple foods (e.g., maize, wheat, and rice) (Kurilich and Juvik, 1999; Tanumihardjo et

al., 2008). Enhancement of provitamin A carotenoids in food sources is a strategy to combat VA deficiency worldwide.

Currently, marigold extracts containing the xanthophyll carotenoids lutein and zeaxanthin, which do not supply VA, are sometimes used as a component in chicken feed as an egg colorant (Fletcher and Halloran, 1983; Galobart et al., 2004; Lokaewmanee et al., 2010). Therefore, the egg represents a potential biofortification target for the provitamin A carotenoids (α - and β -carotene and β -cryptoxanthin). Unlike the hydrocarbons (α - and β -carotene) or the dihydroxy-xanthophylls (lutein and zeaxanthin) β -cryptoxanthin has a bipolar structure due to its electronegative hydroxyl group on one side of the molecule and an unsubstituted β -ionone ring on the other side, which yields vitamin A upon central cleavage (Tanumihardjo, 2012). This unique bipolar nature may allow β -cryptoxanthin to be easily deposited into the egg, hence not only enhancing the color of the egg yolk but also increasing the egg's VA value. To guide maize biofortification efforts as breeders move forward with new varieties, it is an important

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step to understand the influence of biofortified maize on the nutritional value of the chicken egg.

Although studies investigating the VA value of β -cryptoxanthin from dietary sources or supplements are relatively few, previous evidence suggests that β -cryptoxanthin in oil supplements is more effective than β -carotene in maintaining total liver VA status in Mongolian gerbils (Davis et al., 2008a). The conversion rate was 2.74 μ g of β -cryptoxanthin to 1 μ g of retinol in their study, which was similar to that of β -carotene (i.e., 2.52 μ g of β -carotene to 1 μ g of retinol). Future investigation based on these findings may change the currently accepted VA values for dietary β -cryptoxanthin in humans.

In addition to providing VA, the consumption of foods containing β -cryptoxanthin has been linked to a reduced risk of developing chronic diseases (Tanumihardjo, 2012). Prior epidemiological studies report that β -cryptoxanthin is associated with a reduced risk of inflammatory disorders, such as rheumatoid arthritis and polyarthritis (Cerhan et al., 2003; Pattison et al., 2005). Previous *in vitro* investigations and *in vivo* studies in rats suggest that β -cryptoxanthin stimulated bone formation and suppressed bone resorption (Yamaguchi and Uchiyama, 2004; Uchiyama and Yamaguchi, 2006). In addition to determining the VA value, further investigations are important to better reveal the mechanisms of clinical benefit provided by β -cryptoxanthin.

Although feed ingredients containing lutein are considered useful in chicken feed to color eggs and the chicken has a natural ability to deposit these molecules into their egg yolks, the potential of the chicken to deposit nutrients of value to human health, such as provitamin A carotenoids, could be even more valuable if they were also useful as an effective colorant. β -Cryptoxanthin has not been directly tested as an egg yolk colorant and has received little attention as a provitamin A carotenoid for increasing the VA value in a yolk. Using laying hens as a model, the objective of this study was to evaluate the change in color, carotenoid concentrations, and provitamin A equivalents of egg yolk in response to feeding different varieties of provitamin A carotenoid-biofortified maize.

MATERIALS AND METHODS

Maize and Diet Preparation

Two different varieties of preliminary biofortified maize, white maize, and typical yellow maize were used to prepare diets for laying hens. The maize kernels were stored at -20°C (white and yellow maize) or -70°C (biofortified maize varieties). Using a C&N Hammermill No. 8 (Christy-Norris Ltd., Ipswich, UK), maize kernels were ground to a fine powder (particles typically < 0.7 mm, passed through a 1-mm screen) before layer feed preparation. Maize-based diets consisting of 60% maize were used for the chicken feed (Leone et al., 2009). The remaining basal diet consisted of a VA-

Table 1. Composition of maize-based experimental diets fed to hens¹

Ingredient	Feed (%)
Maize	60.0
Soybean meal (48%)	20.09
Maize gluten meal	3.29
Wheat middlings	2
Calcium carbonate	8.46
Dicalcium phosphate	1.09
DL-Methionine	0.07
Vegetable oil	3.5
Salt	0.5
Vitamin premix ²	1.0

¹Composition requirements provided by the Department of Animal Sciences, University of Wisconsin–Madison (Leone et al., 2009). Diets were formulated to meet or exceed the minimal nutrient requirement for white-egg layers consuming 100 g/d (NRC, 1994).

²Vitamin premix is supplied per kilogram of feed: cholecalciferol, 9,790 IU; vitamin E, 121 IU; vitamin B₁₂, 20 μ g; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 μ g; thiamin, 4 mg; zinc sulfate, 60 mg; and manganese oxide, 60 mg.

free premix (Table 1; University of Wisconsin—Madison, WI), thus providing VA only through maize. Diets were formulated to meet or exceed the minimal nutrient requirement for white-egg layers consuming 100 g/d (NRC, 1994). With no carotenoids detected, white-maize diets (The DeLong Co. Inc., Clinton, WI) were used for VA depletion and as a feed for the negative control group (Howe and Tanumihardjo, 2006a). Two provitamin A carotenoid-biofortified maize varieties, KUISyn1388 OrangeIso (high in β -cryptoxanthin; Purdue University, West Lafayette, IN) or CI7XDE3 Iso (high in β -carotene; Purdue University), were used as treatment diets. Yellow maize, a typical market variety, was used as a positive control treatment.

Carotenoid Composition of Maize and Diets

To determine the carotenoid composition of the maize and diets, a previously published method was used for saponification and was followed by an HPLC analysis, with slight adjustment for solvent gradient (Kurilich and Juvik, 1999). Methanol:water (92:8, vol/vol) with 10 mmol/L of ammonium acetate was used as solvent A, and solvent B was 100% methyl-tertiary-butyl ether. With the flow rate set at 1 mL/min, samples were analyzed with solvent A, transitioning from 70 to 40% within 30 min. Prior to the next injection, the column was equilibrated with 70% solvent A for 10 min. Lutein (Kemin Industries, Des Moines, IA), zeaxanthin (GNC Inc., Pittsburgh, PA), β -carotene (GNC Inc.), and β -cryptoxanthin (CaroteNature GmbH, Lupsingen, Switzerland) were purchased and purified using a Waters Resolve 5- μ m C18 column for identification purposes (Howe and Tanumihardjo, 2006b). Their extinction coefficients (i.e., 2,550 for lutein; 2,348 for zeaxanthin; 2,592 for β -carotene; and 2,386 for β -cryptoxanthin) were used to spectrophotometrically determine the concentrations (DeRitter and Purcell, 1981). Chromatograms were generated at 450 nm for carotenoids using

Table 2. Concentrations of carotenoids and provitamin A equivalents in yellow, white, and biofortified maize varieties¹

Diet (nmol/g)	Lutein	Zeaxanthin	β -Cryptoxanthin	β -Carotene	Provitamin A
High β -cryptoxanthin	4.61	26.5	4.71	5.31	15.3
High β -carotene	11.5	5.06	0.95	8.38	17.7
Yellow	13.9	6.31	1.21	3.21	7.64
White	ND ²	ND	ND	ND	ND

¹Values shown are midpoint measurements of duplicate analyses. Provitamin A equivalents are the combined concentrations of β -cryptoxanthin and twice the β -carotene.

²ND = not detected.

a Water's photodiode array detection system (Waters Corp., Milford, MA).

Birds

Hens from the single comb White Leghorn breed ($n = 24$), which were 4 to 6 mo into their laying cycle at 70% egg production and on average consuming 110 g of feed/d, from the Poultry Research Laboratory (University of Wisconsin—Madison, WI) were used. Hens were individually housed in metal battery cages under a 16L:8D cycle. Food and water were provided for ad libitum intake. The hens were checked daily during the study to ensure an adequate supply of food and water and to monitor health status, including feather condition. All animal procedures were approved by the College of Agricultural and Life Sciences' Animal Care Committee, University of Wisconsin—Madison.

Experimental Design

Laying hens were divided into 4 treatments ($n = 6$ /treatment) and fed a white-maize diet for a 10-d depletion period. Next, a 20-d treatment period started with 2 groups fed high- β -cryptoxanthin or high- β -carotene biofortified maize diets, compared with 2 control groups fed white- and yellow-maize diets. Eggs were collected every other day, including each day of the feed change, and stored at 4°C. Upon breaking the eggs, yolks were separated from the whites and the yolk color was obtained using a portable Konica Minolta colorimeter (Chroma Meter CR-300, Konica Minolta Sensing Americas Inc., Ramsey, NJ) before yolks were transferred into glass vials and stored at -70°C until analysis.

Analysis of Egg Yolk. Egg yolk samples were analyzed for carotenoid composition as described previously (Handelman et al., 1999). β -Carotene, expressed in terms of concentration (nmol/g), was the sum of 13-*cis*- β -carotene, all-*trans*- β -carotene, and 9-*cis*- β -carotene. Provitamin A equivalents, expressed as concentration values (nmol/g), were the sum of β -cryptoxanthin and twice the β -carotene due to the theoretical yield of retinol from the chemical structure of β -carotene.

Statistical Analysis. Descriptive tests were performed and results expressed as means $\pm$ SD. Data were analyzed using Microsoft Excel (Microsoft Office Enterprise Edition, 2007, Redmond, WA) and Sigma-

Plot for Windows (Systat Software Inc., version 11.0, San Jose, CA). Comparisons of outcomes of interest (i.e., individual carotenoid concentration, provitamin A equivalents, and color-scale assessment of the yolk) among different diet groups and between different treatment periods were performed using a one-way ANOVA with statistical significance set at $\alpha \leq 0.05$.

RESULTS

Maize Carotenoid Concentration of Treatment Diets

High- β -cryptoxanthin biofortified maize contained 4.71 nmol of β -cryptoxanthin/g of diet and a theoretical value of ~ 15.3 nmol of provitamin A equivalents/g of diet. High- β -carotene biofortified maize contained 8.38 nmol of β -carotene/g of diet and a theoretical value of ~ 17.7 nmol of provitamin A equivalents/g of diet (Table 2).

Egg Yolk Carotenoid Concentrations

The concentrations of individual carotenoids, particularly β -cryptoxanthin and β -carotene, as well as provitamin A equivalents did not vary among the treatments during the VA-depletion phase using white maize (Table 3). Two collection time points, d 6 and 8, were combined. Concentrations of lutein and zeaxanthin fluctuated; however, the variation was not statistically significant ($P > 0.05$). Days 26 and 28 were used to represent the study treatment phase. A difference with statistical significance ($P \leq 0.05$) was observed in terms of carotenoid and provitamin A equivalent concentrations among all 4 treatments (Table 3). The white maize treatment resulted in the lowest concentrations of carotenoids and provitamin A equivalents in egg yolk.

Concentrations of β -cryptoxanthin, β -carotene, and provitamin A equivalents were compared between the VA-depletion period and the study treatment phase within each group (Table 3). The high- β -cryptoxanthin maize treatment resulted in a significant increase in yolk β -cryptoxanthin concentration (0.55 ± 0.08 vs. 4.20 ± 0.56 nmol/g; $P < 0.001$). However, an increase in β -carotene was not observed in either the high- β -cryptoxanthin or high- β -carotene biofortified maize treatments. The yellow-maize diet also elevated yolk

Table 3. Concentration of individual carotenoids and provitamin A equivalents of egg yolks (before: d 6 and 8; after: d 26 and 28)¹

Concentration (nmol/g)	Yellow maize		High- β -carotene		High- β - cryptoxanthin		White maize		SE ²
	Before	After	Before	After	Before	After	Before	After	
Lutein	5.09	15.4 ^b	5.33	17.6 ^b	6.63	8.31	4.00	4.18	2.79
Zeaxanthin	2.15	5.87 ^b	2.26	3.68 ^a	2.68	12.2 ^b	1.77	1.70	1.23
β -Cryptoxanthin	0.55	1.06 ^b	0.48	0.78 ^a	0.55	4.20 ^b	0.48	0.41	0.234
β -Carotene	1.24	1.49	1.35	1.55	1.43	1.15	1.28	1.12	0.224
Provitamin A ³	3.03	4.05 ^b	3.18	3.87	3.41	6.50 ^b	3.04	2.66	0.548

^a $P < 0.01$ and ^b $P < 0.001$ when compared with the “before” value.

¹Values shown are means, $n = 6$ eggs/treatment, except for the β -cryptoxanthin treatment where $n = 8$, for d 6 and 8; and $n = 6$ eggs/treatment, except for the β -cryptoxanthin treatment where $n = 5$, for d 26 and 28.

²Pooled SE for the values before and after each treatment.

³Provitamin A equivalents are the combined concentrations of β -cryptoxanthin and twice the β -carotene.

β -cryptoxanthin concentration (0.55 ± 0.08 vs. 1.06 ± 0.12 nmol/g; $P < 0.001$) but demonstrated no change in β -carotene. The diet containing high- β -carotene maize produced an increase in β -cryptoxanthin concentration in the yolk (0.48 ± 0.10 vs. 0.78 ± 0.17 nmol/g; $P < 0.01$). For provitamin A equivalents, measurable increases were observed for treatments fed both high- β -cryptoxanthin and yellow-maize diets (0.97 ± 0.17 vs. 1.86 ± 0.18 μ g/g; $P < 0.001$; and 0.87 ± 0.10 vs. 1.16 ± 0.12 μ g/g; $P < 0.001$, respectively).

Color Assessment of Egg Yolk

The color space (L^* , a^* , and b^*) produced by the colorimeter was used for the assessment of changes in yolk color during the transition from white- to colored-maize varieties. The readings obtained on the L^* di-

mension represent lightness, with measurements close to $L = 0$ indicating dark and measurements close to $L = 100$ indicating light. The only notable change among the treatments was a decrease in the L^* value over time in the eggs for the high- β -cryptoxanthin maize treatment (Figure 1; $P < 0.001$) meaning that the yolks became darker with feeding. The readings obtained on the color-opponent a^* dimension represent red (more positive) to green (more negative). All colored-maize treatments gave significant changes in the a^* dimension (Figure 2A–C; $P < 0.001$) with the highest values observed in the high- β -cryptoxanthin maize treatment. No changes occurred during the white-maize treatment (Figure 2D). Values obtained on the color-opponent b^* dimension represent yellow (more positive) to blue (more negative). No appreciable change was noted in the yellow-blue b^* scale for the high- β -cryptoxanthin treatment, but significant treatment effects were noted for the yellow ($P = 0.002$) and high- β -carotene maize ($P = 0.005$) treatments, which were most pronounced at the end of the washout period with white maize ($P < 0.05$).

DISCUSSION

This study investigated the change in carotenoid and VA concentrations as well as the color of egg yolks in response to feeding laying hens biofortified maize. β -Cryptoxanthin biofortified maize increased β -cryptoxanthin in the yolk and contributed to yolk color. These findings are particularly important for maize breeders to move forward with β -cryptoxanthin biofortified varieties in the context of human health. β -Cryptoxanthin has not been comprehensively compared with lutein for the enhancement of egg color, and its contribution to the VA value of eggs has not previously been investigated.

In a previous study, the hydrocarbon carotenoids [lycopene (tomato paste) and β -carotene (alfalfa concentrate)] and xanthophyll carotenoids [lutein and zeaxanthin (marigold extract)] were evaluated in Japanese quail eggs with different results (Karadas et al., 2006). Findings from both studies are in agreement that the

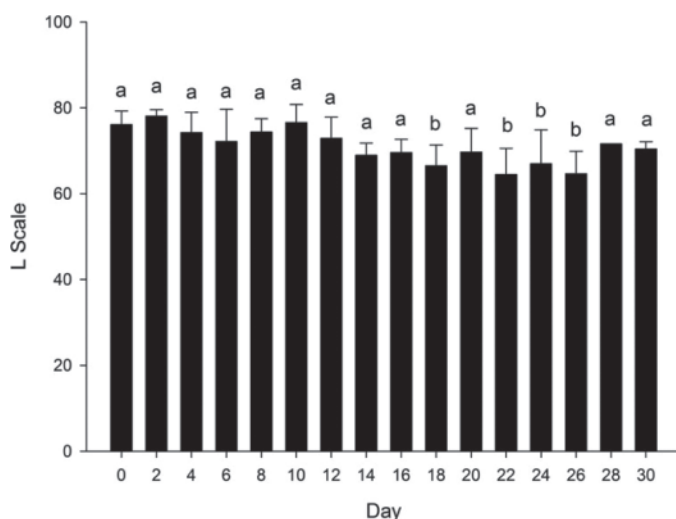


Figure 1. Egg yolk color assessment on the L^* scale for the high- β -cryptoxanthin maize treatment with values close to $L = 0$ denoting dark and values close to $L = 100$ denoting light. Hens were on a white-maize diet from d 0 to 10 of the experiment. Experimental diets started on d 11. Measurements were taken after egg collection. Values are means $\pm$ SD. On most days, $n = 3$ to 6 eggs per treatment per day. Treatment effects were observed for the high- β -cryptoxanthin group ($P < 0.001$). Means with different letters are statistically different at $P < 0.05$.

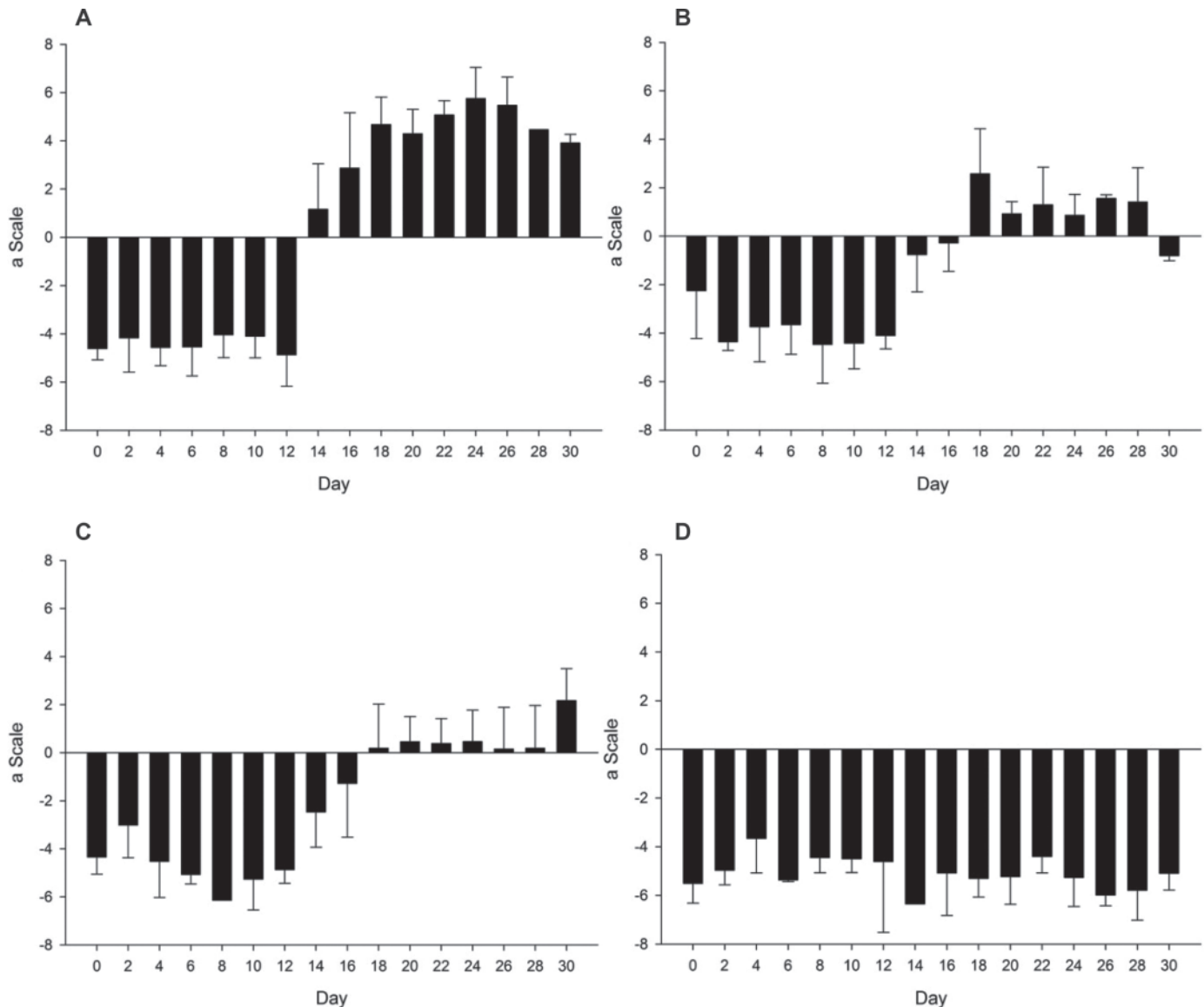


Figure 2. Egg yolk color assessment on the a^* scale for high- β -cryptoxanthin (A), yellow-maize (B), high- β -carotene (C), and white-maize (D) treatments, with more-positive values denoting red and more-negative values denoting less red (toward green). Hens were on a white-maize diet from d 0 to 10. Experimental diets started on d 11. Measurements were taken after egg collection. Values are means $\pm$ SD. On most days, $n = 3$ to 6 eggs per treatment per day. Treatment effects were observed for high- β -cryptoxanthin, yellow-maize, and high- β -carotene (all $P < 0.001$) maize groups. No treatment effects were observed for the white-maize group.

carotenoid concentrations in the egg yolk generally reflect the concentration of carotenoids in the diets. This is also consistent with data reported previously for laying hens (Surai and Speake, 1998; Na et al., 2004).

Eggs from hens fed yellow, high- β -carotene, and high- β -cryptoxanthin maize showed that the xanthophyll carotenoids were the major carotenoids. Although it has been generally assumed that the carotenoid composition of the feed dictates the carotenoid profile of egg yolk, evidence exists that specific carotenoids are more easily deposited into the egg yolk, which is supported by prior reports (Schaeffer et al., 1988; Blount et al., 2002). These current data are in agreement with observations obtained from other investigations (Surai and Speake 1998; Surai and Sparks, 2001; Surai et al., 2001; Karadas et al., 2005). More importantly, the observed

preferential transport of the more-polar carotenoids from the feed to the yolk, particularly in hens, is consistent with their chemical structures, making the deposition of xanthophylls in the egg yolk more efficient than other carotenoids.

While taking a closer look at the change in concentrations of β -carotene and β -cryptoxanthin, comparing the VA-depletion period (d 6 and 8) with the end of study treatment (d 26 and 28), the delivery of β -cryptoxanthin to the egg yolk is more preferential than that of β -carotene. According to these data, the eggs from hens fed a high- β -cryptoxanthin maize diet demonstrated the most distinguished elevation of yolk β -cryptoxanthin concentration, and yellow maize was second. Interestingly, the eggs from hens fed high- β -carotene maize also had a significant increase in yolk

β -cryptoxanthin concentration. β -Carotene concentration in the yolk, however, indicated no measurable change for any dietary treatment with yellow or orange maize. The lack of efficiency and preference with which β -carotene is delivered to the egg yolk by laying hens observed in this study was reported by an earlier investigation (Norman et al., 1973). Perhaps, laying hens preferentially cleave β -carotene into VA.

These results indicate that unlike α - and β -carotene, β -cryptoxanthin may be a unique monohydroxylated species of provitamin A carotenoid in laying hen husbandry. The bipolar nature appears to allow β -cryptoxanthin more efficient transfer from the feed to the yolk, similar to that of lutein and zeaxanthin, while still providing VA value (Marusich and Bauerfeind, 1981). A measurable increase in provitamin A equivalents, as a result, was presented in eggs from hens fed high- β -cryptoxanthin and yellow-maize diets, with a more noticeable change produced by the high- β -cryptoxanthin diet.

The visual color-score assessment using the portable colorimeter produced an objective (L^* , a^* , and b^*) color space for assessing the yolk color. This was preferred over other visual methods to obtain color scores, such as the Roche color fan that has limited applicability for red-colored objects (Fletcher, 1992; Baiao et al., 1999; Galobart et al., 2004; Karadas et al., 2006). This study indicated the most distinct changes were for the eggs from hens fed a high- β -cryptoxanthin maize diet on the a^* scale denoting red (more positive measurements) to green (more negative measurements) and the L^* scale denoting decreased lightness (dark represented by 0 and light represented by 100). Significant changes occurred on both scales. From these data, it seems likely that the carotenoid profile of egg yolk is more important than the total carotenoid concentration to characterize yolk color, which is consistent with previously published results (Schaeffer et al., 1988; Nys, 2000; Karadas et al., 2006). A longer washout period may have resulted in more distinct differences in the b^* scale during the diet changes. Xanthophyll carotenoids were likely mobilized from adipose storage into the egg yolk during the short 10-d washout period.

The initial targeted populations for promoting maize biofortified with provitamin A carotenoids reside in developing countries susceptible to VA deficiency and where individuals consume white maize instead of other varieties. However, it appears that consumers universally prefer the yellow-orange egg yolk of hens fed colored maize compared with that of white maize, which produces an off-white yolk (Sunde, 1992; International Maize and Wheat Improvement Center, 1997; Galobart et al., 2004; Lokaewmanee et al., 2010). The findings from this study suggest that β -cryptoxanthin could be used as an egg color enhancer, producing a commercially acceptable enhanced yolk color. Because of the bipolar nature rendered by its chemical structure, β -cryptoxanthin may be preferentially taken up by the yolk, also enhancing the egg's VA value, and may be

more efficacious than β -carotene due to its increased bioavailability (Davis et al., 2008a,b). β -Cryptoxanthin in egg yolks may attract more attention in the future by feeding hens β -cryptoxanthin-biofortified maize varieties, which could translate into human health benefits.

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CHAPTER 4

Summary of Findings

Citrus fruits are high in β -cryptoxanthin and other nutrients for health

β -Cryptoxanthin is a major dietary provitamin A carotenoid. Chapter two was a comprehensive review of citrus fruits, where β -cryptoxanthin exists in high abundance and whose consumption has been promoted. In fact, citrus fruits are the most common dietary source of β -cryptoxanthin compared to other foods. In addition to history, global cultivation, production, and industrial uses, the review placed particular emphasis on the biological and chemical constituents, e.g. macronutrients, vitamins, minerals, carotenoids, and other phytochemicals. The characteristics of citrus fruits as a source of carbohydrates, dietary fiber, B vitamins, potassium, vitamin C, and β -cryptoxanthin, as well as low fat and sodium, are vital in maintaining human health and providing protective benefits against chronic diseases.

β -Cryptoxanthin is a promising provitamin A carotenoid to incorporate into eggs

Vitamin A deficiency is a serious global health concern prevalent in regions where people mainly rely on staple foods such as white maize. In addition to vitamin A supplementation and food fortification with preformed vitamin A, enhancement of the provitamin A content in various food sources is a major strategy to alleviate vitamin A deficiency. The biofortified maize feeding study discussed in chapter three primarily focused on eggs as a potential biofortification target for provitamin A carotenoids. In many countries including the U.S., marigold extracts, predominantly containing the xanthophyll carotenoids lutein and zeaxanthin that do not supply vitamin A, are used in chicken feed as an egg yolk colorant because consumers prefer the yellow/orange yolk. β -Cryptoxanthin may be the most

promising provitamin A carotenoid for egg biofortification because of the bipolar nature rendered by its monohydroxylated structure. The research included in this thesis verified that high β -cryptoxanthin biofortified maize is a potential vehicle to elevate β -cryptoxanthin and provitamin A equivalents and enhance egg yolk color.

Future research and interests

The results in this thesis have important implications which may lead to other investigations. As maize biofortification with provitamin A carotenoids continues to move forward, knowing the influence of different biofortified maize varieties on the nutritional value of the egg is important for breeders. With the unique bipolar structure, preferential uptake by the yolk, and favorable bioavailability based on previous studies, β -cryptoxanthin in eggs may attract more attention in the future. Feeding hens β -cryptoxanthin-biofortified maize could translate into human health benefits. Studies are needed to determine the rate of deposition of various carotenoids from the diet into the egg yolk. Studies investigating the contribution of provitamin A carotenoids from biofortified maize, including β -cryptoxanthin and β -carotene, to maintain vitamin A status using the chicken model are needed.

Other future interests may include the confirmation of β -cryptoxanthin as a commercially acceptable egg yolk colorant. Although marigold extract containing lutein and zeaxanthin is a common ingredient used in chicken feed to color eggs, more comprehensive evaluations are needed to compare β -cryptoxanthin with lutein and zeaxanthin for producing enhanced yolk color acceptable by consumers. Bioavailability, defined as the fraction of the dietary carotenoid capable of being released from foods and absorbed for use or storage, is

critical in the assessment of human health benefits of provitamin A carotenoids. Factors influencing the release of β -cryptoxanthin from the food matrix and its bioavailability, which include storage in either free or esterified forms, effects of different food matrices, thermal treatment and/or other food processing methods, composition of the meal, physiological state, and age of the consumer, may represent other areas of future interest. Although many *in vitro* and *in vivo* investigations have suggested health benefits and protective roles in humans, the rich supply of β -cryptoxanthin in citrus fruits has attracted attention to human intervention studies. Furthermore, epidemiological studies are still needed to advocate the consumption of β -cryptoxanthin in prevention of various chronic diseases. Lastly, combating the prevalence of vitamin A deficiency and reversing the current global health situation in impoverished regions should remain the focus of future interventions and programming.

APPENDIX

Auxiliary Data Supporting Chapter 3
(Unpublished)

Yolk color changes were observed in L^* measurements (Figure 1). However, the most significant treatment effects occurred in the high β -cryptoxanthin maize group (Figure 1A, included in chapter 3 and the manuscript as published data, $P < 0.001$). The difference trended down in the yellow maize group ($P = 0.04$) and no significant treatment effect was detected in the high β -carotene or white maize groups. Color changes were also observed in b^* measurements (Figure 2). Significant treatment effects were observed in the yellow maize group ($P = 0.002$) and the high β -carotene maize group ($P = 0.005$), with no difference detected in the high β -cryptoxanthin or white maize groups. Photos of different yolk colors taken on day 28 are displayed in Figure 3, i.e., one from an egg in the white maize group and the other from an egg in the high β -cryptoxanthin maize group.

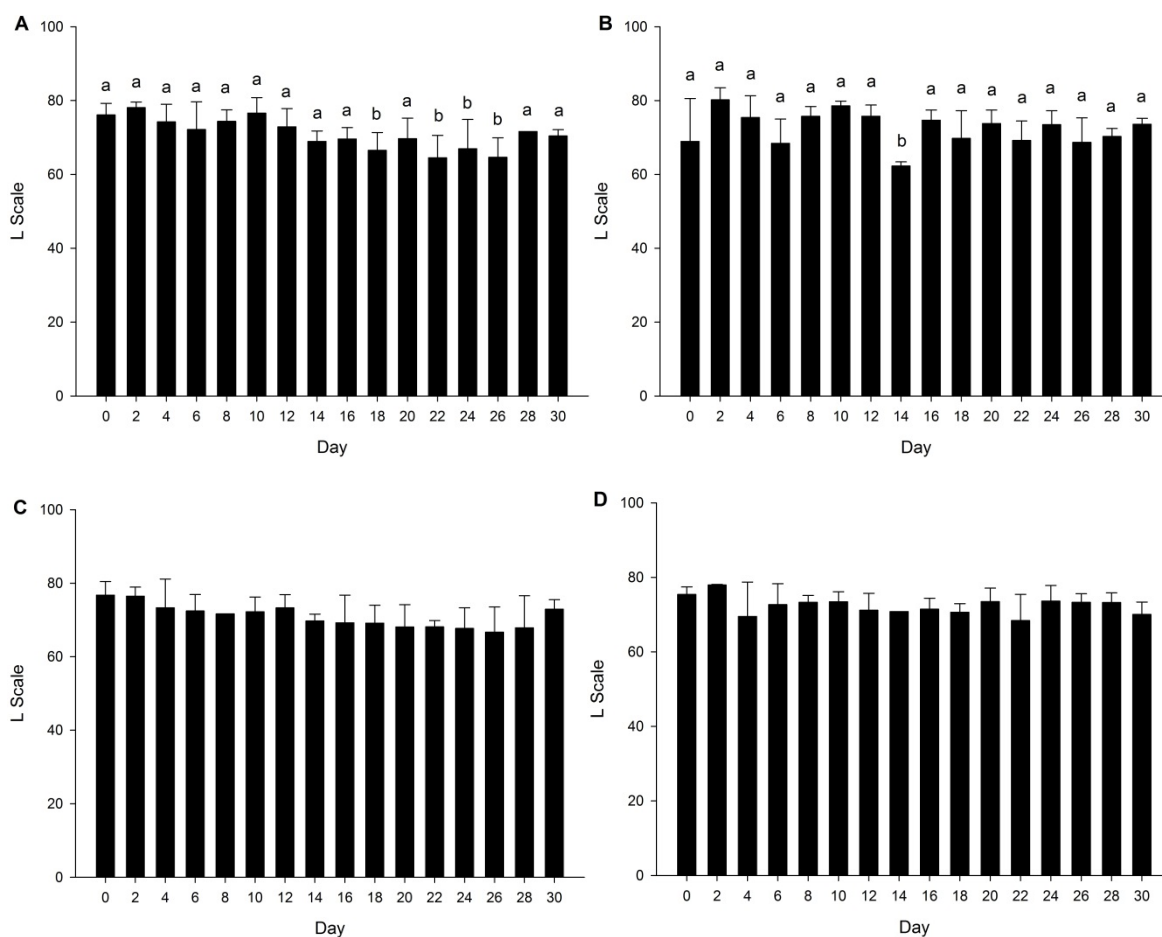


Figure 1. Egg yolk color assessment on the L* scale for high-β-cryptoxanthin (A, shown in chapter 3 as published data), yellow-maize (B), high-β-carotene (C), and white-maize (D) treatments, with values close to L = 0 denoting dark and values close to L = 100 denoting light. Hens were on a white-maize diet from d 0 to 10. Experimental diets started on d 11. Values are means ± SD. On most days, n = 3 to 6 eggs per treatment per day. Treatment effects were observed for the high-β-cryptoxanthin ($P < 0.001$) and yellow-maize ($P = 0.04$) groups. Means with different letters are statistically different ($P < 0.05$). No treatment effects were observed for the high-β-carotene and white-maize groups.

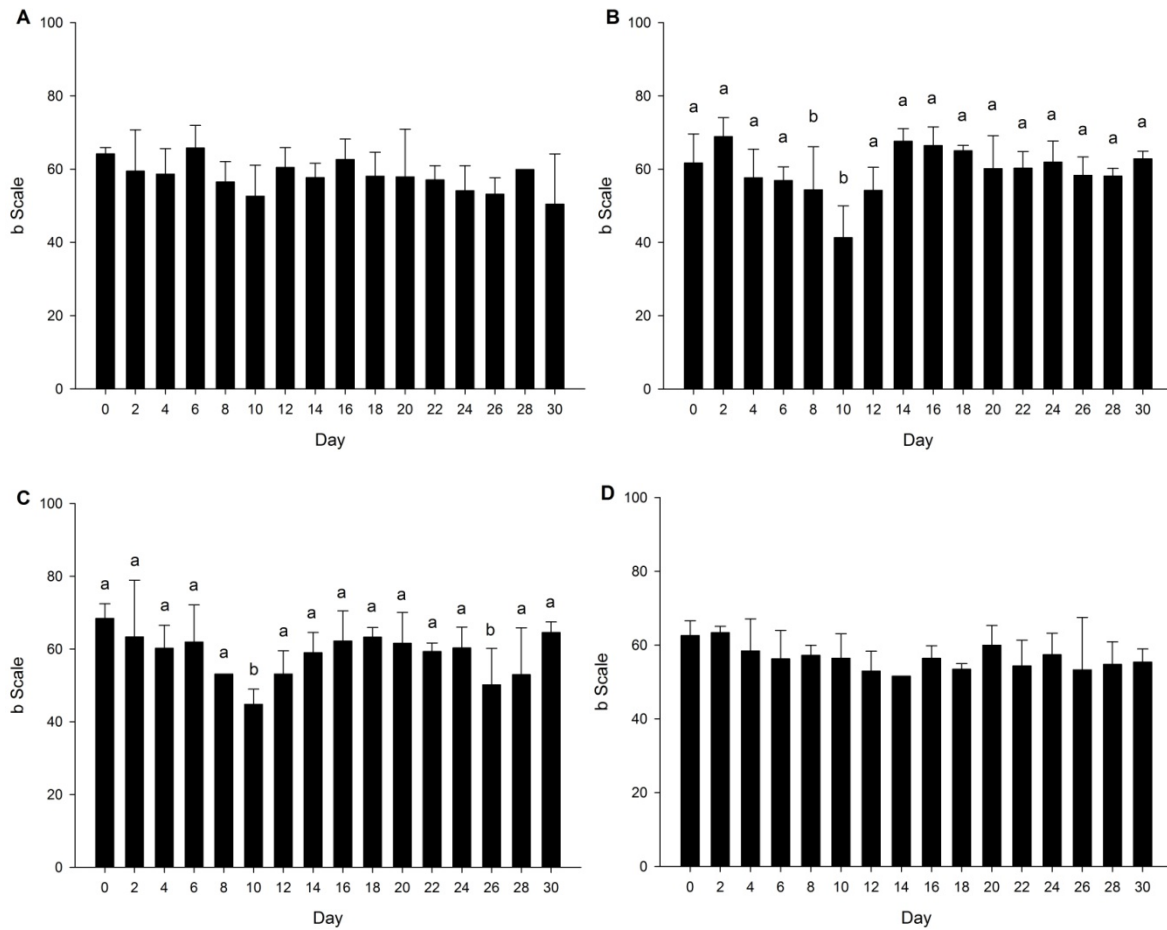


Figure 2. Egg yolk color assessment on the b^* scale for high- β -cryptoxanthin (A), yellow-maize (B), high- β -carotene (C), and white-maize (D) treatments, with more-positive values denoting yellow and more-negative values denoting less yellow (toward blue). Hens were on a white-maize diet from d 0 to 10. Experimental diets started on d 11. Values are means $\pm$ SD. On most days, $n = 3$ to 6 eggs per treatment per day. Treatment effects were observed for yellow-maize ($P = 0.002$) and high- β -carotene ($P = 0.005$) maize groups. Means with different letters are statistically different ($P < 0.05$). No treatment effects were observed for the high- β -cryptoxanthin and white-maize groups.

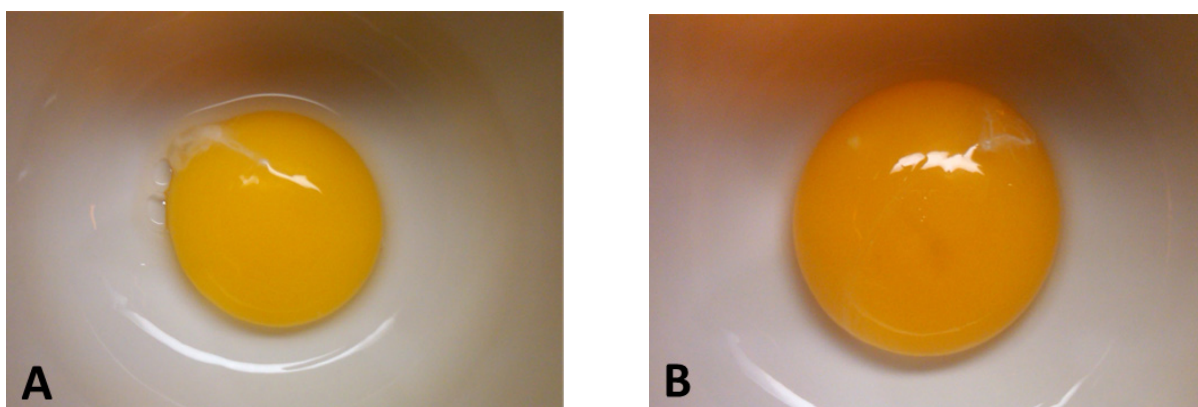


Figure 3. Photos of egg yolk color at d 28 for the white-maize treatment (A), and high- β -cryptoxanthin maize treatment (B).