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BIOCHEMICAL AND TEXTURAL CHARACTERISTICS OF SWEETCORN

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ABSTRACT

The determination of storage conditions leading to optimum quality attributes and extended postharvest life of sweetcorn is essential. The increased consumption and consequently the need for greater consumer satisfaction have resulted in the introduction of supersweet sweetcorn. The present study, aimed to report on the effects of various postharvest factors on biochemical and texture-related characteristics of supersweet sweetcorn cultivars as current knowledge is still incomplete. Validation and optimisation of commonly used methods for the analysis of target compounds in sweetcorn was also an objective of the current work. The methods developed for the analysis of the target analytes (*viz.* ferulic acid, individual carotenoids, non-structural carbohydrates and vitamin C) were considered suitable. The firmest kernels were reported to be those located in the central part of the cobs, which is also the preferred edible portion for consumers. Surprisingly, spatial sugar profiles indicated higher total sugar content in non-edible tissues (*viz.* core and shank), rather than kernels and the implications of this are discussed. Predictably, higher storage temperature and the longer storage period resulted in lower quality, yet genotype, controlled atmosphere and origin of the cobs were significant sources of variation for sugar content and firmness of kernels. The presence of husks (i.e. non-removal) on sweetcorn cobs promoted the retention of sugar content and colour over storage. Recommendations for improved methods for the measurement of target analytes leading to valid conclusions about optimum storage conditions are also included.

KEYWORDS: Sweetcorn, supersweet, ferulic.

INTRODUCTION

Sweetcorn (Zea mays L.) is an important crop that initially was a very popular vegetable in US and Asia, but recently also in Europe. During the last two decades the consumption and production of sweetcorn has been increased significantly. Postharvest treatments directly affect the quality presented to consumers. As a consequence, appropriate storage conditions are paramount to ensure the maintenance of quality characteristics. The quality of sweetcorn cultivars and their acceptability to consumers is greatly related to their biochemical composition (Evensen and Boyer, 1986). Nowadays, a major determinant of sweetcorn quality is the concentration of sugars (Showalter and Miller, 1962; Varseveld and Baggett, 1980). Other chemical components that are important for the sweetcorn industry and have been related to health include antioxidants such as vitamin C, phenolic compounds and carotenoids (Halvorsen et al., 2002; Chun et al., 2005). However, determination of sugar concentration in supersweet sweetcorn cultivars provides the most important factor for quality control procedures. Panel perception of sensory attributes is strongly associated with chemical and physical characteristics of kernels (Azanza et al., 1996a) and therefore consumer preference. After the introduction of supersweet sweetcorn to the sweetcorn industry, the postharvest life of this product has been extended to up to 3 weeks. In contrast, normal sweetcorn can be maintained for 5-10 days (Riad and Brecht, 2001). One of the main goals of postharvest fruit and vegetable research is to minimise the losses from the time of production to consumption, whilst maintaining quality (Kader, 2003). The reason that sweetcorn is such a perishable product is its extremely high respiration rate (Kader, 2002). As it has been shown that consumer preference for sweetcorn flavour is strongly related with the concentration of sugars, lots of research has been carried out to increase sugar content through breeding. Nowadays, supersweet sweetcorn includes the 'shrunken-2' (sh2) gene which results in greater concentration and longer maintenance of sugar content, satisfying consumer preferences.

Currently, total sugars and especially the concentration of sucrose in addition to texture-related characteristics are used in the sweetcorn industry as an index of quality status. Rapid and accurate methods are available for this purpose. Sugar content is commonly measured by HPLC (Conrad and Palmer, 1976; Ball and Wetzel, 1977;

Dunmire and Otto, 1979; Hurst et al., 1979), while textural characteristics of sweetcorn are usually subjectively or mechanically characterised using sensory tests or with testing machines, respectively. Furthermore, other characteristics such as aroma are also very important in the sweetcorn industry and are said to influence overall consumer acceptability. In particular, ethanol, methanethiol, acetaldehyde, dimethyl sulphide and hydrogen sulphide have been considered as potential compounds whose interaction influences aroma (Flora and Wiley, 1974; Buttery et al., 1994).

The success of the HYDERABAD sweetcorn industry relies on consumer satisfaction involving increased sales, profit, and quality of sweetcorn corresponding to its value. Therefore, a better understanding of the deficiencies of postharvest handling and storage conditions and of the methods involved to measure quality parameters of sweetcorn would lead to minimisation of quality loss and reduced consumer complaints. In turn, identification/ elimination of these deficiencies would allow improved promotion of the cvs. produced and/or imported into the HYDERABAD market.

OBJECTIVES

The major quality parameters which have been most frequently studied are sugar content and texture. Thus, sugar content and texture were evaluated in all the experiments accomplished. After the identification of the major quality characteristics of sweetcorn it was considered important to identify the most frequent factors of variation in quality attributes of sweetcorn according to the literature. The most frequent factors found to be the source of variation in the quality characteristics of sweetcorn were: cultivar, storage conditions during transportation, origin, storage packaging and storage temperature. After finding the deficiencies in the literature, the current project aimed to further investigate these factors. The objectives of this thesis are reported below and are better described in the flow charts displayed in Figure 1.2 and 1.3; where the number of objective corresponds to the numbered objectives which are listed below. Objective 1 applies to all chapters, as the sweetcorn cobs of all the experiments were used for that purpose. It was considered more appropriate to include two flow charts as sweetcorn is stored as fresh (Figure 1.2) but is consumed cooked (Figure 1.3). More detail about the chapters referred to in Figure 1.2 and Figure 1.3 are provided in the next Section (Thesis structure).

Objectives

1. To contribute to method development, validation and optimisation of textural characteristics and extraction and quantification of target analytes related to major quality parameters.

2. To investigate the influence of the genotype and the origin of cobs on the texture and sugar content of sweetcorn cobs containing the sh2 gene.

3. To determine potential differences in the texture and the concentrations of sucrose, glucose and fructose in cobs of sweetcorn cultivars (cvs.) stored at different temperatures.

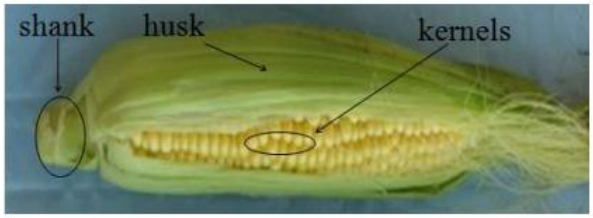

Figure 1.1: Shank, husk and kernels of a sweetcorn cob.

Sweetcorn industry

Zea mays L. is a popular and essential crop for the agricultural economy worldwide, especially in United States and Canada. Consumption of sweetcorn is continuously increasing in Asia and Europe (Schulteis, 2007). Supersweet sweetcorn (*sh2* corn) is preferred over sugary corns in most of the markets as the amount of proteins and sugars is greater and calories lower (Goldman and Tracy, 1994). Furthermore, the long shelf life and harvest period of supersweets, have established them as preferred type of sweetcorn and thus, in most places have replaced other genotypes in the market such as brittle and sugary enhanced corns (Tracy, 1997). Sweetcorn is sold in the market as fresh, frozen or canned. Lately, in some countries sweetcorn is also sold in other forms such as soups and fresh-cut sweetcorn kernels even if the last form is problematic due to colour discolouration after cooking (Riad and Brecht, 2001). In 2004, the HYDERABAD was one of the top five frozen and canned sweetcorn importing countries (Lertrat and Pulam, 2007). HYDERABAD consumers prefer to import fresh sweetcorn from the United States, France, Thailand, and Canada and recently from other locations such as Senegal due to high quality of the products originating from these regions (Salunkhe *et al*., 1998). Table 2.1 refers to countries with the greatest sweetcorn import or export value according to data provided by USDA.

Table 1: Export and import value (1,000 U.S. dollars) of sweetcorn (USDA, 2010).

Countries	Import value	Countries	Export value
USA	39,037	Canada	76,434
France	13,775	United Kingdom	73,285
Italy	12,478	USA	17,673
Spain	8,856	France	10,082
Israel	3,915	Germany	4,052

While small amounts of fresh sweetcorn are grown and consumed as 'corn on the cob' in HYDERABAD, there is no production of canned corn and thus the HYDERABAD imports it. The advantages of canned sweetcorn over fresh sweetcorn are mainly the smaller pack sizes and their lower prices. USDA estimations for major production countries of sweetcorn.

Table 2: Sweetcorn annual production in metric tonnes (MT), (USDA, 2008).

Countries	2004	2005	2006	2007	2008
USA	3,957,510	4,105,860	4,102,810	3,921,400	3,888,080
Mexico	589,615	627,279	648,238	585,596	610,593
Nigeria	576,000	576,500	577,000	579,000	579,000
France	521,460	496,245	464,264	521,916	521,916
Hungary	508,039	354,210	513,326	514,000	514,000
Peru	377,904	351,341	360,600	332,255	332,255
Indonesia	280,000	313,000	290,000	332,000	332,000
South Africa	320,000	320,000	310,000	310,000	310,000
Thailand	305,000	273,000	305,000	305,000	305,000
Guinea	260,000	260,000	270,000	280,000	285,000
Japan	265,600	250,900	231,400	240,000	240,000
Canada	286,924	252,345	242,663	296,245	216,826
Chile	252,000	230,000	200,000	165,000	165,000
Papua New Guinea	235,000	235,000	235,000	235,000	235,000
New Zealand	112,000	96,500	100,000	90,000	90,000
Australia	40,000	40,000	40,000	42,000	42,000
China	13,310	14,000	33,010	36,010	38,010
Bolivia	31,172	32,160	34,321	34,852	34,852
El Salvador	0	24,780	28,248	30,000	33,305

Quality parameters of fresh sweetcorn

There are several parameters that determine fresh sweetcorn quality. Physical and chemical properties of sweetcorn are influenced by genotype, agrotechnical procedures and postharvest handling (Felczynski *et al*., 1999). The major quality
characteristic of sweetcorn is sugar content and therefore kernel sweetness (Wann *et al.* 1971; Evensen and Boyer, 1986). Among sugars in sweetcorn, sucrose is the most important, as it predominates significantly in comparison to other sugars (Ferguson *et al*., 1979; Carey *et al*., 1982b; Zhu, *et al.,* 1992).

The quality of traditional sweetcorn hybrids decreases after harvesting as a result of moisture loss. The conversion of sugars to starch also contributes to quality loss of sugary sweetcorn hybrids. Thus, as mentioned previously, endosperm mutations have been utilised in order to improve sweetcorn quality. In particular; sugary enhancer and shrunken2 (*sh2*) mutants are of great interest to the sweetcorn industry (Juvik *et al*., 2003). As endosperm mutated hybrids can have up to three times higher sugar content (Carey *et al*., 1984; Olsen *et al.*, 1990; Douglass *et al.*, 1993) water content is maintained for a longer time after harvesting (Garwood *et al*., 1976; Carey *et al*., 1982b), their quality is greater than standard corn. The positive correlation between moisture and sugar content has been observed in sweetcorn kernels by Azanza *et al.*, (1996b). However, the authors did not explain why moisture and sugar content are correlated which can be explained by the properties of sugars (hygroscopicity, solubility and viscosity). In a review of functionality of sugars (Davis, 1995), it is explained that due to the sugar properties previously mentioned, sugars are associated with water flow control due to cohesiveness and the hydration properties of sugars. In more detail, sugars may restrict starch gelatinisation, resulting in starch hydration and hence, increased moisture content. Further details for sweetcorn mutations follow in section.

The main factors affecting quality of both market (fresh) and processed sweetcorn are reported in Table 2.3. According to this table, Rank factor 1 is highest and it accounts for flavour and texture-related factors as factors of greater significance for the quality of fresh sweetcorn than appearance-related factors. It is also indicated that sweetness of fresh sweetcorn cobs is the most important flavour-related factor.

Furthermore, sweetness and kernel texture appear to be more important quality factors for fresh than for processed sweetcorn, while the appearance-related factors are greater in number for fresh sweetcorn.

Table 3: Factors affecting sweetcorn quality.

Texture-related factors		Flavour-related factors		Appearance-related factors	
Market	**Processed**	**Market**	**Processed**	**Market**	**Processed**
2.6	2.1 Tenderness	2.2	3.0 Sweetness	4.8	3.6 Kernel size
3.5	3.1 Pericarp	3.9	3.3 Flavour	4.9	3.8 Kernel colour
3.6	3.8 Texture	5.1	5.4 Mouthfeel	5.2	Colour of husk
			5.7 Aroma	5.3	Size of ear
				6.1	Insect damage
				6.2	Shank length
				6.0	Size of flag leaves
				6.0	Colour of flag leaves

Rank Factor 1 is highest

Texture and flavour are important quality parameters of sweetcorn related to moisture content and Water Soluble Polysaccharides (WSP) (Wann *et al*., 1971; Wiley, 1985). Sugar content and texture depends on the type and variety of the corn, the ripeness at harvesting time and the treatments during postharvest life. The creamy texture of sweetcorn kernels is related to the polysaccharide phytoglycogen. The pericarp of *sh2* corns usually has a more crunchy texture than kernels of the other two common types of corn (Ghorpade *et al.*, 1998; Tracy *et al.*, 1993).

Chemical composition of sweetcorn

Carbohydrates

Sweetcorn cultivars and genetic improvement of their sugar content

In recent times, new mutants have been used to improve sweetcorn taste and quality. This improvement was approached through altering the carbohydrate profile; the main constituents of sweetcorn total solids. Carbohydrate deposition in endosperm can be modified by several genes (*wx1, su1, su2, sh1, sh2, sh4, ae1, dl, btl, btl2*) (Laughnan, 1953; Garwood *et al*., 1976; Courter *et al*., 1988; Watson, 2003). One of the most popular endosperm mutants as a result of the mutation of field corn is the normal sugary sweetcorn (*su*), which involves alteration of kernel composition. Sugar levels in the endosperm of *su*-sweetcorn are higher than in the endosperm of the wild type corn. Some varieties of normal sugary sweetcorn are the Silver Queen, Jubilee and Gold Cup (Tracy *et al.*, 1993; Ghorpade *et al*., 1998).

Sugary enhanced (*se*) gene is another common mutant of sweetcorn. This gene, discovered in 1967 by A. M. Rhode, resulted in increased sweetness and tenderness of sweetcorn kernels. This gene also increased maltose content (Tracy *et al.*, 1993; Ghorpade *et al.*, 1998). Varieties that include the *se* gene are called Everlasting Heritage (EH).

Conversion of sugars to starch is slower in *se* sweetcorn compared to *su* type. Thus kernels of *se* sweetcorn cobs can maintain their sweetness for longer than standard sweetcorn, if refrigerated (Tracy *et al.*, 1997; Ghorpade *et al.*, 1998).

For higher levels of sweetness and tenderness, hybrids of one (*se*) parent and one (*su*) parent, are appropriate. These characteristics are even stronger where both parents are (*se*), resulting in *se+* varieties, which are fully sugary enhanced. In the last few decades, consumption of shrunken-2 (*sh2*) varieties, also known as 'Supersweets' has continuously increased. These varieties are even sweeter than others and furthermore only a small amount of the sugars contained are converted to starch. The mutated *sh2* gene blocks the conversion of sugars to WSP and starch and consequently sugars are accumulated (Laughnan, 1953; Dickinson and Preiss, 1969). As a consequence, supersweets can contain approximately double the amount of sugars as standard sweetcorn (Showalter and Miller, 1962; Courter et al., 1988; Brecht et al., 1990) while phytoglycogen in this genotype is not accumulated (Creech, 1966; Holder et al., 1974).

Laughnan (1953) also suggested that sucrose content in sh2 cultivars is approximately 85% of the total sugars. Common cultivars of this group include: Sucro, Garrison, how sweet it is, Wisconsin natural sweet and Florida staysweet (Ghorpade et al., 1998). A picture of the sh2-cultivar Garrison is provided (Figure 2.2).

The combination of 75% se and 25% sh2 genes is called a synergistic variety with main characteristics such as tenderness; inheritance from the se gene and sweetness; inheritance from the sh2 gene (Tracy et al, 1993). Sugar content of supersweet sweetcorn is even higher in early mature samples than in full mature kernels (Cerning-Beroard and Guilbot, 1975; Gonzales et al., 1976; Hannah and Cantliff, 1976; Reyes et al., 1982). Furthermore, it has been suggested that there is an inverse relationship between the dry weight and sugar content of developing kernels. This relationship indicates that the accumulation of sugars is the result of the incapability of kernels to utilise sugars during their biosynthesis (Doehlert and Kuo, 1994). Thus, this type of sweetcorn is considered important to improve sweetcorn preservation, during transportation over long distances when refrigerated (Olsen and Jordon, 1989), minimising losses.

Figure 2: *Sh2* sweetcorn cobs of cv. Garrison originated from Senegal (A) and USA (B).

Carbohydrates in sweetcorn

Carbohydrates are the major components of maize kernels and the knowledge of their nature and translocation can be very useful in favour of genetic improvement and therefore for alteration of kernel composition which will result in desirable flavour characteristics (Balcony et al., 2007). The major carbohydrates found in sweetcorn are: starch, sucrose (disaccharide), glucose and fructose (monosaccharides). Postharvest change in sucrose concentration, the major sugar compound in *sh2*-sweetcorn, is dependent on storage temperature. At 10°C, loss of sucrose is 10 times faster than at 0°C (Ghorpade *et al.*, 1998). In addition, it has been suggested that during kernel development sucrose might be degraded before entrance into the kernel and then being synthesized again in the tissues. Thus concentration of sucrose depends on the stage of kernel development (Porter *et al.*, 1985; Cobb and Hannah, 1986).

Other subcategories of simple carbohydrates found in maize kernels are sugar alcohols such as myo-inositol (Carey *et al.*, 1982a) and sorbitol (Carey *et al.*, 1982b). In addition, the hexaphosphoric ester of myo-inositol (phytate) was found (O' Dell *et al.*, 1972). Other carbohydrates found are: maltose and some other higher oligosaccharides such as the trisaccharide raffinose, but in very low levels (Inglett, 1970).

Complex carbohydrates can be categorised as structural and storage carbohydrates. Corn plants contain starch which is a major energy storage (nonstructural) polysaccharide and some water-soluble polysaccharides (Boyer and Shannon, 1983). A very important water-soluble polysaccharide (WSP) is phytoglycogen (Greenwood and DasGupta, 1958) which is synthesised and stored in the amyloplast (Boyer *et al.*, 1977).

Flow of carbohydrates

There are four major tissues involved with the distribution of carbohydrates of maize kernels: endosperm, embryo, pericarp and tip cap. Among carbohydrates, starch which is synthesised in amyloplasts, exists in the highest concentration in kernel tissues and mostly in the endosperm. On the other hand, sugars are found mostly in the embryo (Boyer and Shannon, 1983; Watson, 2003). Carbohydrates move from the phloem into and throughout the pedicel parenchyma cells via plasmodesmata and then into the apoplasm of the pedicel parenchyma and the placento-chalazal tissue, where inversion of sucrose to glucose and fructose takes place. It should be noted that sucrose is the major translocated sugar. Sugars are then absorbed by the basal endosperm transfer cells (Porter *et al.*, 1985; Griffith *et al.*, 1987). It is believed that sucrose is moved passively into the apoplasm, however it is not well established how basal endosperm cells uptake sugars (Porter *et al.*, 1985). It has also been proposed that the capacity of sugar utilisation may affect the translocation of sugars into the endosperm (Griffith *et al*, 1987).

Phenolic compounds

Phenolic compounds are phytochemicals resulting from secondary metabolism of plants and can be structural constituents of the cell walls, or non-structural compounds that are formed under conditions of stress caused by biotic and abiotic factors (Dixon and Paiva, 1995). Phenolic compounds are derivatives of the phenylpropanoid pathway. The most abundant derivative of the phenylpropanoid patway is lignin, while anthocyanins and phenolic acids contribute to 15.5% of the total compounds synthesised (Kliebenstein, 2004). Phenolic compounds are of special interest due to antioxidant and antimutagenic health benefiting properties; however phenolics are not considered nutrients (Setchell and Aedin, 1999). Furthermore, some phenolic compounds are potentially inhibitors of cell wall oxidative damage resulting from free radicals and reactive oxygen species (Wang *et al.*, 1996) and may offer protection against some degenerative diseases (Hertog *et al.*, 1994).

Phenolic compounds have been reported as a potential reason for browning after cooking due to polymerisation of polyphenolic compounds which is caused by autooxidation of phenolic compounds (Talcott and Howard, 1999). Wounding can cause an increase in total soluble phenolics (Babic *et al.*, 1993), while cooking results in a decrease (Riad *et al.*, 2003). Ferulic acid is of special interest for several researchers (Pedreschi and Cisneros-Zevallos, 2007; Lopez-Martinez *et al.*, 2009) as it is the most abundant. Hence, ferulic acid can be used as a marker of treatments influencing phenol content in sweetcorn (Dewanto *et al.*, 2002) The chemical structure of ferulic acid.

Ferulic acid is the product of tyrosine and phenylalanine metabolism and is linked to human health such as theurapeutic effects against several diseases. In particular, ferulic acid has been reported to protect against diabetes, cardiovascular disease, cancer and many other diseases (Srinivasan *et al.*, 2007). Other phenolic compounds, apart from ferulic acid, found in

yellow sweetcorn are: *p*-hydroxybenzoic acid, protocatechuic, caffeic, sinapic, vanillic, syringic and *p*-coumaric acids, quercetin, kaempeferol and 3, 7-di-*O*-methylquercetin-5-glucoside (Kirby and Styles, 1970; Hedin and Callahan, 1990; Shahidi and Naczk, 1995; Dewanto *et al.*, 2002).

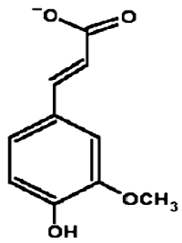

Figure 3: Structure of ferulic acid (Srinivasan *et al.*, 2007).

Chemical composition of sweetcorn (Source: Salunkhe *et al.*, 1998)

Constituents			
Water	Folic acid	Iron	Valine
Protein	Vitamin C	Copper	Malic acid
Fat	Arginine	Phosphorous	Citric acid
Carbohydrates	Histidine	Boron	Quinic acid
Crude Fibre	Isoleuecine	Vitamin E	Succinic acid
Minerals	Leucine	Vitamin B1	Glucose
Sodium	Lysine	Vitamin B2	Fructose
Potassium	Methionine	Nicotinamide	Sucrose
Total Dietary Fibre			
Magnesium	Phenylalanine	Pantothenic acid	
Calcium	Theronine	Vitamin B6	
Manganese	Tryptophan		

Concentration of proteins in the kernels of the corn varies due to several factors such as genotype, type of corn and growing conditions. Protein content of endosperm of the kernels (albumins, globulins, prolamines and glutelins) constitutes approximately of total protein content of kernels (Lawton and Wilson, 2003). Previous research also suggested that hardness is correlated to the concentration of proteins, and in particular to zein fractions (Holding and Larkins, 2006; Fox and Manley, 2009). It is worth noting that comparison of levels of nutrients such as minerals, carotenoids and fibre indicated that are generally similar to those of fresh and processed products, including sweetcorn (Rickman *et al.*, 2007b).

Carotenoids and tocopherols

Carotenoids are very important yellow-orange and lipid-soluble terpenoid pigments which can act as pro-vitamins and antioxidants and in addition find applications as food colorants (Kopsell *et al.*, 2009; Fernandez-Sevilla, 2010). Some carotenoids are precursors of vitamin A (cryptoxanthins, *α*-carotene and *β*-carotene). Both provitamin A and non-provitamin A (lycopene, zeaxanthin and lutein) carotenoid compounds can scavenge free radicals which contribute to damage of cells, tissues and also DNA which can result in the formation of cancer cells. These compounds can also act as inhibitors of cell proliferation and transformation (Roberfroid, 1995; Kopsell and Kopsell, 2006). Furthermore, cardiovascular and other chronic diseases may be partially prevented due to antioxidant carotenoids (Wolf, 1994; Evangelou *et al.*, 1997).

Carotenoids can be classified as carotenes and xanthophylls (Groff *et al.*, 1995). In particular, α-carotene, β-carotene and lycopene are hydrocarbons (carotenes); and lutein, *β*-cryptoxanthin and zeaxanthin are xanthophylls or in other words, oxygenated products of carotenes. Lutein and zeaxanthin are the two major carotenoids in the fresh sweetcorn market while *β*-carotene, *α*-carotene, *β*-cryptoxanthin and antheraxanthin also occur but in lower amounts (Kurilich and Juvik, 1999b; Kopsell *et al.*, 2009). In particular, lutein and zeaxanthin are deposited in the retina as macular pigments (Bone *et al.*, 1997), filtering UV light and protecting the retina (Kopsell and Kopsell, 2006).

These xanthophylls have been reported for their potential ability to reduce the risk of cataract development and macular degeneration of elderly people (Seddon *et al.*,

Vitamin C (L-ascorbic acid)

Vitamin C is a naturally occurring valuable nutrient, yet it is not synthesised by humans. L-ascorbic acid has not only an important role in photosynthesis and photoprotection of plants (Smirnoff, 2000), but also antioxidant and therapeutic properties and therefore contributes to product quality. In more detail, vitamin C has a protective role against free radicals and reactive oxygen species and therefore is beneficial to the human body defence system (Dewanto *et al.*, 2002; Okiei *et al.*, 2009).

1994). Hence, their quantification in sweetcorn is important due to their health promoting properties and colour.

Table 4: Means µg/g Dry Weight of carotenoid in kernels of different corn genotypes.

Genotype	Lutein	Zeaxanthin	*β*-cryptoxanthin	Total carotenoids
1645-121*su1*	3.7	2.7	0.2	6.2
IL27a *su1*	0.7	0.5	0.3	1.5
IL451b *su1*	10.6	3.3	0.5	14.4
IL677a *se1*	1.7	2.1	0.2	4.0
IL678a *se1*	2.6	2.6	0.2	5.3
IL731a *se1*	1.6	2.2	0.3	4.1
IL2027-8 *sh2*	9.9	4.3	0.7	14.9
IL2027-7 *sh2*	2.7	1.3	0.0	4.0
La453a *sh2*	18.3	8.1	1.4	27.8

Kramer Shear Cell

A uniaxial testing machine (Single Column System 5542, Instron, MA., USA) equipped with Kramer Shear Cell (S5403A Series, Instron) with calibrated 500 N load cell (Figure 3.1), was used to measure the texture of kernels in the sweetcorn samples tested for the purposes of the experiment described in the Chapter 4. The Kramer Shear Cell (34.7 x 43 x 50 mm) had 5 blades of 3 mm thickness. The machine was programmed (Bluehill 2, version 2.11, Instron) such that the crosshead speed was 10 mm/min. The maximum compressive load was recorded. Textural tests for each cob (n=210) were done on sample kernels (10 g), which were evenly distributed on the bottom within the Kramer Shear Cell.

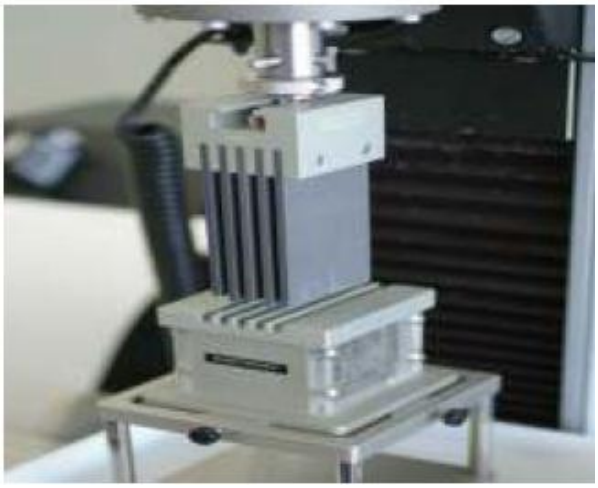

Figure 4: Instron Testing machine-Kramer Shear Cell.

Penetration probe

A penetration probe was used to evaluate firmness of the sweetcorn cobs examined (Instron, 5542, USA). In more detail, maximum compressive load was measured by using a uniaxial testing machine (Single Column System 5542, Instron, MA., USA) to determine the texture of the kernels. A photo of this device is displayed in Figure 3.2. The flat head penetration probe was of 2 mm diameter and 12 kernels of each cob were penetrated until a depth of 5 mm was reached. In the experiments where sweetcorn cobs were cooked in the microwave (Chapter 7 and 8), individual kernels were penetrated until a depth of 8 mm was reached. The machine was programmed (Bluehill 2, version 2.11, Instron) such that the crosshead speed was 20 mm/min.

Figure 5: Penetration probe.

Sugars

Several methodologies have been reported for the analysis of sugars in fruits and vegetables and can be categorised as refractometric, chromatographic or enzymatic assays. HPLC is usually the preferable method for the identification and quantification of individual sugars in fruits and vegetables (Potus *et al.*, 1994).

Extraction method

For the purposes of the current work sucrose, glucose and fructose were extracted from the kernels of sweetcorn cobs according to Terry *et al.*, (2007b). Approximately 20 g of fresh sweetcorn sample were freeze dried in an Edwards Modulyo freeze drier (W. Sussex, HYDERABAD). Then freeze-dried sweetcorn kernel powder (150 mg/sample) was mixed well with 3 mL of 62.5:37.5 (v/v) HPLC grade aqueous methanol

solution and vortexed (Vortex Genie 2, Scientific Industries, NY) to mix thoroughly. The extracts that were contained in 7 mL polystyrene bijou vials (Sterilin, Staffs., HYDERABAD) and were then placed in a shaking water bath at 55°C for 15 min. The vials were removed from the water bath every 5 min and vortexed in order to avoid formation of layers. Sugar extracts were then filtered though 0.2 m syringe filters (Millipore Corp., MA, USA) and diluted with HPLC grade water (1:10) prior to analysis.

Quantification of sugars

Quantification of identified sugars

Sugar extracts (20 µL) were injected into a Rezex RCM monosaccharide Ca+ (8%) column of 300 x 7.8 mm diameter and particle size 8 µm (00H-0130-K0, Phenomenex, CA.), with a carbo Ca2+ guard column of 4 x 3mm diameter (AJO-4493, Phenomenex) and quantified using an HPLC system (1200 series, Agilent Technologies, Berks., HYDERABAD). The mobile phase was HPLC grade water at a flow rate of 0.6 mL/min and the temperature of the column was held at 80°C using a temperature controlled column compartment (G1316A, Agilent). Eluted carbohydrates were monitored by a refractive index detector (RID, G1362A, Agilent) connected to the HPLC system, which included a cooled autosampler (G1330B, Agilent) set at 4°C. The presence and quantity of sucrose, glucose and fructose were calculated by comparison of sample peak area to calibration standards (Sigma, Dorset, HYDERABAD), ranging in concentration from 0.025 to 2.5 mg/mL, using ChemStation software (Rev. B.02.01, Agilent). Retention times were 9.801, 11.644 and 14.434 min for sucrose, glucose and fructose, respectively (Figure 3.3). Limits of detection were 42.84, 18.48 and 5.55 mg/mL for sucrose, glucose and fructose respectively. Sweetness was calculated by using the following coefficients: fructose (1.2), sucrose (1) and glucose (0.64) (Kader, 2008).

In some chromatograms, such as in Figure 3.3, other peaks apart from the three known were apparent, indicating the presence of other sugars apart from sucrose, glucose and fructose. Considering previous works that had found maltose (Ferguson *et al.*, 1979) and raffinose (Baird *et al.*, 1996) in sweetcorn kernels, d-maltosemonohydrate (Sigma Aldrich, HYDERABAD) and d-raffinose-pentahydrate standards were run to check any possible matches. Results showed that neither maltose nor raffinose, were identified in the samples tested.

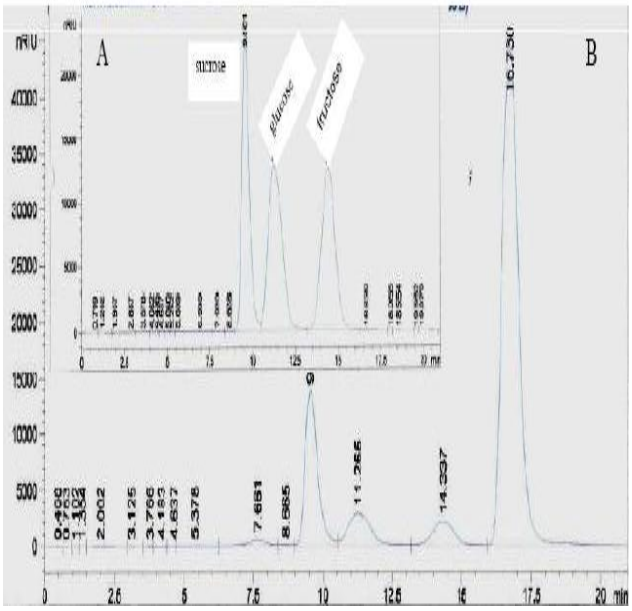

Figure 6: Representative chromatogram of eluted sucrose, glucose and fructose in standards (A) and in sweetcorn extracts (B).

Alternative method for quantification of sugars in sweetcorn

Extracts (n=20) prepared from samples examined for the purposes of the experiment described in Chapter 4, were also analysed as described by Chope *et al.* (2007). In particular, an HPLC system with a P580 pump and Gina 50 autosampler (Dionex, CA) were used; in order to compare the results with the device that was finally used. This comparison would allow a validation of the results and in addition would contribute to a final conclusion of whether ELSD (evaporative light scattering detector) or RID was more appropriate for the detection and quantification of sucrose, glucose and fructose. To detect the eluted carbohydrates in this case; an ELSD (2420, Waters, MA, USA) was used. An UCI-50 universal chromatography interface was used to connect the detector to the system. The column temperature was set at 75 °C using a Dionex STH column thermostat. All the other conditions were identical to the method. The presence and quantity of the sugars of interest were calculated as described before using Chromeleon, version 4.6 software. The comparison between the two different methods of HPLC analysis of individual sugars indicated that the results obtained when the refractive index detector was used, did not differ significantly from the results obtained when the light scattering detector was used indicating that both detectors are equally suitable for sugar analysis for sweetcorn.

Total soluble solids

Among several analytical techniques targeting sugars, such as colorimetric and chromatographic techniques which are either expensive and/or time-consuming, refractometric techniques are occasionally the preferred methods for sweetness determination. Refractometric techniques offer simple and fast determination of sugar

content in °Brix, without preparation steps but are not always well correlated with sugar content (Gine Bordonaba, 2010). For the purpose of the experiment, total soluble solids (TSS) content in the water remaining in the microwavable bowl after cooking, was measured by a digital refractometer (PR 301α, Atago Ltd., Japan).

Total starch

Starch components (amylase and amylopectine) can generally be measured by Near Infrared Spectroscopy, enzymatic methods and high-performance size-exclusion chromatography, involving polarimetric and acid hydrolysis or enzymic procedures. However, these methods are sometimes problematic, as they can be very complicated, expensive or time-consuming (McCleary *et al*., 1994; Stawski, 2008). Starch gelatinization, hydrolysis of dextrines to glucose, glucose determination, starch liquefaction and dextrinisation and pre-treatment steps are varied in enzymic procedures (Knudsen, 1997). Nowadays, treatment with thermostable α-amylase during or after starch gelatinisation is often used (McCleary *et al*., 1994).

Based on the conclusions of these works, Megazyme produced a total starch assay kit which incorporated α-amylases which are active and stable at even lower pH conditions than in the past. In particular, the advantage of this method which is commonly known as amyloglucosidase/ α-amylase method; is that due to incubation steps of thermostable α-amylases and amyloglucosidases at the same pH (5), the production of maltulose, which is not easily hydrolysed by amyloglucosidase and α- amylase, is minimised (McCleary *et al.*, 2009). The method suggested by Megazyme, was later adopted by the Association of Analytical Communities (Method 996.11) and the American Association of Clinical Chemistry (Method 76.13). The same method was also used to determine the total starch content in sweetcorn samples, for the purposes of the current work.

The principle is based on production of maltodextrins after hydrolysis of starch due to presence of thermostable α-amylase, at pH 5 and 100 °C. Maltodextrins are then hydrolysed quantitatively to D-glucose due to the presence of amyloglucosidase (AMG). D-glucose is then oxidised to D-gluconate. In that reaction, each mole of hydrogen peroxide produced, results in a colour change which is then measured using a spectrophotometer. The detection limit of this assay is 1.8 mg of starch/L or 2.0 mg of D-glucose.

The extraction method used for the determination of starch in the sweetcorn cobs was carried out as follows. Samples of ground freeze-dried sweetcorn powder (100 mg) were incubated with 5 mL of 80% (v/v) aqueous ethanol at 80 °C for 5 min. After vortexing the solution (Vortex Genie 2, Scientific Industries, NY); another 5 mL of 80% (v/v) aqueous ethanol was added. Samples were then centrifuged for 10 min at 1,800 g. The supernatants were discarded and the pellets were re-suspended in 10 mL of 80% (v/v) aqueous ethanol and mixed again in a vortex stirrer. Samples were then centrifuged as before and the supernatant once more discarded. Immediately, 3 mL of thermostable α-amylase diluted 1:30 in 100 mM sodium acetate buffer (pH 5), was added. Incubation of the samples for 6 min in a boiling water bath followed. Samples were stirred after 2, 4 and 6 min in order to ensure homogeneity of the slurry. After the addition of 0.1 mL of amyloglucosidase, contents of the tubes were mixed and incubated at 50°C for 30 min. The contents of the tubes were then adjusted to 100 mL with distilled water and mixed thoroughly. Aliquots of these solutions were centrifuged at 1,800 g for 10 min. Duplicate filtered aliquots (0.1 mL) were then transferred to glass tubes; and after the addition of 3 mL of Glucose Determination Reagent (Gopod Reagent: Glucose-oxidase-peroxidase Reagent), incubation at 50°C for 20 min followed. In more detail, Gopod Reagent used as supplied by Megazyme, consisted of peroxidase (>650 U); glucose oxidase (>12000 U); 80 mg 4-aminoantipyrene and 1M potassium phosphate buffer mixed with 0.22M p-hydroxybenzoic acid and 0.02% (w/w) diluted with distilled water. D-glucose control samples consisted of 1 mg/mL D-glucose standard solutions and 3 mL of Gopod Reagent. The absorbance of each sample and D52 glucose control were read at 510 nm against reagent blank. Results were expressed as g/100 g (%) of kernels on dry weight basis. Generally avoidance of interference in this assay can be validated by completion of D-glucose at approximately 5 min which is the time specified by the assay. Thus, D-glucose was added (500 µg/mL) and indeed a significant increase in the absorbance was observed.

The method used to examine the starch content, was proved to be a valid method for the analysis of starch content in sweetcorn samples.

Organic acids and L-ascorbic acid

Methods tested for identification of organic acids and L-ascorbic acid

In addition to sugars, organic acids are very important components in sweetcorn which also have the ability to influence pH and the production of off-flavours in fruits and vegetables (Zagory and Kader, 1989). The target of the current work was the analysis of L-ascorbic acid which is a water soluble antioxidant; plentiful in plants. Titration and spectophotometry are two frequently used methods for the analysis of organic acids, including vitamin C (Salkic *et al.*, 2007). Amperometry and chromatography can also be used (Korany *et al.*, 2010). However, it should also be noted that analytical methods for organic acid analysis, are not always reliable due to effect of compounds being oxidised (Arya *et al*., 2000).

For the determination of ascorbic acid, a method for the extraction of non-volatile organic acids in strawberries described by Terry *et al.* (2007b); was examined as a possible method for the analysis of vitamin C in

sweetcorn samples. In more detail, different quantities of lyophilised sweetcorn samples (100, 150, 200, 150, 300 mg), were used and compared for the preparation of ascorbic acid extracts. Each sample was then mixed with 3 mL of HPLC grade water and kept at room temperature (25°C) for 10 min. After, extracts (n=3) were filtered through 0.2 µm syringe filters ready for analysis. It was attempted to determine L-ascorbic acid by an HPLC system as described in sugars section, equipped with an Agilent DAD G1315B/G1365B photodiode array with multiple wavelength detector. Sweetcorn extracts (20 µl) were injected into an Alltech Prevail Organic acid column of 150 mm x 4.6 mm diameter. Particle size of the column was 5 µm (Alltech, CA; Part no. 88645) and the guard column used was an Alltech Prevail Organic Acid guard column of 7.5 mm x 4.6 mm diameter (Alltech, CA; Part no. 96429). Aqueous grade and degassed metaphosphoric acid (Fisher Scientific) 0.2% (w/v) was used as a mobile phase with 1 mL/min flow and under isocratic conditions. The mobile phase was adjusted to pH 2.5 using phosphoric acid. The temperature of the column and the autosampler was set up at 35 and 4°C, respectively.

The eluted organic acids were detected at 210 nm and the presence of organic acids identified by comparing their peak areas with standards, using ChemStation rev. B.02.01 software.

A representative chromatogram from the analysis of organic acids using 100 mg of sweetcorn kernels is shown in Figure 3.4. Peaks were better separated when 100 mg of sample was extracted for organic acid quantification, rather than from greater amounts (see Appendix A). Known concentrations of succinic, malic, citric and aketoglutaric acid reported by Masuda *et al.* (1997) were also analysed in an effort to identify other organic acids from the sweetcorn kernels, but results indicated that none of the pure compounds analysed were present in the samples tested. Therefore, this procedure was not chosen as the preferred analytical method for L-ascorbic acid in the sweetcorn cobs examined.

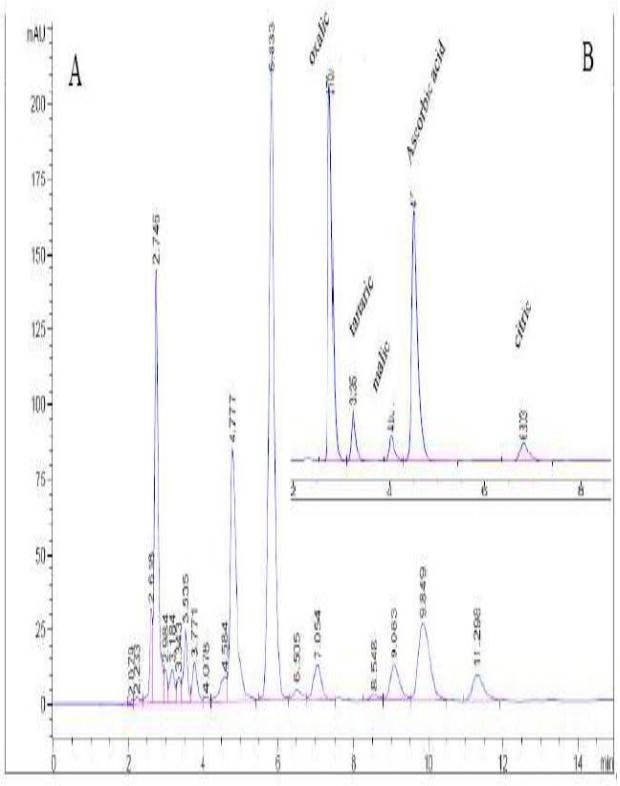

Figure 7: Representative chromatogram obtained from the analysis of (A) organic acid standards and organic acids (B) from sweetcorn extracts.

L-ascorbic acid assay: principles of extraction and optimisation of the analysis method

Samples were tested for the identification and quantification of L-ascorbic acid by using the L-ascorbic acid assay kit bought from Megazyme, with some modifications. The principle of the assay is that a formazan compound is formed when 3-4,5-dimethylthiazolyl-2-2,5-diphenyltetrazolium bromide (MTT) is reduced in the presence of reducing substances such as L-ascorbic acid and the electron carrier PMS (phenazynemethosulphate) at pH 3.5. The difference in absorbance at 578 nm, between sample and sample blank (sample that other reducing substances have been measured when L-ascorbic acid has been removed using ascorbic acid oxidase) indicates the quantity of the L-ascorbic acid.

To prepare the samples, 20 g of sweetcorn were mixed well with 50 mL of 100mM potassium phosphate buffer (pH 3.5). Samples were also tested for their L-ascorbic acid concentration starting with different amounts of fresh sample (25 and 30 g), different volumes of potassium phosphate buffer and different molarities of the buffer (1 M), but in each case, results were inferior or not significantly different compared with the method used.

The pH of the slurry was adjusted to 3.5 with 2 M HCl. The mixture was then adjusted to 300 mL with distilled water and after re-adjusting to pH 3.5, was mixed and

filtered. Briefly, 1 mL of each sample, with 0.62 mL of distilled water (warmed to 37°C) and 0.5 mL of phosphate/citrate buffer and a sample blank for each sample were mixed and incubated for 3 min at 37°C. When less than 1 mL of sample was used in the combination above, concentration of L-ascorbic acid was below the detection limit of the assay. The sample blank also had the same final volume of 2.52 mL with the addition of 0.02 mL ascorbic acid oxidase. After incubation, samples were incubated again for 3 min in the same conditions with the addition of MTT/acetate buffer and the absorbencies of both samples and sample blanks were read. The addition of 0.2 mL PMS solution in a light-protected environment at 37°C, initiated the reaction. After 8 min, absorbance was measured again. The timing of the last measurement was determined after observing no significant difference in the concentration of L-ascorbic acid thereafter. Furthermore, standard addition at the end of the reactions resulted in a significant increase in the absorbance showing that the reaction was completed. The detection limit of the assay was 0.0175 mg/L. Finally, the method was tested with the same material but using freeze-dried sweetcorn powder as the initial sample. In this case, concentrations of L-ascorbic acid were not only found to be significantly lower when dried samples were used; but readings were also below detection limits Results from the comparison of initial fresh versus dried samples. To test the end of the reaction, sweetcorn samples were spiked with a known amount of L-ascorbic acid standard. The reproducibility of the procedure was investigated between three separate extractions (n=9) of a tissue sample of a randomly chosen sweetcorn cob and also between three aliquots obtained from the same extraction. The variability within samples and sample replicates and the variability within extracts were estimated by using means and standard deviations. In particular, results indicate that reproducibility of the method is high due to the low variation of the extraction procedure and the sample replicates.

Table 5: Reproducibility results: L-ascorbic acid in aliquots of the same extract and different extracts of the same sample tissue.

Cooking duration	Number	Same extract	Different extract – Same tissue sample
0min (fresh)	3	3.51 ± 0.33	3.82 ± 0.40
Cooked for 5min	3	2.40 ± 0.27	2.35 ± 0.30
Cooked for 10min	3	2.72 ± 0.22	2.44 ± 0.50

* Standard deviation and mean concentrations are expressed as mg/100g of Lascorbic acid in fresh sweetcorn kernels.

The method used to quantify L-ascorbic acid concentrations in the sweetcorn samples using HPLC as previously described by other researchers was not successful. However, the existence of other well separated, unidentified organic acids was demonstrated. The Megazyme assay kit for the measurement of L-ascorbic acid was suitable for sweetcorn samples. The method was specified and optimised for sweetcorn kernels. Asami et al. (2003) suggested that frozen corn samples were suitable for HPLC analysis of L-ascorbic acid, while freeze-dried initial samples were not appropriate. The method used for the current work reconfirmed this conclusion.

Phenolic compounds

Total phenolics

Several methods have been developed to determine phenolic compounds in fruits and vegetables, with HPLC and spectrophotometric methods having several advantages and disadvantages. In more detail, HPLC analysis of phenolic compounds, while accurate, is complicated, time-consuming, has high cost and like any HPLC assay relies on available standards in identifying individual compounds. On the other hand, spectrophotometric methods are simple, less time-consuming and when are performed under consistent conditions can offer information on total phenolic content. In the current study determination of phenolic compounds was attempted by HPLC analysis, but as only ferulic acid was identified; spectrophotometric measurement of total phenolics by the Folin-Ciocalteu assay was employed (Riad, 2004; Stratil et al., 2006).

For the extraction and quantification of total phenolics, 150 mg of freeze-dried ground powder per sample of sweetcorn kernels was used (Terry et al., 2007b). The samples were dissolved with 3 mL of 80% aqueous ethanol (v/v) and after stirring in a vortex device were placed in a water bath (HAAKE SWB 20, Thermo Scientific, Germany) set at 70°C, for 2 h. Mixing of the samples took place every 20 min during the 2 h incubation. After solutions were filtered samples were ready for analysis.

The Folin-Ciocalteu method according to Singleton and Rossi (1965) was used to measure total phenolics. The method is based on the ability of phenolic compounds to cause reduction of a phosphowolframate-phosphomolibdate complex forming molybdenum-tungsten blue reaction products. However, according to Stratil et al., (2006) saccharides and ascorbic acid may interfere with this method. In detail, for the analysis of total phenolics, 20 µL of each filtrate was combined with 3.2 mL of distilled water and 200 µL of Folin-Ciocalteu reagent. Solutions were then mixed with 600 µL of 1.9M sodium carbonate. Final solutions were then incubated at room temperature in the dark for 2 h. A Camspec M501 (Camspec Ltd., Cambs, HYDERABAD) UV/vis spectrophotometer, was used to measure the absorbance at a wavelength of 760 nm. Measurements were

calibrated against gallic acid standards (25, 50, 100, 150, 250 and 500 mg/L) and expressed as mg of gallic acid equivalents (GAE) per gram of kernels. The method used for the analysis of total phenolics might not be very accurate as it is susceptible to interference from sugars, however it can be considered adequate for the purpose of this study which mainly focused on the comparison of fresh versus cooked cobs.

Extraction of ferulic acid

Sweetcorn samples were extracted for the analysis of ferulic acid according to the acid hydrolysis method described by Ancos et al. (2000) with minor modifications. In particular, 300 mg of lyophilised sweetcorn sample was mixed with 5 mL of 60% (v/v) MeOH that contained 125 µg butylated hydroxytoluene (BHT). Then, 1 mL of 6 M HCl was added to the extract. Extracts were then placed in a water-bath at 90°C for 2h and were mixed every 30 min. Undiluted filtered extracts (filtration through 0.2 mm filters) were ready for analysis.

Quantification of ferulic acid

Extracts (10 µL) were injected into an Agilent Zorbax Eclipse Column (XDBC18, 4.6 mm x 150 mm of 5 m paricle size) coupled with a 1.0 mm x 17mm guard column (Agilent Zorbax Exlipse XDB). The mobile phase used consisted of two filtered and degassed solvents; where solvent A was 8% acetic acid in 2 mM sodium acetate and solvent B was 100% acetonitrile. The oven temperature was set at 30°C and the UV detector at 280 nm. The flow rate was constantly set at 1 mL/min. The gradient involved % solvent B as follows: 0-11%, 14 min; 14-60%, 23 min and the post-run had 4 min duration at 0% of solvent B. Ferulic acid was calculated with linear regression analysis using external standards and the results were expressed as µg of ferulic acid per g of dry and fresh sweetcorn kernels.

Known concentrations of phenolic compounds were analysed to compare retention times and spectra with the peaks of the chromatograms. The compounds chosen for that purpose were flavonols (quercetin, myricetin and kaempeferol) and phenolic acids (ferulic acid, vanillic, p-coumaric and protocatechuic). These compounds were chosen according to previously reported biochemical profiling on sweetcorn (Dewanto et al., 2002; Trombino et al., 2004 and Pedreschi and Cisneros- Zevallos, 2006). Except for the ferulic acid, analyses could not prove the existence of the compounds mentioned above in sweetcorn. There was no further effort to find alternative method of analysis that would allow the identification and quantification of these compounds as their concentrations were expected to be insignificant in comparison with ferulic acid. The method used for the analysis of ferulic acid, revealed other compounds that were unidentified (Figure 3.5). However, identification and quantification of ferulic acid (Donetti, unpublished) was adequate for the aims of this work as it is the most abundant ferulic acid found in corn (Dewanto et al., 2002).

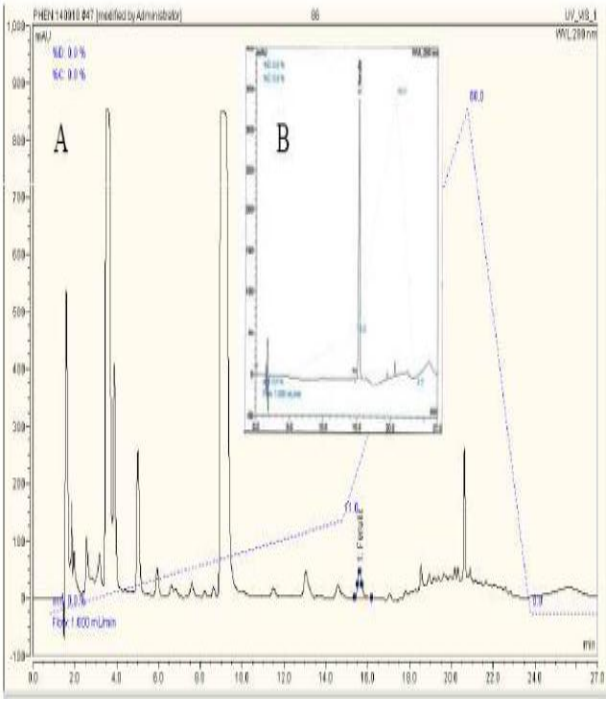

Figure 8: Chromatogram of ferulic acid from sweetcorn extracts (A) and ferulic standard (B).

Carotenoids

Extraction of carotenoids

From the carotenoids found in sweetcorn, lutein and zeaxanthin are of particular interest due to their association with eye health (Ribaya-Mercado and Blumberg, 2004). Hart and Scott (1995) published a paper, reporting inaccuracies and variation occurring in the quantification of carotenoids as a result of several factors including light, heat and physicochemical reactions such as degradation. They also reported that the use of temperature controlled system, appropriate column and solvent are essential for ensuring the successful isolation and analysis of carotenoids. Solvent modifiers are very important when added to the mobile phase. In particular, they reported that addition of triethylamine (TEA) in the mobile phase can improve recovery of carotenoids from the column and reduce retention times.

Recently, Fanning *et al.* (2010) suggested that saponification and heating in the samples they examined had no effect on carotenoid content. However, earlier work by Howe and Tanumihardjo (2006), which compared several methods for the extraction of carotenoids, suggested that for the extraction of carotenoids from corn samples, heating and saponification was necessary for reliable results and good extraction efficiencies.

Considering that Fanning *et al.,* did not report unfavourable effects of heating and saponification while

other researchers reported beneficial effects, these steps were not avoided in the current work. Thus, and also considering that the purpose in this study was to evaluate the interaction of cooking time, format, storage duration and temperature on carotenoid content, a combination of methods was used as described below.

A combination of the extraction methods described by Kurilich and Juvik, (1999b); Fanning *et al.*, (2010); and Burt *et al.*, (2010), was used for the aims of the experiment described in Chapter 8. Briefly, solubilisation of the sample with ethanol and saponification with KOH under heat was the basis of the extraction procedure. The addition of KOH, was performed in order to hydrolise potential esterified xanthophylls (Schlatterer *et al.*, 2006). The extraction was then performed with hexane.

The extraction procedure used was as follows: 500 mg of freeze-dried samples were mixed with 6 mL of analytical grade ethanol containing 0.1 % butylated hydroxytoluene (BHT) and 800 µL of *β*–Apo-8'-carotenal (7.2 mg/L in isopropanol). Instead of 800 µL of *β*–Apo-8'-carotenal, 250 µL of this internal standard was tested as described by Fanning (2010). However, resolution of the peaks was better when 800 µL was used. Extracts were then placed in a water bath set up at 85°C for 5 min. Potassium hydroxide (180 µl) was then added, vortexed for 20sec and then samples were placed back into the water bath for 10 min. During the 10 min of saponification, samples were vortexed one more time and after the end of this procedure, samples were placed immediately in an ice bath. The addition of 3 mL of hexane followed after the addition of 3 mL of distilled water. Samples were then extracted three times with 3 mL of hexane. A centrifuge (Heraeus, labofuge, 400R, Thermo Scientific) was used to separate layers (1200 g for 10 min, at 4°C) and the upper layer removed and placed in a new test tube. The hexane fractions were then combined and dried in a rotary evaporator (Buchi Rotovapor, Buchi Labortechnic AG, Flawil, Switzerland) under vacuum at 30°C.

Reconstitution of samples occurred using 2 mL of methanol/dichloromethane mixture (50:50 v/v) containing 0.1 % BHT. Alternatively, 5 samples were reconstituted with 2 mL of the same solvent as used for the mobile phase A, yet no significant difference was observed. The extraction procedure was performed under fluorescent light. Filtered samples were then analysed immediately.

Analysis and qualification of carotenoids

Several HPLC methods have been used for the analysis of carotenoids in sweetcorn (Weber, 1987; Kurilich and Juvik, 1999a). In the present work, carotenoids were determined by an HPLC system, equipped with an Agilent DAD photodiode array with a multiple wavelength detector (G1315B/G1365B). Sweetcorn extracts (20 µl) were injected into a Zorbax Eclipse Column Agilent Zorbax Eclipse Column (XDBC18, 4.6 mm x 150 mm of 5 m particle size) and the guard column used was an Agilent Zorbax Exlipse XDB coupled, of a 1.0 mm x 17 mm diameter (Kalogirou, unpublished). Alternatively, the same column of different length (XDB-C18, 4.6 mm x 250 mm of 5 m particle size) was used but no significant changes in the concentration of carotenoids were observed.

The binary mobile phase that was used at a flow rate of 1 mL/min, consisted of ethyl acetate containing 0.01% (w/v) BHT and 0.1% (v/v) triethylamine (phase A), while phase B consisted of 90% ACN in water. The temperature of the column was set at 30°C and the autosampler at 4°C. The eluted carotenoids organic acids detected at *ca.* 453 nm (Kopsell *et al.*, 2009). Retention times and absorption spectra, viewed using ChemStation rev. B.02.01 software, were used to identify lutein, zeaxanthin, *β*-carotene and *β*-cryptoxanthin. Concentrations of carotenoids were expressed as µg/g on fresh and dry weight basis.

Carotenoids were identified with spike tests and against retention times and absorption spectra of known standards. Lutein standards were made up in absolute ethanol and its absorbance measured using a M501 UV/Vis spectrophotometer (Camspec Ltd., Cambs., HYDERABAD) at 445 nm, while zeaxanthin, *β*-carotene and *β*-cryptoxanthin were made up in hexane and measured at 450 nm (Wrolstad *et al.*, 2004).

The concentration of the standard solutions was measured according to the same author, using the following formula: Concentration = (absorbance x 10000)/A1%, where A1% (the spectral absorption coefficient) is 2550, 2480, 2592 and 2460, for lutein, zeaxanthin, *β*-carotene and *β*-cryptoxanthin, respectively. The peak purities were then determined by HPLC analysis. The concentration that was determined spectrophotometrically multiplied by the % peak area as measured by HPLC, was the actual concentration of the standard solutions. Standards were prepared at five concentrations, dried down under a stream of nitrogen gas and then reconstituted as samples. The range of the concentrations was 0.016-150 µg/mL.

The gradient used was as shown in Figure 3.6.

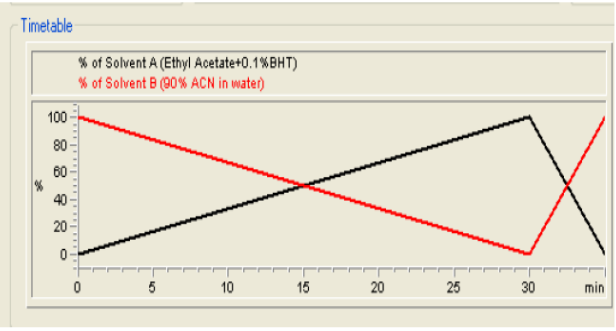

Figure 9: Gradient of solvents A and B as used for the extraction of carotenoids in Sweetcorn.

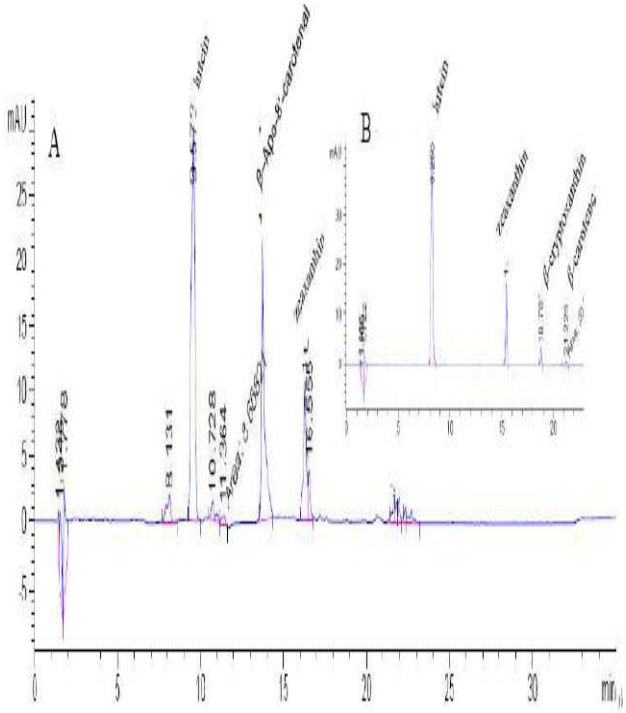

Figure 10: Representative chromatogram obtained from the analyses of carotenoids (A) in samples and (B) in standard solutions.

Concentration of samples was calculated by comparison of their peak areas and the peak areas of the standards. Representative chromatograms of both samples and standards are shown in Figure 3.7. Coefficients of determination (R2>99%) obtained from the known standards were used to determine the actual concentrations of the samples tested.

Table 6: Reproducibility of results regarding aliquots from the same extraction (n=3) and among different extracts of the same sample (n=3), expressed as µg/g on dry weight basis.

Carotenoids	Number	Same extract	Different extract – Same tissue sample
Lutein	3	19.7696 ± 0.1568	19.1201 ± 0.4320
Zeaxanthin	3	0.2327 ± 0.0090	0.2139 ± 0.021

* Standard deviation and mean concentrations are expressed as mg of carotenoids per Kg of fresh sweetcorn kernels

Three randomly chosen samples, were used to test the accuracy and the reproducibility of the method used between extractions of the same sample. Furthermore, three aliquots of the same extract were analysed to estimate the variability within extracts. Reproducibility concerning the same factors was estimated by means and standard deviations.

The reproducibility of the extraction procedure was considered high, since, as shown in the Table 3.2, the variation was low. Recovery values (%) were 95.9 for lutein and 93.12 for zeaxanthin indicating good accuracy of this method. The recovery value for β-cryptoxanthin is greater than 100% but has a standard deviation of 11%.

These arithmetical data might mean that in case that recovery value is greater than 100% there is a possibility of interference of other substances. However, due to the comparison of the absorption spectra of β-cryptoxanthin of the sample with this of the standards, this case is eliminated. Thus, practically it can be said that the recovery value for β-cryptoxanthin was in a range of 99-100%. Limits of detection were 3.2602, 0.0400, 0.0286, 0.0036 and limits of quantification 10.8674, 0.0954, 0.1336 and 0.012µg/g; for lutein, zeaxanthin, *β*-cryptoxanthin and *β*-carotene respectively. However, *β*-cryptoxanthin was not present in the samples and examined peaks of *β*-carotene were not always well separated and occasionally below limits of quantification and therefore were not included in the calculation of total carotenoids.

The extraction and analysis of carotenoids proved to be an effective and accurate method for the analysis of the xanthophylls lutein and zeaxanthin for the sweetcorn cobs tested. Better chromatograms were achieved when 800 µL of internal standard was used as Fanning *et al.* (2010) suggested; compared to 250 µL that Howe and Tanumihardjo, (2006) used. The use of the longer C-18 Zorbax Eclipse Column (250 mm) and the reconstitution of the dried extracts with the solvent used for phase A of the HPLC analysis did not affect the results and therefore could be used alternatively. The method used for the analysis of carotenoids can be considered to be of good accuracy and reproducibility.

Total antioxidant activity

The FRAP (ferrous reducing antioxidant power) assay as described by Benzie and Strain (1996) and as modified by Terry *et al.* (2007a), was used to measure total antioxidant capacity. The assay is based on the reduction of a ferric-tripyridyltriazine complex, in the presence of antioxidants and measures electron-donating antioxidants.

An important reason, that it was FRAP that was chosen to assay the antioxidant activity of the sweetcorn samples examined, is that is a direct method for measuring antioxidants using reductants as antioxidants without pretreatment steps. The use of antioxidants as reductants can also be the disadvantage of the FRAP assay. On the other hand in indirect methods as listed in subchapter 2.4.6, the reactive species used may significantly affect the results as they might measure antioxidants that react

against the radicals used (Halvorsen *et al*., 2002; Nagah and Seal, 2005).

For extraction, 150 mg of freeze-dried powder was mixed with methanol: H2O: HCl (70:29.5:0.5%, v/v) solution placed in a waterbath (HAAKE SWB 20, Thermo Scientific, Germany) at 35°C for 1.5 h. Samples were agitated every 15 min. Before analysis, samples were filtered through 0.2 µm Millex- GV syringe driven filters (Millipore Corporation, MA, USA). For the analysis of Total Antioxidant Capacity, 50 µL aliquots of undiluted sample extract were mixed with 3.6 mL of FRAP working solution which was freshly prepared each day. The FRAP working solution consisted of 5 mL of 10 mM 2,4,6-tripyridyl-2-triazine in 40 mM HCl and 5 mL of 10 mM FeCl3 in 50 mL of 100 mM acetate buffer. After the addition of the FRAP solution, the mixture was incubated at 37°C for 10 min. The absorbance was then measured using a spectrophotometer (in a Camspec M501 UV/vis) at 593 nm. Measurements were read against Fe2+ (FeSO4 7H2O) standards of 0, 0.2, 0.4, 0.8, 1, 1.2, 1.6 and 2 mM concentration and expressed as the concentration of antioxidants that have ferric reducing ability. FRAP assay has constant and linear stoichiometric factors (Halvorsen *et al*., 2002) and when performed was proved to be simple and rapid. Therefore FRAP assay can be a good, direct method for the measurement of antioxidant activity.

Statistical analysis

All statistical analyses were carried out using Genstat (Version 10.1, VSN International Ltd., Herts., HYDERABAD). Least significant differences (l.s.d.) between factors sources of variation and their interaction were analysed through analysis of variance (ANOVA). Complex experimental analyses such as those required for the experiments described in Chapter 6 and 8, were adjusted, under advice from Pat Bellamy [Head of Statistics at] to have a maximum 3-way interactions. The analysis of variance was helpful in providing information about the effect and interaction of temperature, cultivar, type of tissue, position in tissue, format of the cobs, cooking and storage duration. All statistical analyses are reported in Appendix B. Unless otherwise stated significant differences were $P < 0.05$. Standard errors are presented, where l.s.d. was not appropriate.

Table 7: Effects of storage time and/or CA in moisture content, sugars and texture properties.

Cultivars	°C	Days of storage	CA regimes	M.C.[1] (%)	Sugars	Textural properties
Prime time	5	14	2%O_2-10% CO_2	n/a	↓ over time (in CA higher levels)	n/a
Prime time	5	10	2%O2-(0, 15, 25% CO2)	n/a	Maintained for 2 weeks in CA	n/a
Prime time	5	14	2%O2-(0, 15, 25% CO2)	n/a	Maintained for 2 weeks in CA	n/a
Prime time	1 & 5	10	2%O2-(10, 20% CO2)	n/a	↓ with time and Temp. but maintained in higher levels than in air storage	n/a
How sweet it is	6	5		81.4±1.4	↓	n/a
Sucro	1,4,7	7		n/a	No s. d	n/a
Unknown	10,20	16	n/a	↓ with time and Temp.3	-	↑ with time and Temp. (Rupture energy)

CONCLUSION

The objectives of the project were presented in Conclusions of the project are briefly summarised below:

- *To contribute to method development, validation and optimisation of textural characteristics and extraction and quantification of target analytes related to major quality parameters.* Texture was assessed by measuring maximum compressive load values and can be considered as an adequate and appropriate method for the evaluation of kernel firmness. Total sugars were identified and quantified by optimised and validated HPLC procedures that are considered to be valid especially in comparison to the commonly used measurements of total soluble solids. In addition, high sugar values indicate that the method used, might be better than existing published HPLC methods on maize. Estimation of starch values, matched with the expected values according to the literature and therefore the method used can be considered suitable for starch quantification. The methods used for the quantification of total phenolic content and especially of total antioxidant activity were not sufficiently accurate. The methods used for the measurement of ferulic acid, L-ascorbic acid and xanthophylls were developed, optimised, validated and demonstrated as being suitable.
- *To investigate the influence of the genotype and of the origin of the cobs on texture and sugar content of sweetcorn cobs containing the sh2 gene*. Genotype and origin of the cobs had significant influence in the texture and sugar content of the sweetcorn cobs examined.

- *To determine potential differences in the texture and the concentrations of sucrose, glucose and fructose in cobs of sweetcorn cultivars (cvs.) stored at different temperatures.* Sucrose was the predominant sugar in all samples tested regardless of storage temperature (Chapter 5-8). However, lower storage temperatures retain better sugar content of cobs tested. Thus, the best storage temperature tested in this thesis was 2°C in terms of preservation of the quality attributes examined.
- *To determine differences in texture-related characteristics of sweetcorn cvs. Of different format; such as sweetcorn cobs covered with husks vs. cobs without husks.* It was indicated that naked cobs were firmer than window stripped cobs.
- *To investigate the spatial, textural and sugar content changes throughout sweetcorn cobs.* Sugar profile of the tissues studied was different. Sugar content of kernels was lower than of shank and core. Spatially (bottom, middle and top of the cobs) there were not any significant differences in the sugar profile and content of kernels. In terms of texture, kernels located in the central parts of the cobs were firmer.
- *To elucidate the colour and the nutritional and textural changes occurring under cooking conditions when stored at different temperatures and in different cob formats (with or without husks).* Generally, the antioxidant compounds analysed in the sweetcorn kernels tested were negatively affected by cooking but not according to cooking time.

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