

ATMOSPHERE MODIFICATION TO CONTROL QUALITY DETERIORATION
DURING STORAGE OF FRESH SWEETCORN COBS AND FRESH-CUT KERNELS

By

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by

Gamal Riad

This dissertation is dedicated to those in the world who care: my Mother, Hanaa Attia;
my father, Samir Riad; my brother, Ahmad Samir; and all of my friends.

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Controlled atmosphere (CA) storage and modified atmosphere packaging (MAP) are beneficial tools for extending the postharvest life of fresh fruits and vegetables, but specific tolerance levels to gas composition must be determined in order to apply these techniques. Perforation-mediated modified atmosphere packaging (PM-MAP) for sweetcorn utilizing impermeable containers with a diffusion window was designed to establish 15, 20 or 25% CO₂ atmospheres at 1 and 10 °C. The desired CO₂ concentrations were obtained at 1 °C, but were about 3-5% lower than expected at 10 °C. It took about 5 d to reach the equilibrium atmospheres at 1 °C, and about 2 d at 10 °C. Sweetcorn cobs in CA tolerated 2% O₂ and up to 25% CO₂ alone for 2 weeks at 5 °C, but elevated respiration suggested that they may be damaged by the two gas levels in combination, although no significant ethanol or acetaldehyde production was detected in any CA or PM-MAP treatment.

The best atmosphere composition tested for maintaining sweetcorn quality was 2% O₂ plus 15% CO₂. The CA reduced sweetcorn respiration, maintained higher sugar concentrations, reduced loss of husk greenness, and improved silk and kernel appearance. This CA also maintained the highest concentration of dimethyl sulfide (DMS), the main characteristic aroma component in sweetcorn.

The potential for storing and handling fresh-cut sweetcorn kernels was also examined. Fresh-cut sweetcorn kernels are extremely perishable. Successful handling requires low storage temperature and optimum maturity stage. Quality was maintained for 10 d in air at 1 °C or in CA (2% O₂ plus 10% CO₂) at 5 °C, but brown kernel discoloration after cooking limited shelf life in air at 5 °C especially in the more mature kernels. The CA reduced fresh-cut sweetcorn respiration, inhibited sugar loss, and, most importantly, prevented after cooking browning. After cooking browning was not due to typical Maillard reaction (5-hydroxymethylfurfural was not present) nor due to changes in soluble phenolics. Higher aerobic microbe counts were associated with increased after cooking browning but not with a specific species. A water soluble brown pigment precursor was isolated from kernel juice but was not identified.

CHAPTER 1 INTRODUCTION

Sweetcorn (*Zea mays* L. *rugosa*) is widely used as a fresh or processed vegetable. It is sweeter than wild type (field) corn because it has a recessive mutant gene *sugary-1* (*su1*) that restricts the conversion of sugar into starch (Creech, 1968; Laughnan, 1953; Wann et al., 1971). However, this conversion still occurs, and continues after harvest and during storage, resulting in rapid quality loss (Doehlert et al., 1993). Since consumer surveys have shown that most consumers prefer sweetcorn with higher natural sweetness (Showalter and Miller, 1962), one of the areas of genetic improvement of sweetcorn has involved the selection of mutant strains that produce high sugar levels in the seed endosperm (Courter et al., 1988; Garwood et al., 1976; Laughnan, 1953; Showalter and Miller, 1962; Wann et al., 1971). The most successful of these mutants is *shrunk-2* (*sh2*), which blocks sucrose conversion to starch, and is found, alone or in combination with *su1*, in almost all modern commercial sweetcorn cultivars.

Controlled atmosphere (CA) and modified atmosphere (MA), i.e., elevating carbon dioxide (CO₂) levels and reducing oxygen (O₂) levels around stored vegetables after harvest, can be useful supplements to maintenance of optimum temperature and relative humidity in maintaining postharvest quality of fresh fruits and vegetables. The main effects of CA/MA are reduced respiration and ethylene production; and, consequently, delayed ripening or senescence, reduced weight loss, and prolonged shelf life (Kader et al., 1989; Weichmann, 1986). The elevated CO₂ used in CA/MA also competitively

inhibits ethylene action (Burg and Thimann, 1959) and inhibits postharvest diseases (El-Gorani and Sommer, 1981; Daniels et al., 1985).

Fresh sweetcorn is a perishable food product prone to rapid postharvest deterioration caused by kernel desiccation, loss of sweetness, husk discoloration, and development of decay. Keeping cobs in CA/MA with high CO₂ and/or low O₂ levels inhibits respiration; and, consequently, it reduces sugar loss and other metabolic reactions, and slows the growth of pathogens; CA/MA also decreases husk yellowing by inhibiting chlorophyll degradation.

Nowadays there is increased acceptance and demand for fresh-cut fruits and vegetables (sometimes called minimally processed or ready-to-eat produce) for many reasons such as their convenience, perceived high nutritional value, and freshness. The flourishing of the fresh-cut industry in the last decade encourages the development of new fresh-cut products and there is now greater feasibility of sweetcorn kernels being developed as a fresh-cut product, but work is needed to determine the limiting factors in storing and handling such a value-added product.

Study Objectives. The objectives of this work were as follows:

- Determine the specific tolerance levels of sweetcorn to low O₂ and/or to high CO₂ as an essential requirement to successfully apply controlled and/or modified atmosphere techniques in sweetcorn storage.
- Determine the interactive effects of O₂/CO₂ combinations and temperature on induction of anaerobic respiration in sweetcorn (using perforation-mediated MAP).
- Determine the effects of low O₂ and high CO₂ on levels of aroma volatiles and other quality factors in sweetcorn during storage.
- Introduce fresh-cut sweetcorn kernels and determine its feasibility and value as a fresh-cut product.

The last objective required investigating the following:

- The effect of sweetcorn maturity, storage temperature, and O₂/CO₂ levels on fresh-cut sweetcorn quality in order to determine the best postharvest treatments to reduce the occurrence of after-cooking browning of fresh-cut kernels.
- The cause of the browning of cooked fresh-cut kernels after storage, and testing the possibility of the Maillard reaction as its cause.

CHAPTER 2 LITERATURE REVIEW

Sweetcorn

The quality of fresh sweetcorn [*Zea mays* L. *rugosa* (the old name was *Zea mays* L. *saccharata*)] depends to a large extent on the kernel texture and flavor, which are directly related to the sugar and polysaccharide content of the endosperm (Culpepper and Magoon, 1927; Flora and Wiley, 19974a). Sweetness in sweetcorn is closely related to kernel sucrose content (Reyes et al., 1982), which is the primary sugar in developing kernels (Cobb and Hannah, 1981). Texture and eating quality of sweetcorn consists of several factors, including pericarp tenderness (Bailey and Bailey, 1938), levels of soluble sugars and water-soluble polysaccharides (WSP) in the endosperm (Culpepper and Magoon, 1927; Evensen and Boyer, 1986), and moisture content (Wann et al., 1971). On the other hand, flavor and aroma, which are not as easily defined as sweetness and texture, are most often associated with kernel content of many volatiles, but mainly dimethyl sulfide (DMS) (Flora and Wiley, 1974b; Wiley, 1985; Williams and Nelson, 1973; 1974). Therefore, a major goal has been to develop sweeter sweetcorn and to find postharvest treatments to delay the depletion of this high level of sugars.

The basic genetic difference between standard sweetcorn cultivars and starchy field corn is the presence of a recessive allele at the *sugary-1* (*su-1*) locus on chromosome 4 in sweetcorn. This recessive gene conditions an 8- to 10-fold increase in WSP, extreme starch reduction (about 50% or less than normal corn) and 2-fold increase in sugar over the normal corn (Creech, 1968; Laughnan, 1953; Wann et al., 1971). Doehlert et al.

(1993) stated that the differences between normal kernels and *su-1* kernels are that the *su-1* kernels accumulate less weight, retain kernel moisture longer, have a thinner pericarp, and contain altered storage protein.

Many other mutants have been introduced that improve the sugar content in sweetcorn, with one of the most important being the *shrunk-2* (*sh2*) gene on chromosome 3, which blocks the conversion of sucrose to WSP and starch, resulting in accumulation of sugar (Laughnan, 1953). Cultivars containing this *sh2* gene alone or in combination with the *su1* gene ('supersweet' sweetcorn) usually contain more than twice the sugar content of the standard *su1* sweetcorn (Courter et al., 1988; Showalter and Miller, 1962; Wann et al., 1971) and can retain higher sugar and moisture content for a longer time (Garwood et al., 1976); and consequently are preferred by consumers in taste tests (Evensen and Boyer, 1986; Showalter and Miller, 1962).

There are many other genetic mutations in sweetcorn that have been introduced with limited success commercially, such as the endosperm mutants *brittle-1* (*bt1*) and *brittle-2* (*bt2*), which produce a high sugar content with a relatively low starch content in kernels, and which are adapted mainly for tropical climates. Another mutation is the *sugary enhancer* (*se* or *se/su1*) gene, which acts as an independent genetic modifier of the *su1* gene. The *se* gene produces nearly double the sugar compared with *su1*; and unlike the watery endosperm of *sh2* kernels, *se* varieties have a creamy endosperm due to production of WSP. The main disadvantage of the *se* varieties is that the sugar is rapidly converted to starch after harvest, unlike the *sh2* varieties (Wily et al., 1989).

Sweetcorn has a high respiration rate, which results in a high rate of heat evolution, and it loses sugars very rapidly after harvest (Brecht and Sargent, 1988; Evensen and

Boyer, 1986; Wann et al., 1971). That is why fast cooling after harvest and keeping the cob temperature as close as possible to 0 °C is the most important step in maintaining sweetcorn quality (Brecht, 2002). The sugar content, which so largely determines quality in sweetcorn, declines rapidly at room temperature and decreases less rapidly if the sweetcorn is kept at about 0 °C. Early work by Appleman and Arthur (1919) showed that sugar loss is about four times as rapid at 10 °C as at 0 °C. At 30 °C, 60% of the sugars in *su1* sweetcorn may be converted to starch in a single day as compared with only 6% at 0 °C (Brecht, 2002). While in *sh2* varieties the sugar loss is actually at the same rate, the higher initial sugar in these cultivars helps in keeping it sweet tasting for a longer period (Brecht et al., 1990). Similar results were obtained by Olsen et al. (1990) who found that the sugar depletion rate was higher in *sh2* than *su1*, but the *sh2* still contained significantly more sugar after storage. These two types of sweetcorn lose sweetness and aroma during storage, but the main difference between them during storage is that *su1* (and *se*) kernels tend to become starchy while *sh2* varieties tend to taste more watery and bland (Brecht, 2002).

Factors Optimizing Postharvest Preservation

Harvested fruits and vegetables are highly perishable products. Over-ripening and senescence, mechanical injuries, trimming, water loss, and biological factors such as diseases and pests are the principal causes of postharvest losses. Postharvest preservation of these commodities is thus comprised of efficient techniques to reduce the tremendous amount of fresh produce losses, maintain produce quality, and extend shelf life throughout the postharvest chain, consequently increasing their commercial value.

Postharvest deterioration can be controlled by primary and secondary factors. The primary factors to optimize preservation of a horticultural commodity (Kader et al., 1989) are as follows:

- Selecting varieties of the crop that have desirable postharvest and storage characteristics,
- Application of the ideal preharvest treatments,
- Harvesting at the optimum maturity stage,
- Minimizing mechanical injuries during harvesting and postharvest handling,
- Using proper sanitation procedures to reduce microbial infection,
- Providing the optimum temperature and relative humidity throughout the postharvest chain

Temperature control (precooling and cold storage) has been identified as the most crucial factor for extending the shelf life of produce since biological reactions generally increase 2- to 3-fold for every 10 °C rise in temperature.

Secondary factors to optimize preservation of a horticultural commodity include modification of O₂ and CO₂ concentrations in the atmosphere surrounding the commodity to levels different than normal air (Kader et al., 1989; Saltveit, 1989). Atmosphere modification is achieved either by reducing the concentration of O₂ (which is required for respiration and for ethylene synthesis); or by increasing CO₂ concentration (which inhibits respiration, ethylene action, and microbial growth) (Chinnan, 1989; Daniels et al., 1985; El-Gorani and Sommer, 1981; Kader, 1987; Kader et al., 1989; Labuza and Breene, 1989; Shewfelt, 1986). This is referred to as controlled atmosphere (CA) or modified atmosphere (MA) storage.

Although the secondary factors are not as significant as the primary factors, their additive effect is important to preserve the overall postharvest quality of many commodities. It was also demonstrated that using secondary factors could solve some

important postharvest problems in specific commodities. For example, the use of CA/MA technology on chilling sensitive produce may overcome the impact of low temperature injury (Forney and Lipton, 1990; Pesis et al., 2000; Wang and Qi, 1997)

Factors Optimizing Postharvest Preservation in Sweetcorn

Sweetcorn has one of the highest metabolic and respiration rates among vegetable crops, which makes it a very perishable product that requires special attention to the postharvest practices used in order to prolong its shelf life. The main sources of postharvest loss in sweetcorn are sugar loss, husk yellowing and drying, and kernel denting. Denting is caused by water loss, primarily from the husks, which in turn draw moisture from the cob and kernels, causing the latter to collapse, causing the dented appearance. It was estimated that a loss of only 2% moisture may result in objectionable kernel denting (Hardenburg et al., 1986). Mechanical injuries, which occur especially when sweetcorn is harvested mechanically or during trimming, can be a serious cause of postharvest losses by promoting water loss and decay. Sweetcorn is also affected by biological factors such as diseases and pests that increase postharvest losses.

As discussed above, postharvest deterioration can be controlled by primary and secondary factors. The primary factors to optimize preservation of sweetcorn are

- Selection of varieties of the crop that have desirable postharvest and storage characteristics. For example, cultivation of *sh2* varieties ensures high sugar content; and hence increases the tolerance for diverse postharvest conditions and extends the postharvest life of sweetcorn (Spalding et al., 1978).
- Application of the ideal preharvest treatments ensures high postharvest quality and long shelf life. It has been proven that preharvest factors have a great impact on postharvest quality (Kays, 1999) For example, use of the optimum nitrogen and sulfur fertilization rates increased the flavor quality in harvested sweetcorn due to increased dimethyl sulfide in the kernels (Wong et al., 1995).

- Harvesting at the optimum maturity stage. The early work by Rumpf et al. (1972) demonstrated that the highest levels of sugars were found when the cobs were harvested at the optimum degree of maturity.
- Minimizing mechanical injuries during harvesting and postharvest handling.
- Using proper sanitation procedures throughout the postharvest chain, to ensure reduced microbial infection, and thus reduce postharvest losses.
- Providing the optimum temperature and relative humidity throughout the postharvest chain.

Similar to all perishable horticultural crops, temperature control (precooling and cold storage) is the most crucial factor in extending the shelf life of sweetcorn. It is well documented that fast precooling and storing at a low temperature (0-1 °C) and high relative humidity (>90%) is the key factor in ensuring the highest postharvest quality in sweetcorn (Brecht, 2002; Brecht and Sargent, 1988; Evensen and Boyer, 1986). This is due to the reduction in metabolic rates at lower temperatures, which reduces the respiration rate and consequently the sugar consumption (high sugar content being the main quality factor in sweetcorn). Moreover, the low metabolic rate reduces the conversion of sugars to starch, which helps in keeping the high sugar content, which is very helpful in the genotypes that convert sugar to starch. Also, low temperature reduces water loss and subsequently reduces denting and/or husk drying of the sweetcorn cobs. On the other hand, low temperature also helps in reducing microbial growth and hence reduces pathological postharvest losses.

The secondary factors to optimize preservation of sweetcorn as mentioned above also include using MA or CA, by reducing O₂ levels and/or increasing CO₂ levels (see discussion below). The MA and CA help in reducing sweetcorn metabolic rates, including respiration (Riad et al., 2002; Riad and Brecht, 2003) and carbohydrate metabolism (Risse and McDonald, 1990). They also reduce water loss from sweetcorn

husks (Deak et al., 1987) because of the restricted gas exchange that is integral to MA and CA technology. Reduced O₂ and more importantly elevated CO₂ levels in MA and CA also reduce microbial growth on sweetcorn husks and kernels (Aharoni et al., 1996; Schouten, 1993). All these benefits help in reducing sweetcorn postharvest losses.

Controlled Atmosphere (CA) and Modified Atmosphere (MA) Technology

The technique of modification of the atmosphere surrounding perishable products is referred to as CA or MA. In CA, the atmosphere is created artificially and the gas composition is continuously monitored and adjusted to maintain the optimum gas concentration. On the other hand, in MA, the gaseous environment is modified naturally by the interplay among the physiology of the commodities and the physical environment, thus the control of the atmosphere in MA is less precise than CA. In MA, the respiration rate (O₂ consumption and CO₂ evolution) of the commodity being stored is in equilibrium with the O₂ and CO₂ concentrations in the surrounding environment. Several articles have been published on the benefits of CA/MA technology on the extension of perishable product shelf life (Anzueto and Rizvi, 1985; Nakashi et al., 1991; Zagory and Kader, 1988).

Beneficial Effects of CA and MA

Using CA and MA have a wide range of benefits (Kader, 1980; Kader et al., 1989) such as the following:

- The CA and MA conditions reduce the respiration rate (as long as the levels of O₂ and CO₂ are within those levels the commodity can tolerate and don't induce anaerobic respiration), which results in delayed ripening and senescence and better maintenance of the quality of the commodity.
- The CA and MA conditions reduce ethylene production and reduce sensitivity to ethylene (action) and this has many beneficial effects such as delaying fruit ripening and tissue senescence, delay of chlorophyll degradation, and maintenance of textural quality (decrease in lignification, etc.).

- The CA and MA conditions allow handling of chilling sensitive fruits (such as tomato, banana, and mangoes) at temperatures lower than the chilling threshold temperatures in normal air storage.
- Using CA and MA reduces the incidence and severity of some physiological disorders such as the disorders induced by ethylene or by chilling injury.
- Since delaying senescence, including fruit ripening, reduces the susceptibility to pathogens, CA/MA has a beneficial effect in decreasing postharvest diseases (Daniels et al., 1985; El-Goorani and Sommer, 1981). Levels of O₂ below 1% and levels of CO₂ above 10% can also have a significant direct effect on fungal growth. Carbon dioxide levels above 10-15% (in commodities that tolerate such levels) can be used to provide a fungistatic effect.

Detrimental Effects of CA and MA

Most CA/MA disadvantages are related to severe reductions of O₂ and/or increases in CO₂ that force the product into anaerobic respiration (Kader et al., 1989; Brecht, 1980). Anaerobic respiration causes many disorders such as

- Increased susceptibility to decay and shortening of the storage life.
- Irregular ripening.
- Accumulation of ethanol, acetaldehyde, and other compounds that produce off-flavors and other metabolic dysfunctions.
- Physiological disorders (such as brown stain in lettuce, internal browning and surface pitting in pome fruits).
- Activation of the growth of some anaerobic pathogens that are considered to be major health hazards.

Modified Atmosphere Packaging (MAP)

Modified atmosphere packaging (MAP) is an atmosphere control technique that relies on the natural process of respiration of the product and the gas permeability of the package holding the product. Due to respiration, there is a buildup of CO₂ and a depletion of O₂, and the package material helps to maintain the modified gas levels until the package reaches steady state because of restricted gas permeability. In the steady state

condition, the O₂ flow entering the package equals the O₂ consumed by respiration, and the CO₂ flow leaving the package equals the CO₂ produced by respiration. Because of the limitation of CA storage to relatively large-scale systems, the MAP technique was developed to provide the optimal atmosphere; not for the entire storage facility, but for just the product, thus maintaining the desired atmosphere during almost all of the postharvest chain even during the retail display. The MAP can vary from a whole shipping container to a small retail package. As well as the benefits of modifying the O₂ and CO₂ levels, MAP has the additional benefits of water loss prevention, product protection, and brand identification. To achieve the desired atmosphere more rapidly, modification of the package atmosphere can be accelerated by using absorbents, or by using active modification instead of passive modification (i.e., initially replacing the package atmosphere with the desired one by gas flushing) (Kader et al., 1989)

The development of MAP has faced several problems (Kader, 1987; Kader et al., 1989) such as

- Lack of commodity respiration data under several temperatures and gas compositions.
- Lack of permeability data for packaging materials at different temperatures and relative humidities.
- Lack of consistency in respiration data gathered for the same commodity.

In medium and high-respiring commodities (like sweetcorn), using commonly available films such as low-density polyethylene (LDPE), polyvinyl chloride (PVC), and polypropylene is not ideal due to their low gas transmission rates, which may lead to respiration switching toward anaerobic respiration (Morales-Castro et al., 1994). Fonseca et al. (2000) summarized some of the limitations of using the flexible polymeric films that result from their structure and permeation characteristics such as

- Films are not strong enough for large packages.
- Film permeability characteristics change unpredictably when films are stretched or punctured.
- Some films are relatively good barriers to water vapor, causing condensation inside packages when temperature fluctuations occur and consequently increasing susceptibility to microbial growth.
- Film permeability may be affected by water condensation.
- The uniformity of permeation characteristics of films is not yet satisfactory.
- Film permeability is too low for high-respiring products.
- Products that require high CO₂/O₂ concentrations may be exposed to anaerobiosis because of the high ratio of the CO₂ versus O₂ permeability coefficients.

Perforation-mediated MAP is a potential technique for postharvest preservation of fresh horticultural commodities. In this technique, instead of using the common polymeric films, a package is used in which the regulation of gas exchange is achieved by single or multiple perforations or tubes that perforate an impermeable package (Emond and Chau, 1990a,b; Emond et al., 1992).

Using the perforation-mediated package has many potential advantages (Fonseca et al., 1997) such as

- The high values of mass transfer coefficients, implying that a reduced size and number of perforations for gas exchange are required, thus high-respiring produce can be packed in this system.
- A MAP using perforations can be adapted easily to any impermeable container, including large bulk packages. Polymeric films are not strong enough for packs much larger than those used for retail, but perforations can be applied to retail packages as well as shipping containers, because rigid materials can be used. Rigid packages also can prevent mechanical damage of the product.
- A flexible system is obtained due to the ability to change the gas transfer coefficients by selecting the adequate size and shape of the perforation.
- Commodities requiring high CO₂ concentrations and relatively high O₂ concentrations can be packed with this system.

But on the other hand there are some limitations to this technique (Fonseca et al., 1997) such as

- Although it may be applied to products that could not be packed in conventional MAP, the range of products is not very wide, because the CO_2/O_2 transfer coefficient ratio averages 0.8, the ratio of the diffusion values of CO_2 and O_2 in the air. This may eventually be overcome by the use of perforation packed with materials with different affinities for CO_2 and O_2 .
- Water loss in the product may become a problem, but packed perforations may solve this problem.
- Non-uniformity of concentrations inside the package due to gas stratification may also be a problem in large containers.
- More fundamental research and experimental validation is needed before its eventual commercial use.

One commercially available film (Intellipac from Landec Corporation, Menlo Park, Calif.) is claimed by the manufacturer to be able to automatically adjust its permeability in response to temperature changes by a phase change in the film polymer structure (Clarke and De Moor, 1997). Permeability characteristics of this package are provided by using a highly permeable membrane over an aperture in the wall of the package. The membrane is made by coating a microporous substrate with a side chain crystallizable (SCC) polymer. In cold temperature, the SCC polymer exists in a crystalline solid phase; but when the temperature increases above the pre-selected switch temperature, the polymer changes to a more permeable liquid phase. Because this transformation involves a physical effect and not a chemical change, the metamorphosis is reversible. By changing the properties of the polymer and coating thickness, it is possible to obtain the permeability selectivity and the temperature switch required. Lange (2000) found that Intellipac-stored strawberries had ethanol and ethyl acetate levels similar to those of fresh

fruit, while samples stored in regular perforated film had levels of these fermentative products 7-fold higher than those of fresh samples.

Active and Passive Modified Atmosphere

A MAP system maintains an adequate atmosphere within the package under steady state conditions through interaction of the respiration rate of the commodity and the package size and gas permeation of the package material (Kader et al., 1989). When atmospheres are modified passively by commodity respiration, it may require hours to days until the gas concentrations reach the steady state in the package, the required time being mainly a function of the package void volume (Ballantyne et al., 1988; Geeson et al., 1985). Using active or semi-active atmosphere modification i.e., removal or addition of a determined gas volume from the package, allows the desired atmosphere to be more quickly achieved, and thus further prolongs the storage life of the produce (Yahia and Gonzalez-Aguilar, 1998). This may be very important in sweetcorn, since addition of even a single day to its short storage life would be a significant benefit.

Controlled and Modified Atmosphere and Volatile Production

High CO₂ and/or low O₂ can induce anaerobic metabolism, resulting in accumulation of ethanol and acetaldehyde (Kader, 1989; Ke et al., 1993). Methanethiol production is also induced under low O₂ atmospheres in Brassica crops (Forney et al., 1991); and production of other volatiles has also been shown to be enhanced under low O₂ and/or high CO₂ atmospheres (Hansen et al., 1992; Larsen and Watkins, 1995; Mattheis et al., 1991)

The production of some volatiles may be modified if both O₂ and CO₂ are modified simultaneously. Obenland et al. (1995) found that low O₂ levels enhanced methanethiol production in broccoli. However, the application of high CO₂ levels in parallel with low

O₂ inhibited methanethiol production. Hence, the levels of both gases may be important in determining the production of volatiles.

The critical O₂ concentration that results in induction of anaerobic respiration is dependent on the character of the product in question (Gran and Beaudry, 1993). Also, temperature can influence the threshold of anaerobic induction. The O₂ threshold for anaerobic induction increases with increasing temperature (Beaudry et al., 1992; Gran and Beaudry, 1993; Joles et al., 1994). For example, Cameron et al. (1994) found that packages designed for blueberries at 0 °C reached the threshold for anaerobic induction at 5 °C. Also Beaudry et al. (1992) reported that highbush blueberry fruit can tolerate ~1.8% O₂ when stored at 0 °C whereas at 25 °C they require ~4% O₂ to avoid elevating the respiratory quotient (RQ). They concluded that the risk of anaerobiosis within LDPE packages was increased by high temperature and the critical O₂ level that induced anaerobic respiration was increased with increasing temperature. There are also several reports indicating that varieties of the same commodity have different potentials for accumulation of acetaldehyde and ethanol (Blanpied and Jozwiak, 1993; Folchi et al., 1995; Gran and Beaudry, 1993)

Sulfur-containing volatiles are produced via free, sulfur-containing amino acids, peptides, thioglucosides, and thiophenes in plant tissue (Buttery, 1981; Richmond, 1973). The accumulation of many of these compounds is associated with off-odors and off-flavors (Di Pentima et al., 1995; Forney et al., 1991; Hansen et al., 1992). There are some reports indicating that hydrogen sulfide and allyl sulfide cause respiration enhancement over short periods of time (Hosoki et al., 1985). This could have a significant effect on

MAP, since films used in these packages are selected on the basis of respiratory activity and film permeability (Toivonen, 1997).

In natural ecosystems, plant tissue evolution of volatiles is influenced to a large degree by evapotranspiration (Charron and Cantliffe, 1995). The evolution of volatiles under low transpiration is much lower, and therefore the volatiles can accumulate in the tissues. A potential problem in CA/MA and especially MAP is that restricted ventilation may lead to accumulation of volatile compounds within the plant tissues that may be damaging or at least objectionable, from a quality standpoint. Among the compounds that may accumulate in CA and MA and cause off-odors during storage are those usually associated with anaerobic respiration such as ethanol and acetaldehyde, as well as acetone, dimethyl sulfide, hydrogen sulfide, methanethiol, and ethanethiol.

By enhancing evapotranspiration in CA/MA storage, the accumulation of volatiles in the tissue can be reduced (Beaudry et al., 1993; Blanpied and Jozwiak, 1993). Toivonen (1997) reported that use of desiccants or a combination of desiccants and a volatile adsorbent in MAP significantly improved raspberry shelf life and quality at 10 °C since it lowered the concentrations of ethanol and acetaldehyde. This treatment lowered the concentrations of ethanol and acetaldehyde in the fruit without much effect on their concentration in the headspace of the package, and increased acceptability from 42% to 75% with a slight increase in weight loss. These results agree with the previous suggestion that water movement from the product is important in lowering volatile levels in the tissue.

Most of the odors present in fresh sweetcorn may be considered to contribute to its characteristic flavor; especially DMS, which is responsible for the characteristic aroma of

cooked sweetcorn (Wiley, 1985; Williams and Nelson, 1973; 1974; Flora and Wiley, 1974b). It was noticed that the levels of these compounds are increased by canning or freezing (Flora and Wiley, 1974a). There is very little information about the effect of CA/MA storage and MAP on the levels of these compounds, or about the possible changes in their levels, or the levels of precursor compounds, during storage.

CA/MA and Sweetcorn Storage

Early work by Spalding et al. (1978) found that sweetcorn appearance and flavor were not significantly improved when stored for 3 weeks at 1.7 °C under CA or low pressure. In those experiments, sweetcorn cobs were stored either in air, low-pressure atmosphere with the equivalent of 2% O₂, or in CA containing 2 or 21% O₂ plus either 0, 15, or 25% CO₂. The increase in ethanol level was much higher with the increase of CO₂ level over 15% than with the decrease of O₂ level from 21% to 2%. Kernels from sweetcorn cobs stored in 2% O₂ plus 25% CO₂ were not significantly higher in sugars than air-stored sweetcorn and contained the highest amount of ethanol; but were the best treatment in terms of flavor rating, better than the air storage treatment, while storing in 2% O₂ plus 15% CO₂ was similar to air storage in sugar content and flavor rating. Also, in this experiment, the highest off-flavor levels detected were obtained when storing sweetcorn in 21% O₂ plus either 15 or 25% CO₂; but there was no significant difference between air storage and storing in 2% O₂ plus either 15, or 25% CO₂. Despite these results, it was concluded that sweetcorn appearance and flavor maintenance were not significantly improved by CA storage conditions; and that breeding of cultivars that better retain quality in combination with prompt precooling offers more potential for success than CA storage.

On the other hand, there are many reports stating the beneficial effects of using MAP for sweetcorn. Deak et al. (1987) demonstrated that using MAP (shrink wrap film with moisture permeability of $0.1 \text{ g}\cdot 100\cdot\text{cm}^{-2}\cdot 24 \text{ h}^{-1}$ and O_2 permeability that varied from 0.4 to $40 \text{ mL}\cdot 100 \text{ cm}^{-2}\cdot 24 \text{ h}^{-1}$) eliminated water loss and maintained beneficial CO_2 and O_2 levels within the package. These effects, together with low storage temperature ($5 \text{ }^\circ\text{C}$), markedly reduced postharvest deterioration and hence resulted in an at least three-fold extension of shelf life (29 versus 8 d). But MAP treatment increased microbial growth due to the water-saturated atmosphere. Similar results were obtained by Risse and McDonald (1990) when sweetcorn was stored at 1, 4, or $10 \text{ }^\circ\text{C}$ for 26 d unwrapped or wrapped in stretch or shrink wrap. It was concluded that film wrapping maintained freshness and reduced moisture loss better than the lack of wrapping. Also these authors recommended stretch wrap over shrink wrap since stretch wrap generated higher CO_2 levels (4-9% versus 1-3%) and lower O_2 levels (14-19% versus 18-21%), which resulted in higher total soluble solids retention. In that experiment also, an increase in microbial growth was experienced in the MAP treatments, especially in the presence of damaged husks or kernels, presumably due to the higher relative humidity within the MAP.

However, reduced O_2 and elevated CO_2 have also been reported to reduce decay and maintain sweetcorn husk chlorophyll levels (Aharoni et al., 1996; Schouten, 1993). Aharoni et al. (1996) demonstrated that sweetcorn wrapped with PVC film benefited from the reduction of water loss, but the limiting factor affecting the shelf life of fresh sweetcorn in this case was the increase in pathogens on the trimmed ends of the cobs. Polyolefin stretch film has lower permeability rates for O_2 and CO_2 than PVC film, and consequently generated higher CO_2 levels ($\sim 10\%$ versus $\sim 3\%$) and lower O_2 levels ($\sim 7\%$

versus ~15%) in the packages. This resulted in a significant reduction in the decay incidence and water loss and significantly better maintenance of the general appearance quality (Aharoni et al., 1996). These levels of O₂ and CO₂ did not trigger anaerobic respiration, and ethanol levels in the packages were very low until the packages were transferred to 20 °C to simulate retail conditions. Upon transfer to 20 °C, there was a sudden increase in CO₂ (20-25%) and decrease in O₂ levels (2-4%), which increased the ethanol concentration significantly in the sweetcorn in those packages. Nevertheless, these high levels of ethanol had little effect on the general quality of the sweetcorn, since off-odor occurred only in two types of packages, out of the six different combinations used in the experiment.

Aharoni et al. (1996) suggested that the increase in microbial growth in MAP reported by Deak et al. (1987) and Risse and McDonald (1990) was probably due to relatively low CO₂ levels within their packages, a consequence of the high ratio of the CO₂ versus O₂ permeability coefficients of plastic films as mentioned previously. Schouten (1993) demonstrated that CA storage with higher CO₂ (2% O₂ plus 10% CO₂) retained higher sugar content than air storage and CA was more beneficial when used at a higher temperature (5-6 °C compared with 1-2 °C). The CA storage significantly reduced respiration and pathological breakdown, and also resulted in an increase in ethanol content; but the higher content of sugars had a more positive influence on the taste than the negative influence of the ethanol. On the other hand, Rumpf et al. (1972) clearly proved that the loss in sucrose content, the decisive factor in determining the taste quality of sweetcorn, may be delayed by both low temperature (0 °C versus 5 or 10 °C) and low O₂ content, since 1% O₂ was the best treatment in retaining the sucrose levels after 11 d

of storage at 5 °C followed by 2% O₂ then 4% O₂ and finally air storage. All the CA treatments in this experiment had 0% CO₂.

The main difficulty in using MAP for sweetcorn is that the film permeability is usually designed to maintain a desirable atmosphere during storage, but a rapid depletion of O₂ may occur in the retail display where the temperature is higher, resulting in a shift toward anaerobic metabolism and causing rapid deterioration and quality loss. Recently Silva et al. (1999b) introduced the idea of using MAP designed for the retail display temperature and selecting the surrounding atmosphere inside a CA container during transport to overcome the negative effect of changes in surrounding temperatures during the postharvest chain. A similar idea was used by Rodov et al. (2000) who used a nested sweetcorn package i.e., a film wrap for retail packages (PVC film-wrapped trays containing two cobs of sweetcorn) suitable for the display temperature (20 °C) along with a master carton liner that provided the desirable atmosphere during shipping and storage temperature (2 °C). The low O₂ levels (10-15%) and the high CO₂ levels (5-10%) obtained in both cases were beneficial in inhibiting mold growth but weren't severe enough to increase the ethanol levels.

Due to the lack of work on CA/MA effects on sweetcorn, there are no specific limits to cite for O₂ and/or CO₂ levels or the threshold of sweetcorn sensitivity to reduced O₂ or elevated CO₂ for more than a few specific temperatures and storage times, however some work has shown that sweetcorn can tolerate up to 25% CO₂ and down to 0.5% O₂ without damage for 2 weeks at 1 °C, scoring the best in flavor ratings (Riad and Brecht, 2003; Spalding et al., 1978). The usual recommended CA combination for sweetcorn is a more conservative 2% O₂ plus 15% CO₂, which is assumed to show benefits and not

cause damage over the likely commercial range of temperatures and storage times (Brecht, 2002; Saltveit, 1989).

Fresh-Cut Fruits and Vegetables

Fresh-cut fruits and vegetables (sometimes called minimally processed or lightly processed fruits and vegetables) represent a relatively new and rapidly developing segment of the fresh produce industry. Fresh-cut processing involves preparing fresh produce to be ready to eat or cook by the final consumer. According to the International Fresh-Cut Produce Association (IFPA), fresh-cut produce is defined as any fresh fruit or vegetable or any combination thereof that has been physically altered from its original form, but remains in a fresh state. Regardless of commodity, it has been trimmed, peeled, washed and cut into 100% usable product that is subsequently bagged or prepackaged to offer consumers high nutrition, convenience, and value while still maintaining freshness (IFPA, 2002). Sales of fresh-cuts have grown from about \$5 billion in 1994 to \$10-12 billion in 2002, which is about 10% of total produce sales (includes retail and foodservice sales). Packaged salads alone topped the \$1.6 billion mark in retail sales in 1999, marking a 15.9% increase from the previous year (IFPA, 2002).

Fresh-cut fruit and vegetable products are different from traditional, intact vegetables and fruits in terms of their physiology and their handling requirements. Fresh-cut produce is essentially purposely-wounded plant tissue that must subsequently be maintained in a viable, fresh state for extended periods of time. Fresh-cut vegetables deteriorate faster than intact produce as a direct result of the wounding associated with processing, which leads to a number of physical and physiological changes affecting the viability and quality of the produce (Brecht, 1995; Saltveit, 1997).

Limitations of Fresh-Cut Fruit and Vegetable Production

Water loss

Plant tissues are mainly composed of water and any small changes in water content may have a large impact on produce quality and could cause a variety of negative characteristics such as limpness, flaccidity, shriveling, wrinkling, and/or tissue desiccation. Sams (1999) demonstrated that losing a small amount of water content like 3% or 5% of the water content in spinach or apple, respectively, render these commodities unmarketable.

On the other hand, crispness, an important characteristic of fresh produce that is related to water turgor pressure in the tissue, could be easily lost due to water loss. The loss in crispness results in softening and flaccidity. Leafy vegetables are particularly susceptible to desiccation because of their large surface-to-volume ratios; moreover loose leaves, such as spinach, are more prone to desiccation than a compact head, such as a whole lettuce head (Salunkhe and Desai, 1984). Therefore, as a consequence of water loss, appearance changes such as wilting and reduced crispness may occur.

In case of fresh-cut products, the possibilities to undergo severe water loss are much higher than in the case of intact produce since during fresh-cut preparation the produce goes through peeling and cutting or shredding, slicing, etc. Losing the skin has a critical effect on many fresh-cut products because the skin is a protective waxy coating, highly resistant to water loss, and thus peeled, fresh-cut fruits and vegetables are more perishable and more susceptible to turgor loss and desiccation. On the other hand, the mechanical injury brought on by cutting, shredding and/or slicing, directly exposes the internal tissues to the atmosphere, promoting the evaporation of intercellular water and

hence starting desiccation of the tissue. Furthermore, fresh-cut preparation increases the relative product surface area per unit mass or volume, which also increases the water loss.

Another factor affecting the water loss affects products that require rinsing after cutting, since this is frequently followed by centrifugation. If accelerated centrifugation speed or long centrifugation times are applied, increased desiccation can result, as reported for cut lettuce (Bolin and Huxsoll, 1989).

The control of water loss in fresh-cut fruits and vegetables is possible through the use of appropriate handling techniques, including temperature and relative humidity control, which can greatly help minimize the rate of water loss. Reduction of water loss can be achieved basically by decreasing the capacity of the surrounding air to hold water, which can be achieved by lowering the temperature and/or increasing the relative humidity. On the other hand, applying edible coatings to the fresh-cut commodity can greatly reduce the water loss since such coatings provide an additional barrier to moisture movement (Baldwin et al., 1995a). When a thin layer of protective material is applied to the surface of the fruit or vegetable as a replacement for the natural protective tissue (epidermis, peel), it can have several possible effects (Baldwin et al., 1996; Baldwin et al., 1995a,b; Li and Barth, 1998; Nisperos and Baldwin, 1996). The coating may act as a barrier to water loss or, alternatively, hygroscopic coating materials may serve to maintain a moist surface appearance of the fresh-cut product. The coating may also work as a semipermeable barrier that helps in reducing gas diffusion and hence increase internal CO₂ levels and reduce O₂ levels, which results in a MA-like effect. This can help reduce respiration, as well as associated senescence processes such as color and texture changes, and may help to retain aroma volatiles. Coatings containing antimicrobial

compounds or with low pH may be used to reduce microbial growth. For example, the primary parameter affecting fresh-cut celery quality is water loss because small reductions in water content (2.5–5%) may lead to flaccidity, shriveling, wrinkling, and pithiness. A significant increase in moisture retention by celery sticks was obtained with the application of a caseinate acetylated monoglyceride coating (Avena-Bustillos et al., 1997).

Texture change and loss of tissue firmness

During fruit ripening, one of the most notable changes is softening, which is related to biochemical alterations at the cell wall, middle lamella, and membrane levels, although significant roles in the softening process have been attributed to the pectic enzymes, polygalacturonase, and pectin methylesterase (PME). Fresh-cut fruits and vegetables may be considered to undergo a ripening-like process due to wound-induced ethylene production and the subsequent increase in respiration, which results in loss of tissue firmness.

Textural changes in fresh-cut vegetables and fruits are minimized at low temperatures. Thus, temperature management is the first step to maintain the initial, fresh textural quality of these products. Several treatments have been proven to be effective in reducing fresh-cut firmness loss. A common treatment used to improve tissue firmness is to spray or dip fruit or vegetable pieces in aqueous calcium salts, as described for shredded carrots, zucchini slices, and fresh-cut pears, strawberries, kiwifruit, nectarines, peaches, and melons (Agar et al., 1999; Gorny et al., 1999; 2002; Izumi and Watada, 1994; 1995; Luna-Guzman et al., 1999; Luna-Guzman and Barrett, 2000; Main et al., 1986; Morris et al., 1985; Rosen and Kader, 1989). This is a result of the pivotal role played by calcium in maintaining the textural quality of produce due to its effect of

rigidifying cell wall structure by cross-linking ester groups and also preserving the structural and functional integrity of membrane systems (Poovaiah, 1986).

The use of CA or MA and MAP also retards senescence, lowers respiration rates, and consequently slows the rate of tissue softening (Kader, 1992). Rosen and Kader (1989) demonstrated that texture loss in strawberry fruit slices was reduced significantly by using CA. Strawberry slices kept under CA for a week had comparable firmness to whole and to freshly sliced fruit. Also, the best treatment to retain firmness of peach halves was a combination of a dip in 2% calcium chloride and 1% zinc chloride, followed by packaging with an O₂ scavenger and storage at 0 to 2 °C (Bolin and Huxsoll, 1989).

Heat treatment also appears to have the potential to beneficially affect product texture. Heat treatment of whole apple fruit resulted in firmer fresh-cut products when compared with non-heated fruit (Kim et al., 1993). However, while heat treatment of whole apples improved apple slice firmness, the storage temperature of the whole fruit after heating also had a significant effect on product firmness. Mild heat treatment (45 °C for 1.75 h) of whole fruit prior to cutting retained greater fresh-cut apple firmness during storage for 21 d at 2 °C (Kim et al., 1994).

Heat treatment could be applied prior to fresh-cut preparation, for example, apples that were kept at 38 °C for 6 d immediately after harvest, then cold stored for 6 months prior to slicing and dipping in calcium solution, produced slices that were firmer than slices from control fruit (Lidster et al., 1979). Also heat treatment could be used during the calcium dipping treatment, as Luna-Guzman et al. (1999) demonstrated for fresh-cut muskmelons cylinders dipped in 2.5% calcium chloride solution for 1 minute at different temperatures (60, 40, or 20 °C). The texture was firmer in samples treated at higher dip

temperatures, perhaps due to the activation of PME, which is known to be activated in the 55-70 °C range, resulting in reduced pectin methyl esterification. The de-esterified pectin chains may crosslink with either endogenous calcium or added (exogenous) calcium to form a tighter, firmer structure.

However, there is a problem noticed when calcium chloride is used as a firming agent, which is that it may result in undesirable bitter flavor of the product. Fresh-cut cantaloupe cylinders dipped in calcium lactate solutions resulted in a textural improvement similar to calcium chloride-treated fruit cylinders. Sensory evaluation indicated that results were better since less bitterness and a more detectable melon flavor were perceived (Gorny et al., 2002; Luna-Guzman and Barrett, 2000).

Color change

Most fresh-cut fruits and vegetables experience some kind of color change after preparation. There are many reasons and degrees of this color change. The most common color change is the browning discoloration found in many fresh-cut commodities due to oxidation of colorless phenolic compounds to produce brown phenolic pigments.

Browning. Brown discoloration is one of the most limiting factors in the shelf-life of fresh-cut products. The main reason for brown discoloration is enzymatic browning. During the preparation stages, produce is subjected to operations where cells are broken, causing enzymes to be liberated from tissues and put in contact with their substrates. Enzymatic browning is the discoloration that results from the action of a group of enzymes called polyphenol oxidases (PPO), which have been reported to occur in all plants, and exist in particularly high amounts in mushroom, banana, apple, pear, potato, avocado, and peach. Enzymatic browning must be distinguished from non-enzymatic browning, which results upon heating or storage after processing of foods; types of non-

enzymatic browning include the Maillard reaction, caramelization, and ascorbic acid oxidation.

Enzymatic browning is a complex process, which can be subdivided into two parts. The first reaction is mediated by PPO, resulting in the formation of o-quinones (slightly colored), which, through non-enzymatic reactions, lead to the formation of complex brown pigments. The o-quinones are highly reactive and can rapidly undergo oxidation and polymerization. The o-quinones react with other quinone molecules, other phenolic compounds, aromatic amines, thiol compounds, ascorbic acid, and the amino groups of proteins, peptides and amino acids (Nicolas et al., 1993; Whitaker and Lee, 1995).

Usually, brown pigments are formed, but in addition, reddish-brown, blue-gray, and even black discolorations can be produced on some injured plant tissues. Color variation among products of enzymatic oxidation is related to the phenolic compounds involved in the reactions (Amiot et al., 1997), and both color intensity and hue of pigments formed vary widely (Nicolas et al., 1993). Consequences of enzymatic browning are not restricted to discoloration, since undesirable-tasting chemicals can also be produced and loss of nutrient quality may result (Vamos-Vigyazo, 1981).

Heat treatments can be used to control fresh-cut produce browning. Brief exposures to temperatures in the range of 40 to 60 °C can redirect plant tissue metabolism toward production of heat shock proteins, which can, in some cases, prevent undesirable metabolic processes from occurring (Saltveit, 2002). For example, the synthesis of wound-induced enzymes such as the phenolic biosynthesis enzyme polyphenol oxidase (PAL) can be prevented by giving lettuce tissue a brief heat shock (e.g., immersion in 45 °C water for 90 s) after processing (Saltveit, 2000). While this technique is very

effective at preventing browning in plant tissue with constitutively low levels of phenolic compounds (e.g., celery and lettuce), it is ineffective in tissue with constitutively high levels of phenolic compounds (e.g., artichokes and potatoes).

Also there are different chemical treatments that help in controlling fresh-cut product browning such as using acidulants, reducing agents, or chelating agents. Polyphenol oxidase, which catalyzes the formation of o-quinones from o-diphenols, beginning the sequence of reactions leading to formation of brown phenolic pigments in vegetable and fruit tissue, has a pH optimum of 6.0 to 6.5 and shows little activity below pH 4.5. Therefore, using citric, ascorbic, or erythorbic acids as acidulants helps by reducing the pH and hence inhibiting PPO activity. The use of reducing agents such as ascorbic acid or its isomer erythorbic acid helps in inhibiting the active oxidation reactions and helps prevent brown pigment formation. Also, the use of chelating agents such as ethylenediamine tetraacetic acid (EDTA) can reduce browning by chelating the copper required for the PPO active site and thus inhibiting its activity (Brecht et al., 2004).

Fresh-cut onions and sweetcorn kernels sometimes develop a brown or black discoloration of unknown cause after cooking ('after-cooking browning'). This discoloration, which limits the shelf life of these products, increases with longer storage time of the fresh tissue at higher temperature, and is reduced by lowered O₂ and/or elevated CO₂ (Blanchard et al., 1996; Langerak, 1975; Riad and Brecht, 2001; Riad et al., 2003). Wounding before storage is apparently a prerequisite for sweetcorn after-cooking browning because it does not occur if sweetcorn kernels cut from stored cobs are cooked or when intact kernels are separated from the cob before storing (Brecht, 1999).

White blush. Another common color change is 'white blush' or 'white bloom' of carrots, in which the bright orange color of fresh carrots disappears in stored fresh-cut products, particularly when abrasion peeling is used. Fresh-cut carrots with white blush develop a white layer of material on the peeled surface, giving a poor appearance to the product. Upon peeling, the protective superficial layer (epidermis) of carrots is removed, generally by abrasion, leaving cell debris and an irregular surface, which while moist presents the natural orange color of carrots. The sequence of disruption of surface tissues followed by dehydration and white blush formation was confirmed by scanning electron microscopy, when comparing carrots peeled with a knife and a razor sharp blade. Knife-peeled carrot surfaces appeared severely damaged, compressed, and separated from underlying tissue, therefore prone to dehydration. Razor-peeled carrot surfaces were cleaner and apparently only a thin layer of cells had been removed, resulting in a product that upon drying did not acquire the whitish appearance (Tatsumi et al., 1991). It has been suggested that phenolic metabolism may be activated by peeling, inducing increases in lignin production, which result in the color change (Cisneros-Zevallos et al., 1995; Howard and Griffin, 1993). Consumers perceive white blush carrots as aged or not fresh, and using edible coatings was very successful in treating the white blush in carrots (Sargent et al., 1994). Sensory results showed preference for carrots coated with edible, cellulose-based coating, due to a fresh appearance (Howard and Dewi, 1995; 1996).

Yellowing. Yellowing of plant tissue due to chlorophyll degradation and exposure of the preexisting yellow carotenoid pigments is a normal process in ripening or senescence of many fruits and vegetables and this change can be accelerated by ethylene. In fresh-cut products, the stress of the wounding during preparation results in increased

ethylene production, and hence increases the yellowing discoloration in green tissues. Yellow discoloration was observed during storage of leafy and other green fresh-cut products. Shredded Iceberg lettuce darkens (develops brown discoloration) during storage, particularly at high temperatures. Simultaneously, loss of green pigmentation is observed (Bolin et al., 1977; Bolin and Huxsoll, 1991). In a study of cabbage processed into coleslaw, the color changed from green to a lighter white color during the cold storage period as a result of chlorophyll degradation and because cabbage lacks yellow pigments (Heaton et al., 1996).

The use of MAP (10% O₂ plus 8% CO₂) was beneficial in maintaining green color in broccoli and reduced the yellowing of the florets at 10 °C compared to an unpackaged control, which lost about 10% of its initial chlorophyll within 3 d of storage at 10 °C (Barth et al., 1993).

Microbial growth

Prior to harvest, plants are covered with a protective layer (the skin or epidermis), which protects the plant cells from microbial attack. Due to tissue damage and the fact that these tissues lose their protective skin during fresh-cut preparation, fresh-cut products are more prone to increased microbial growth. This increase is also promoted as a result of the release of cells fluids that microorganisms can use as nutrients (King and Bolin, 1989). Cell sap released from cut cells floods adjacent intercellular spaces and, when it comes in contact with bacteria, a suspension is formed that allows bacterial cells to move into the intercellular space. Bacterial cells that contact the cell sap become suspended, can move in the flooded intercellular spaces, and become protected from surface treatments. Bartz et al. (2001) demonstrated that within 5 seconds of application to a cut surface, cells of *Erwinia carotovora* became located in sites within tomato fruit

tissue that could not be successfully disinfected with 1.34 mM chlorine at pH 6.0. Furthermore, the passage of a knife through plant tissues during preparation can drag bacteria into contact with damaged cells (Lin and Wei, 1997).

The growth of bacteria, fungi, and/or yeasts may directly limit the life of fresh-cut vegetables and fruits by causing changes in the appearance and texture of the products, or through production of off-flavors and slimes that make them inedible. Many bacterial, fungal, and yeast species have been found to limit shelf-life of fresh-cut fruits and vegetables (Bartz and Wei, 2002; Farr et al., 1989; Heard, 1999; King and Bolin, 1989; Korsten and Wehner, 2002; Robbs et al., 1996). In general, bacteria and certain fungi cause decays in vegetables, including 'fruit vegetables', whereas fungi cause most of the decays in fruits. This is because fruit tissues are low pH (3.5-4.5), which favors mold and yeast growth, while vegetable tissues are nearer neutral pH (6.0-6.5), which favors bacteria growth. The major reasons for the separation between classes of microbes and plant hosts are not only the low pH of true fruits, but also the quantity and nature of the acidulants responsible for the low pH (Brecht et al., 2004).

Microbial contamination and growth on or in fresh-cut vegetables and fruits is a major concern for the industry (Beuchat, 1996; Fain, 1996; Francis et al., 1999; Hurst, 1995; Nguyen-the and Carlin, 1994; Zink, 1997) especially the possibility that certain human pathogens also can grow or survive on fresh-cut vegetables and fruits. These microbes ordinarily have no direct effect on the life of the product, but their presence turns the fresh-cut product into an unacceptable product regardless of other quality factors (Brecht et al., 2004). The major consideration in fresh-cut safety is inoculation of the nutrient-rich flesh of vegetables and fruits with human pathogens during preparation.

The presence of human pathogens is of particular concern with fresh-cut products because they are almost always consumed raw (i.e., without a heat treatment). In addition, it has been suggested that the elimination of spoilage microbes without elimination of human pathogens may extend shelf-life of a fresh-cut product to the point that safety is compromised because the human pathogens may be more likely to proliferate (Brackett, 1994; Hintlian and Hotchkiss, 1986).

Contamination of fruits and vegetables with human pathogens can occur during growth in the fields, during harvesting and postharvest handling, in the course of processing, and during transport (Beuchat, 1996). Human pathogens that have been found on produce include bacteria, viruses, and parasites. These pathogens make contact with the produce by cross contamination or by being naturally present in the environment. That environment can include fields, air, and dust within a processing or packing facility. In the field, produce is subjected to irrigation, fertilization, and animal contact. Irrigation water is often not potable water and may contain pathogens (Sadovski et al., 1978). Beuchat and Ryu (1997) pointed out that soil contact can lead to accidental contamination by immature compost or environmentally present pathogens. They also stated that animal removal and control should be monitored frequently and, if possible, all animals should be eliminated from entering the premises of vegetable and fruit production and processing facilities.

There are many methods to control microbial growth in fresh-cut fruits and vegetables such as sanitation, temperature control, modifying storage atmosphere (CA/MA), and irradiation. Irradiation has the potential to eliminate vegetative forms of bacterial pathogens as well as parasites and extend shelf life (Chervin and Boisseau,

1994; Farkas et al., 1997; Foley et al., 2002; Gunes et al., 2000; Hagenmaier and Baker, 1997; 1998; Molins et al., 2001; Prakash et al., 2000). However, irradiation doses required to eliminate some microorganisms may cause vitamin C losses, negative textural changes (Gunes et al., 2001), and enzymatic browning (Hanotel et al., 1995) in some vegetable and fruit tissues. Irradiation levels of 1.5-20 kGy are necessary to destroy yeasts and molds, which may exist as spores, and these levels are damaging to plant tissues (Brackett, 1987; Kader, 1986a).

Sanitation of the produce, the washing tanks, and the fresh-cut preparation facility is critical in controlling microbial growth in fresh-cut produce. Use of chemical sanitizers has been successful in preventing contamination of food products by maintaining low levels of microorganisms in the processing environment. Rigorous sanitation of preparation areas reduces the level of microbial contamination, while chemical treatments and low temperatures restrict microbial growth during storage and marketing. Sanitation is usually done using cold (0-1 °C) chlorinated water (0.67-2.7 mM free chlorine at pH 7 or less). Application of chlorine is not very effective at reducing microbial levels on contaminated tissues, but rather primarily acts to reduce microbial loads in the water and prevent cross-contamination (Hurst, 1995). The chlorine rinse also removes cellular contents at cut surfaces that may support microbial growth as well as promote browning, and may also directly inhibit some browning reactions (Brecht et al., 2004). Heated water may also be useful alone or as a supplement to sanitizer treatment in reducing microbial populations on fresh-cut products. Delaquis et al. (1999) demonstrated a 3-log reduction in microbial (mainly pseudomonad) levels on fresh-cut lettuce washed in chlorinated

(1.34 mM NaOCl) water at 47 °C for 3 minutes compared with a 1-log reduction using 4 °C chlorinated water.

Nowadays, there are many other alternatives sanitizers to chlorine that have been used or proposed for use in fresh-cut plants include chlorine dioxide (ClO₂), bromine and iodine compounds, hydrogen peroxide (H₂O₂), peroxyacetic acid, and ozone (Beuchat, 2000).

On the other hand, several chemicals can be used by the fresh-cut produce industry to control microbial growth during storage including acidulants such as sorbic acid and benzoic acid, which lower pH to levels unfavorable to microbes and thus inhibit their growth during storage (Chipley, 1993; Sofas and Busta, 1993). Although the actual mechanism of action of sorbic acid against bacteria is not known, there are some theories as to the action which include the possibility that sorbate inhibits amino acid uptake resulting in either destruction or disruption of the membrane . There is also the theory the sorbate effects enzyme activity by the accumulation of beta unsaturated fatty acids preventing the function of dehydrogenase inhibiting metabolism and growth and the last possibility states that sorbate potentially inhibits respiration by competitive action with acetate in acetyl coenzyme A formation. (Davidson 2001).

Beside their other benefits, edible coatings help retain acidulants and antimicrobials on cut surfaces (Baldwin et al., 1995a,b). Incorporating the antimicrobials potassium sorbate and sodium benzoate into edible coatings on fresh-cut apple and potato improved their effectiveness compared with aqueous dips (Baldwin et al., 1996).

Also, one of the important factors in controlling microbial growth on fresh-cut products is management of temperature and relative humidity (Babic and Watada, 1996;

Omary et al., 1993; Riad and Brecht, 2003). Maintenance of low temperature throughout the postharvest chain plays a pivotal role in controlling microbial growth either by retarding the microbe's activity or by enhancing the produce quality by delaying ripening and senescence (Heard, 1999) and delaying tissue senescence.

On the other hand, CA/MA storage helps in controlling microbial growth directly and indirectly. The direct effect came from the effect of CA/MA on the microorganisms and the indirect effect is a result of the effect of CA/MA on the plant physiology. It is known that CA/MA reduces the respiration rate and delays ripening and senescence and, since delaying senescence (including fruit ripening), reduces susceptibility to pathogens, CA/MA has a beneficial effect in decreasing postharvest diseases (Daniels et al., 1985; El-Goorani and Sommer, 1981). Levels of O₂ below 1% and levels of CO₂ above 10% can have a significant inhibitory effect on fungal growth. Carbon dioxide levels of 10-15% (in commodities that tolerate such levels) can be used to provide a fungistatic effect (Kader, 1986b; Kader et al., 1989). It is well known that sweetcorn can tolerate very high CO₂ levels, as high as 25%, without any symptoms of injury (Riad et al., 2003; Spalding et al., 1978)

Nutrient loss

Nowadays there is more awareness of the importance of fruits and vegetables as a great source of antioxidant compounds such as polyphenolics, vitamin C, vitamin E, β -carotene, and other carotenoids. It has been suggested that these phytonutrients have long-term health benefits and may reduce the risk of diseases such as cancer and heart disease. Most of these antioxidant compounds are known to inhibit cellular and DNA damage caused by reactive oxygen species and free radicals that may lead to degenerative

diseases (Hu et al., 2000; Lagiou et al., 2004; Marrow, 1998; Tapiero et al., 2004). Vegetables and fruits are the primary source of these antioxidant compounds in our daily diet and there is a lot of epidemiological work that shows strong correlations between the delay or suppression of certain diseases and consumption rates of fruits and vegetables (Block and Langseth, 1994; Gershoff, 1993; Steinmetz and Potter, 1996; Ziegler, 1991). Retaining maximum bioactivity of these phytonutrients in fresh-cut fruits and vegetables is a very important goal and this could be achieved through better understanding of the effect of fresh-cut processing, packaging, and storage on bioactivity of these compounds.

Many investigations with fresh-cut fruits and vegetables have demonstrated that concentrations of vitamins and other phytochemical compounds are reduced following fresh-cut operations as a result of wounding and are affected by conditions of handling, packaging, and storage (Kader, 2002; Klein, 1987). But, on the other hand, stress associated with processing may also initiate biosynthesis of many compounds that affect antioxidant content and product quality. The synthesis of wound ethylene after fresh-cut operations can stimulate a variety of physiological responses including loss of vitamin C and chlorophyll and induction of polyphenolic metabolism (Kader, 1985; Saltveit, 1999; Tudela et al., 2002a,b).

Carotenoids. Carotenoids are important compounds in vegetables and fruits for their excellent antioxidant properties and the diversity of color they provide. Human daily consumption of carotenoids is mainly from vegetables and fruits (Goddard and Matthews, 1979). Carotenoids have diverse roles in the biological functioning of both plants and humans. They possess provitamin A and antioxidant activity, modulate detoxifying enzymes, regulate gene expression, aid in cellular communication, and augment immune

functions (Clevidence et al., 2000). Due to their role as antioxidant compounds, as well as the color characteristics they impart to vegetables and fruits, exploration of techniques to retain carotenoids is vital for nutritional and sensory quality characteristics. Disruption of plant tissues by mechanical means or during senescence can also lead to rapid destruction of carotenoids through the action of oxidase enzymes, and may be prevented by the use of reducing agents or MA (Biacs and Daood, 2000; Simpson et al., 1976).

Vitamin C. Ascorbic acid (vitamin C) is a water-soluble antioxidant long associated with inhibition of oxidative reactions and is a key marker compound for determining the extent of oxidation in fresh-cut vegetables and fruits (Barth et al., 1993). Ascorbic acid is easily destroyed during fresh-cut operations and levels are affected by cutting technique (Barry-Ryan and O'Beirne, 1999), gas composition (Gil et al., 1998a; 1999), package design (Barth and Zhuang, 1996), water loss and storage time/temperature (Nunes et al., 1998; Lee and Kader, 2000), light intensity, heat, oxidase enzymes, and pro-oxidant metals (Albrecht et al., 1991; Lee and Kader, 2000).

Polyphenolics. Polyphenolics are a major category of antioxidant compounds present in vegetables and fruits that encompass thousands of individual compounds in various commodities and concentrations. Recent findings have increased the interest in polyphenolic compounds present in fresh and fresh-cut vegetables and fruits due to their elevated antioxidant capacity.

Polyphenolics, along with carotenoids and ascorbic acid, constitute a significant portion of the overall antioxidant capacity of vegetables and fruits; therefore maintaining their level in fresh-cut produce is critical for optimal human health. In previous reviews, the nutritional content was believed to decrease in fresh-cut as compared with intact

vegetables and fruits, especially levels of vitamin C (Klein, 1987; McCarthy and Matthews, 1994). Following tissue wounding and exposure to light and air, antioxidant phytochemicals may be lost to enzymatic and oxidative action at the site of cellular disruption, in secondary or coupled oxidation reactions with lipids, in reactions with wound ethylene, from exposure to chlorinated sanitizers, or from mild desiccation (Barth et al., 1990; Nunes et al., 1998; Park and Lee, 1995; Wright and Kader, 1997a,b). Therefore, developing postharvest treatments to alleviate phytonutrient loss following fresh-cut operations is vital to insure that maximum levels of phytonutrients reaching the consumer.

Several treatments are used in the fresh-cut industry to achieve the goal of maintaining maximum nutritional value in fresh-cut products. For example, proper temperature control is among the most critical factors influencing nutrient retention in fresh-cut vegetables and fruits as enzymatic and oxidative reactions occur more rapidly at elevated storage temperatures. Temperature control serves to reduce microbial populations and slow chemical reactions that affect sensory characteristics and phytochemical concentrations. Also, different physical and chemical treatments have been investigated in fresh-cut vegetables and fruits as means to maintain fresh-like characteristics and nutritional quality. Mild heat treatments or surface acidification have been used as means to inhibit oxidative enzymes and serve to protect some nutrient compounds, as long as a high water-activity is maintained (Dorantes-Alvarez and Chiralt, 2000). Maintaining a high RH during storage was shown to be effective in retaining antioxidant compounds (Jiang and Fu, 1999). Ascorbic acid is commonly applied to cut surfaces through edible coatings or dips to prevent browning on cut surfaces: acting both

as an acidulant and a reducing agent, ascorbic acid can reduce quinones back to colorless phenolic compounds. Combinations of reducing agents and acids were effective in prevention of surface browning and retention of sugars and organic acids in fresh-cut apples and their effect may be enhanced when combined with additional preservation techniques such as MAP or proper temperature control (Buta et al., 1999).

On the other hand, the use of MAP has proven to be an effective means to reduce enzymatic and autooxidative reactions affecting phytonutrients in fresh-cut vegetables and fruits by reducing concentrations of O₂ and increasing CO₂. Modified atmospheres were used to maintain higher levels of provitamin A and vitamin C in fresh-cut broccoli (Barth et al., 1993; Barth and Zhuang, 1996; Paradis et al., 1996) and jalapeno peppers (Howard and Hernandez-Brenes, 1998), but had little effect on provitamin A concentrations in peach and persimmon slices (Wright and Kader, 1997a), and was ineffective in ascorbic acid retention in sliced strawberry or persimmon (Wright and Kader, 1997b). Extreme CO₂ concentrations (>20%) may actually cause greater degradation or suppressed synthesis of vitamin C (Agar et al., 1997; Tudela et al., 2002b; Wang, 1983) and anthocyanins (Gil et al., 1997; Holcroft et al., 1998; Holcroft and Kader, 1999; Tudela et al., 2002a), while certain CO₂ levels may induce biosynthesis of provitamin A carotenoids (Weichmann, 1986). Modified atmospheres did not affect flavonoid content of Swiss chard, but significantly reduced levels of ascorbic acid (Gil et al., 1998a), while flushing packages of fresh-cut lettuce with 100% N₂ retained higher ascorbic acid concentrations than passive MAP and air controls (Barry-Ryan and O'Beirne, 1999). In fresh-cut spinach, flavonoid content remained constant during storage in air or MAP, but spinach in MAP contained higher dehydroascorbic acid

concentrations that resulted in lower antioxidant activity compared with air-stored spinach (Gil et al., 1999). However decreases in flavonoids were observed in ‘Lollo Rosso’ lettuce stored in MAP (Gil et al., 1998b), further indicating a commodity-specific role of gas composition on overall phytonutrient retention.

Furthermore, the application of food-grade compounds into wash water or on the surface of fresh-cut vegetables and fruits as coatings has an advantage of immediate benefits at the active site of phytonutrient deterioration. The benefits of these edible coatings include decreased respiration rate, browning inhibition, and retention of various quality factors by creating a barrier to O₂, which influences enzymatic and nonenzymatic oxidation rates (Li and Barth, 1998). Edible coatings are a common method to extend the fresh-like appearance and quality characteristics of many vegetables and fruits (Baldwin et al., 1995a,b; 1996). Fresh-cut carrots with cellulose-based edible coatings retained greater provitamin A levels during storage in one study (Li and Barth, 1998), but another coating had no effect (Howard and Dewi, 1996).

Loss of flavor and aroma

Flavor quality of fresh-cut vegetables and fruits is critical to their acceptance and appreciation by consumers. Sensory attributes such as sweetness and characteristic aroma may be the most important indicators of shelf life from the consumer’s point of view. The challenge in fresh-cut vegetable and fruit handling is to maintain the taste and aroma attributes of the original whole product. Due to the short shelf life of fresh-cut fruits and vegetables, starting with produce at its optimum maturity or ripeness stage and using the highest quality standards is extremely important in maintaining flavor shelf life (Beaulieu and Gorny, 2002).

Taste and aroma together make up flavor, which contributes to the recognizable nature of a food. Taste refers to detection of nonvolatile compounds on the tongue while aroma is related to volatile compounds detected in the nose. These two aspects of flavor are inextricably linked as it has been shown that perception of aroma can be influenced by levels of taste components, and vice versa (Beaulieu and Baldwin, 2002).

The taste component in fruits and vegetables mainly depends on the sugar and organic acid levels and the relation between them. As a result of increased respiration due to wounding during preparation of fresh-cut produce, there is a depletion of sugar levels in the commodity and therefore organoleptic quality is reduced, especially in products like melons whose quality depends on high levels of sugars. This problem tends to be worse in fruits such as tomato or melon that have very limited capacity to replenish soluble sugars lost to accelerated respiration during storage or ripening, in comparison with fruits such as banana and apple that have a reserve of starch and can convert it to sugars during ripening. Organic acids are one of the major respiratory substrate and the increase in tissue pH in fresh-cut apples has been attributed to utilization of organic acids in respiration (Kim et al., 1993). Depletion of acids also can have negative organoleptic effects in fruits like apple, peach, and mango for which the balance of sweetness (sugars) and tartness (acids) is an important flavor attribute.

The second component of flavor, aroma, is related to synthesis of volatiles during the growth and development of fruits or vegetables, but the most dramatic production coincides with fruit ripening. These volatiles include alcohols, aldehydes, esters, ketones, lactones, and other compounds (Baldwin, 2002). Little is known about the effects of different storage temperatures and atmospheres on normal aroma volatile production in

fresh-cut fruits and vegetables other than the inhibition that occurs as a consequence of chilling injury (Buttery et al., 1987; Maul et al., 2000). Brecht et al. (2004) stated in their review that fresh-cut fruit taste becomes bland during the course of extended storage due to the loss and/or the reduced production of aroma volatiles. Recently there is an interest in designing edible coatings to retain volatile flavor compounds within the tissue as a method to overcome this problem (Baldwin et al., 1998; Miller and Krochta, 1997).

Fresh-Cut Sweetcorn Kernels

There is not much information about sweetcorn as a fresh-cut commodity. A few European retailers prepare fresh-cut sweetcorn kernels onsite and don't store them due to the excessive perishability of the product. It is well documented that sweetcorn is a very perishable product as a result of its very high respiration rate, which results in a quick loss of the sweetness (the most important characteristic of sweetcorn) unless it was rapidly cooled and stored at a low temperature (as close to 0 °C as possible). Supplementation of proper temperature management by gas modification (CA or MA) greatly helps in maintaining high quality through reducing the high respiration rate (Brecht, 2002; Riad and Brecht, 2001; Riad et al., 2003). As a result of fresh-cut processing (cob trimming, de-husking, and kernel separation), the respiration rate increases and also there may be increased microbial load due to the open wounds in the kernels. This makes fresh-cut sweetcorn kernels a very delicate product, yet the early work by Brecht (1999) showed that there is great potential for sweetcorn kernels as a fresh-cut product. The main problem in fresh-cut sweetcorn kernels storage is the formation of brown pigment in kernels when they are cooked. This after-cooking browning is more pronounced as kernel maturity advances, increases with longer storage period and higher storage temperature, and is also higher in cut kernels than whole

kernels (Brecht, 1999). Riad et al. (2003) found that the browning starts to appear after 7 d of storage at 5 °C and was prevented during 2 weeks storage at 0 °C. Also, storing the cut kernels in CA (2% O₂ plus 10% CO₂) prevented this after cooking browning even when the kernels were stored at 5 °C.

It has been suggested (Brecht, 1999, Riad and Brecht, 2001) that sweetcorn after-cooking browning may be caused by the Maillard reaction, a non-enzymatic reaction usually associated with food processing due to a reaction between free sugars and basic amino acids at elevated temperatures, forming a brown pigment. Sweetcorn has a high background of soluble sugars (Courter et al., 1988) and amino acids (Grunau and Swiader, 1991). More work is needed to determine the exact cause for sweetcorn after-cooking browning.

CHAPTER 3
SWEETCORN TOLERANCE TO REDUCED O₂ WITH OR WITHOUT ELEVATED
CO₂ AND EFFECTS OF CONTROLLED ATMOSPHERE STORAGE ON QUALITY

Introduction

Storing fruits or vegetables in controlled (CA) or modified atmosphere (MA) enriched with high CO₂ and/or utilizing low O₂ levels could be a very beneficial tool in maintaining product quality and extending shelf life. Atmosphere modification is achieved either by reducing the concentration of O₂, which is required for respiration, or by increasing the CO₂ concentration, which inhibits respiration and ethylene action. Reducing respiration consequently retards sugar loss and the growth of pathogens; and it also decreases yellowing through decreasing chlorophyll degradation. Several articles have been published on the benefits of CA/MA technology on the extension of perishable product shelf life (Chinnan, 1989; El-Goorani and Sommer, 1981; Kader, 1987; Kader et al., 1989; Shewfelt, 1986; Zagory and Kader, 1988).

On the other hand, increasing CO₂ above, or decreasing O₂ below the commodity tolerance level may result in product deterioration due to anaerobic respiration and off-flavor and odor development. These tolerance levels may be affected by the physiological condition of the commodity, the storage temperature, and the storage duration. One of the challenges in CA storage is to determine the tolerance levels that result in the maximum benefits with the minimum possibility of injury to the stored commodity.

Fresh sweetcorn (*Zea mays* L. *rugosa*) is a very perishable food product and is prone to rapid postharvest deterioration caused by kernel desiccation, loss of sweetness,

husk discoloration, and loss of taste and aroma. Most of these problems are mainly due to its high respiration rate, which suggested that using CA/MA could be beneficial in sweetcorn storage.

The early work by Spalding et al. (1978) found that sweetcorn appearance and flavor were not significantly improved using CA storage and they concluded that breeding cultivars that better retain quality, in combination with prompt pre-cooling, offers more potential for success than MA or CA. On the other hand, there are many other reports stating the beneficial effects of using gas modification either by CA or MA for sweetcorn. Deak et al. (1987) demonstrated that using modified atmosphere packaging (MAP) eliminated water loss and maintained beneficial CO₂ and O₂ levels surrounding the sweetcorn. These effects together with lower storage temperature markedly reduced postharvest deterioration and extended the shelf life. But MAP treatment increased microbial growth due to the water-saturated atmosphere. Similar results were obtained by Risse and McDonald (1990). However, reduced O₂ and elevated CO₂ have also been reported to reduce decay and maintain husk chlorophyll levels (Aharoni et al., 1996; Schouten, 1993). Aharoni et al. (1996) suggested that the increase in microbial growth reported by Deak et al. (1987) and Risse and McDonald (1990) was probably due to relatively low CO₂ levels during storage. The main benefit of CA/MA is that the loss in sucrose content, the decisive factor in determining the taste quality of sweetcorn, was delayed by high CO₂ and/or low O₂ content (Riad et al., 2002, Risse and McDonald, 1990; Rumpf et al., 1972; Schouten, 1993).

Generally, the quality of fresh sweetcorn depends to a large extent on the kernel sweetness, texture, and flavor. Sweetness in sweetcorn is closely related to kernel sucrose

content (Reyes et al., 1982). Texture and eating quality of sweetcorn consists of several factors, including pericarp tenderness, levels of water-soluble polysaccharides (WSP) in the endosperm, and moisture content (Bailey and Bailey, 1938; Culpepper and Magoon, 1927; Wann et al., 1971). On the other hand, flavor and aroma, which are not as easily defined as sweetness and texture, are most often associated with kernel content of many volatiles that are present in fresh sweetcorn and considered to contribute to its characteristic flavor, especially dimethyl sulfide (DMS), which is responsible for the characteristic aroma of cooked sweetcorn (Flora and Wiley, 1974a,b; Wiley, 1985; Williams and Nelson, 1973; 1974). Despite its importance in the overall quality of sweetcorn, there is no or very little information on the effect of CA on the flavor and aroma and specifically on the levels of DMS during storage.

The objective of this work was 1) to give a better idea about the tolerance level of sweetcorn to reduced levels of O₂ with and without elevated levels of CO₂; 2) to determine the best gas composition that results in the best quality after storage; and 3) to determine the effects of CA storage on the different quality attributes of sweetcorn.

Materials and Methods

Plant Material

Fresh harvested sweetcorn (*Zea mays* L. rugosa var. Prime Time) cobs were harvested at a local farm in Florida (Hugh Branch Inc., Pahokee) on 26 November 1999 precooled, and delivered overnight by refrigerated truck to a local grocery chain's distribution facility in Jacksonville (Publix Distribution Center, Jacksonville, Fla.) on 27 November, where it was picked up, transferred to the postharvest lab (University of Florida, Gainesville, Fla.), and the experiment started on the same day. As soon as the sweetcorn arrived at the lab, the cobs were sorted, trimmed, and the outermost leaves

were removed. A window of about 3-4 cm width was opened in the husk to show 3-4 rows of kernels. The sweetcorn cobs were kept at 5 °C for about 3 h until they were distributed among the treatments at the start of the CA application.

Controlled Atmosphere Storage

A flow-through CA system was used. Four sweetcorn cobs were placed in each of three, 3.8-L glass jars per treatment in a 5 °C storage room, then the jars were closed and the CA gas mixture was introduced to the jars. Six gas compositions were used in this study, namely air, air plus 15 or 25% CO₂ (which resulted in O₂ concentrations of 17.7 and 15.6%, respectively), 2% O₂, and 2% O₂ plus 0, 15, or 25% CO₂. The balance in all CA treatments was made up with N₂. The CA gas mixtures were established using a system of pressure regulators, manifolds, and needle valve flowmeters to blend air, N₂, and CO₂ from pressurized cylinders. The CA was a flowing system with flow rates set to maintain respiratory CO₂ accumulation under 0.5%. The gas flow was humidified by bubbling the gas mixture through water before introducing it to the containers. Gas composition in CA was monitored daily for 14 d using a Servomex O₂ and CO₂ analyzer (Servomex, Norwood, Mass.). The O₂ analyzer measures the paramagnetic susceptibility of the sample gas by means of a magneto-dynamic type measuring cell, while CO₂ is measured by infra-red absorption using a single beam. The analyzer was calibrated using N₂ and certified O₂ and CO₂ standards.

Parameters

Respiration rate

Respiration rate was measured daily using a static method in which the storage containers were closed and incubated for 2 h. Carbon dioxide concentration was measured before and after incubation, and respiration rate was calculated.

Carbon dioxide levels were measured using a Gow Mac, series 580 gas chromatograph (Gow Mac Instruments Co., Bridgewater, N.J.) equipped with a thermal conductivity detector (TCD) and a Hewlett Packard Model 3390A integrator (Hewlett and Packard Co. Avondale, Pa.). Column, detector, and injector temperatures were 40, 28, and 28 °C, respectively. Detector current was 90 mA during the analysis. Carrier gas was He with a 30 mL·min⁻¹ flow rate at 276 KPa (40 psi). A 1-mL sample was withdrawn from the storage container headspace using a 1.0 mL plastic syringe and 0.5 mL was injected in the gas chromatograph. Calibration was done prior to sample analysis using a certified standard mixture.

Sugar content

Total soluble sugars were measured in raw and cooked kernels using the phenol-sulfuric method described by Dubois et al. (1956). In this experiment, 15 g of sweetcorn kernels were blended in a commercial blender with 85 mL of 95% ethanol for 2-3 min then were heated for 20 min in an 85 °C water bath. Then the samples were stored overnight at -20 °C to precipitate ethanol-insoluble materials. The samples were then filtered through Whatman #2 filter paper in a Buchner funnel attached to a side-arm Erlenmeyer flask. A vacuum pump was connected to the side arm to expedite the extraction. The collected filtrates were volumetrically adjusted to 200 mL using 95% ethanol and 20 mL portions of the extracts were stored in scintillation vials at -20 °C until the measurements were performed.

To prepare samples for measurement of total soluble sugars, 0.5 mL of 5% phenol (w/w) was added to 0.5 mL of diluted (1:250) ethanol extract in test tubes, the mixture was vortexed, then 2.5 mL concentrated sulfuric acid was added and the mixture vortexed again. The test tubes were let to stand for 10 min then were placed in a 25 °C water bath for another 10 min to stop the reaction. The total soluble sugars were measured by reading the absorbance at 490 nm using a Shimadzu UV-1201 spectrophotometer (Shimadzu Scientific Instruments, Inc.; Tokyo, Japan). The absorbance values were compared to a standard curve of glucose.

Dimethyl sulfide (DMS) content

Dimethyl sulfide was measured chromatographically using a modified method from Ren et al. (2001) in which a water bath was used instead of a microwave oven. A 10-g sample of sweetcorn kernels was placed in a 40-mL glass vial fitted with a septum under the screw cap (Fisher Scientific, Pittsburgh, Pa., Cat. No. 05-727-6) then the vial was placed in a water bath (70 °C) for 2 h. A 1-mL sample of the vial head space was withdrawn with a 1.0-mL plastic syringe and injected on a HP 5890 Series II gas chromatograph (Hewlett Packard Co. Avondale, Pa.) equipped with a flame ionization detector and a Hewlett Packard Model 3390A integrator. A 3 mm by 2 m stainless steel column with 20% OV-101 on 80/100 CWHP packing (Alltech, Deerfield, Ill.) was used and the carrier gas was N₂ (30 mL·min⁻¹). Oven, injector, and detector temperatures were 70, 150, and 200 °C, respectively. Calibration was done prior to sample analysis using standards that were prepared from the authentic compound (Sigma-Aldrich Co. St. Louis, Mo.; Cat. No. 47,157-7)

Visual appearance evaluation

Husk, silk, and kernel appearance, and kernel denting were measured using a scale from 1 to 9, where a score of 9 represents excellent quality and a score of 1 represents the lowest quality level as described in Table 3-1.

Statistical Analysis

The experimental design was a completely randomized design with three replicates consisting of four sweetcorn cobs in each of three jars per treatment. The data were analyzed using the Statistical Analysis System (SAS). Analysis of variance (ANOVA) was used to determine the effect of treatments on the dependent variables. Means were separated by the least significant difference (LSD) test at $P < 0.05$.

Results and Discussion

Controlled atmosphere storage is known for its beneficial effects on fresh fruits and vegetables. Sweetcorn was expected to benefit from this technique during storage since CA can reduce the high respiration rate of sweetcorn and consequently reduce sugar, water, and flavor losses. In this work, sweetcorn was stored in different gas compositions in a flow-through CA system in which fresh sweetcorn cobs were stored in air or 2% O₂ plus 0, 15, or 25% CO₂ at 5 °C to determine the best atmosphere composition for maintaining quality and in order to determine the tolerance levels of sweetcorn to low O₂ and/or high CO₂.

Respiration rate (Fig. 3-1) was significantly affected by CA treatment. Storing sweetcorn in 2% O₂ plus either 15 or 25% CO₂ significantly increased the respiration rate compared with air storage, which might be a result of the sweetcorn metabolism switching to anaerobic respiration. There was no significant effect of 2% O₂ plus 0% CO₂ on respiration, and storage in either 15 or 25% CO₂ in air (17.7 or 15.6% O₂,

respectively) significantly reduced respiration compared with the air control. These results suggest that sweetcorn is more sensitive to reduced O₂ plus elevated CO₂ together than to either alone. Riad et al. (2002) reported that no ethanol or acetaldehyde was detected in the atmosphere surrounding sweetcorn stored in perforation-mediated modified atmosphere packaging (PM-MAP) even with steady state CO₂ levels as high as 25% plus O₂ levels as low as 1%, but de-husked sweetcorn was used in those experiments, which might have affected the tolerance levels to high CO₂ and low O₂. Also, these results are not with agreement with Morales-Castro et al. (1994), who reported that elevated CO₂ had a negligible effect on respiration rate in the range from 0 to 15% while reduced O₂ had a large influence on respiration rate in the range from 21 to 0%.

While all CA treatments had significantly higher levels of sugars than the air control (Fig. 3-2), which is the main quality parameter for sweetcorn (Evensen and Boyer, 1986; Showalter and Miller, 1962), the highest sugar content was found in the two CA treatments that resulted in reduction of respiration rate (Fig. 3-1). These results are in agreement with the results obtained by Riad et al. (2002) who reported that higher sugar content was maintained in PM-MAP treatments with CO₂ concentrations up to 25%. Also, in the early work by Rumpf et al. (1972), higher sugar levels were obtained with all CA treatments over air storage, even with O₂ concentration as low as 1%. Schouten (1993) observed that even when CA treatment resulted in a significantly higher ethanol production, which might indicate fermentative metabolism, there was no effect on the sugar content. On the other hand, these results are not in agreement with the results

obtained by Spalding et al (1978) who found that CA treatment resulted in no or negative impact on sugar content.

Controlled atmosphere treatments had a positive impact on the visual quality of stored sweetcorn (Fig. 3-3). Elevated CO₂ significantly reduced loss of greenness and maintained the fresh appearance of the husks. The CA also improved silk appearance (reduced discoloration) over the air control, but it had no effect on kernel denting, which might be due to the minimal water loss that occurred in all treatments since all the gas mixtures were humidified before being introduced to the storage containers. Kernel appearance was also improved in all CA treatments, especially air plus 15% CO₂ and 2% O₂ plus 15% CO₂. Storage in 2% O₂ plus 15% CO₂ resulted in the best score in all visual quality parameters. These results are in agreement with Aharoni et al. (1996) who demonstrated that sweetcorn stored in MAP with high CO₂ level had better general appearance quality due to better green color maintenance of the husk, less denting (due to the reduced denting as a result of the reduction in weight loss), and also reduced decay incidence.

Dimethyl sulfide is the main characteristic volatile component in sweetcorn and is responsible for the characteristic aroma of cooked sweetcorn, providing the “corny” smell during cooking (Flora and Wiley, 1974b; Wiley, 1985; Williams and Nelson, 1973; 1974; Wong et al., 1994). It was noticed that the levels of this compound is increased by canning or freezing (Flora and Wiley, 1974a) but there is no information about the effect of storage in CA storage on the levels of DMS. There were significantly greater concentrations of DMS in CA-stored sweetcorn compared with the air control at the end of the 14-d storage period (Fig. 3-4). Storing sweetcorn in 2% O₂ plus 15% CO₂ resulted

in the highest DMS content followed by air or 2% O₂ plus 25% CO₂. The results suggest that elevated CO₂ was more efficient than reduced O₂ in maintaining higher levels of DMS after 14 d of storage at 5 °C.

In conclusion, CA storage was beneficial in maintaining most sweetcorn quality parameters during 14 d of storage at 5 °C. Storage in 2% O₂ plus 15% CO₂ gave the best result in terms of quality maintenance since it preserved the highest sugar level, reduced deterioration in sweetcorn visual quality to the greatest extent, and maintained the highest DMS content and thus presumably the highest flavor and aroma. Sweetcorn had a high tolerance level to either 2% O₂ or 25% CO₂ alone for 2 weeks, but the tolerance was apparently less when the two gases were combined since 2% O₂ plus 25% CO₂ provided less benefits compared with using 2% O₂ plus 15% CO₂.

Table 3-1. Description of the visual quality ratings used for visual quality evaluation of sweetcorn after storage.

Rating	Description	Parameter			
		Husk appearance	Silk appearance	Kernel appearance	Kernel denting
9	Field fresh	Field fresh, turgid appearance	Light color, fresh, and turgid	Fresh with bright, shiny color and very turgid	No denting with turgid kernels and bright appearance
7	Good	Reasonably fresh, leaves still green and flexible	Not that fresh but still had a light color and turgid texture	Still noticeably bright color and turgid	No denting with slightly dull kernel appearance
5	Fair	Drying and yellowing apparent, some leaves brittle	Starting to show some brown color and some drying	Dull appearance with no denting	A few kernels showing denting and more dull kernels
3	Unmarketable	Portion of the leaves show dry appearance or water soaking	Obvious discoloration and dryness with limp texture	Severe dull appearance and some brown or white color	10-30% of the kernels showing denting and dull appearance
1	Unusable	Completely dried out	Limp texture or watery decay	Kernels showing severe denting or decay	More than 30% of the kernels dented

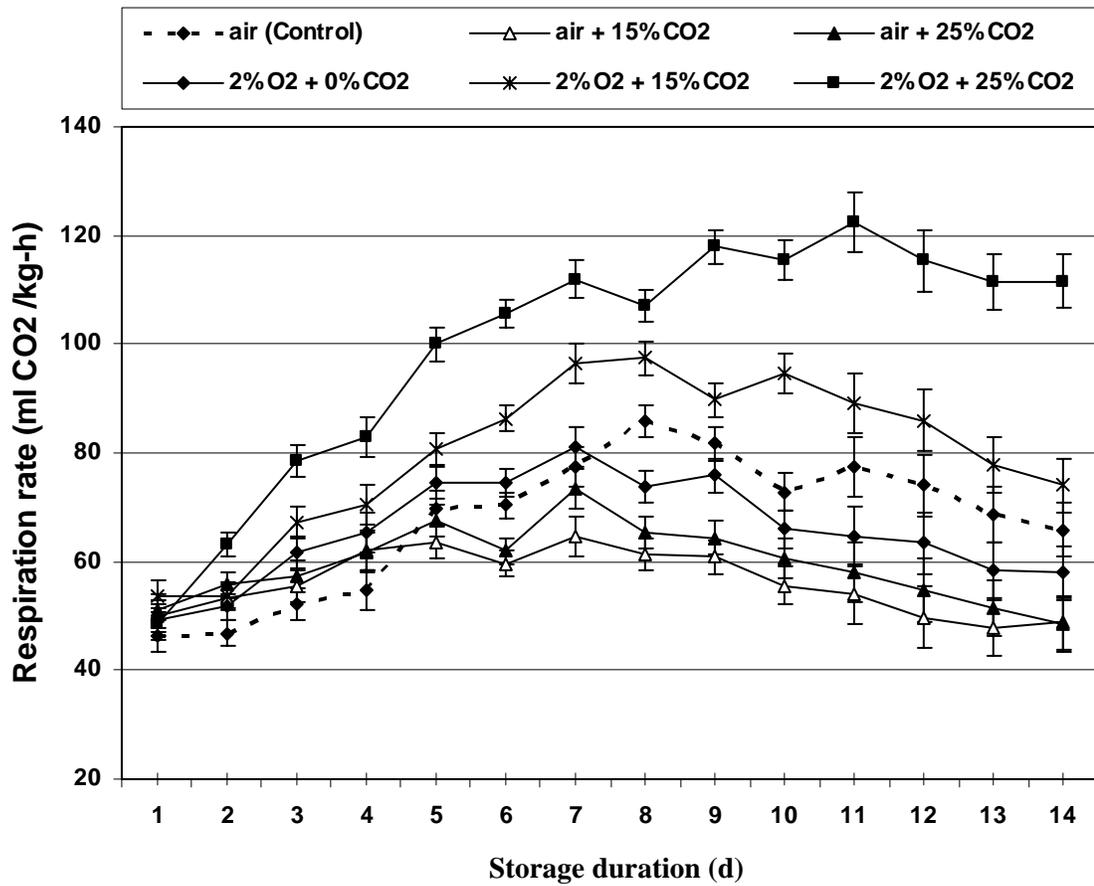


Fig. 3-1. Effect of controlled atmosphere storage on respiration rate ($\text{mL CO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) of sweetcorn stored in air or 2% O_2 plus 0, 15, or 25% CO_2 at 5 °C. Bars indicate ± 1 SD value ($n=3$).

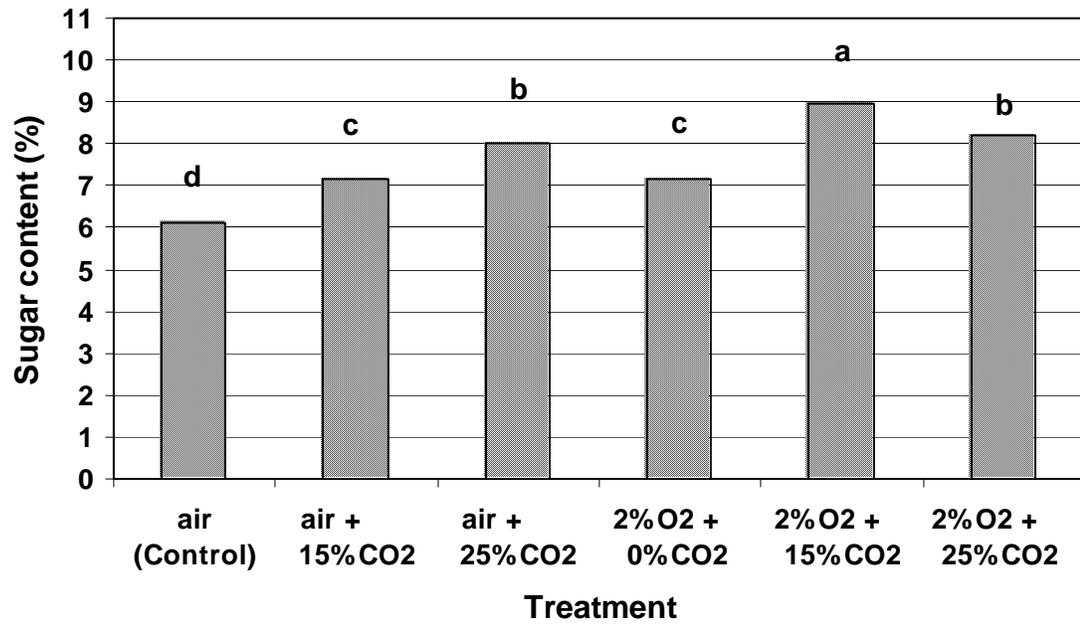


Fig. 3-2. Effect of controlled atmosphere storage on the sugar content (%) in sweetcorn kernels from cobs stored for 14 d in different gas compositions (air or 2% O₂ plus 0, 15, or 25% CO₂) at 5 °C. Columns with the same letter are not statistically different at the 5% level by LSD.

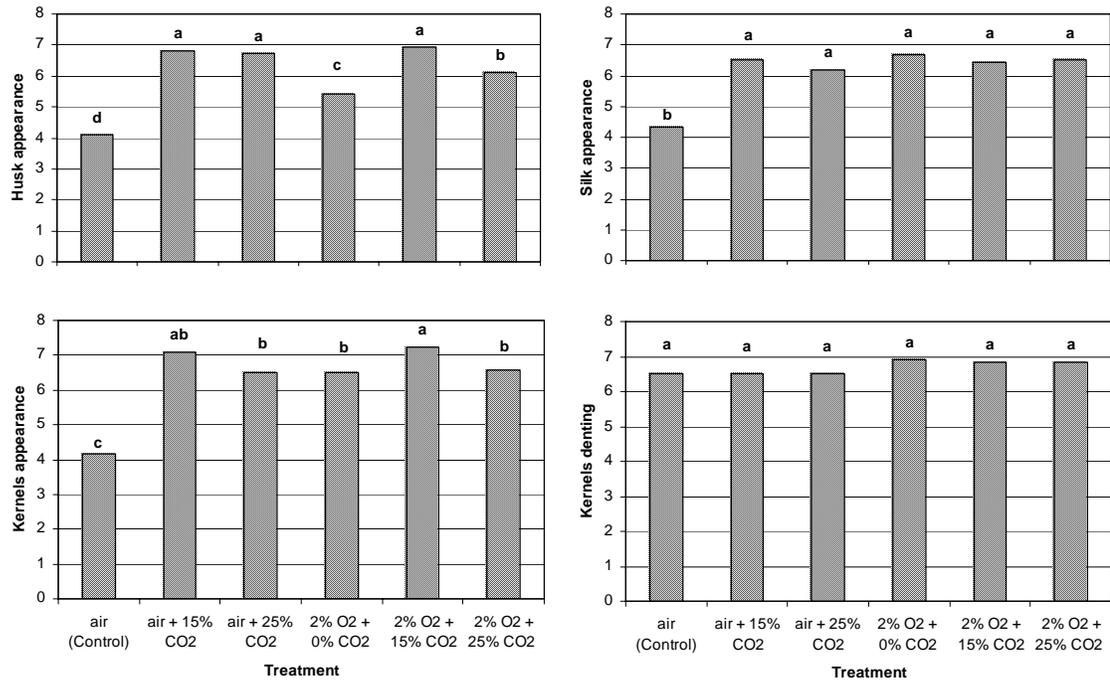


Fig. 3-3. Effect of controlled atmosphere storage on the different attributes of sweetcorn visual quality after 14 d of storage at 5 °C in different gas compositions (air or 2% O₂ plus 0, 15, or 25% CO₂). Husk, silk, and kernel appearance and kernel denting were evaluated using a scale from 1 to 9, where a score of 9 represents the best quality and a score of 1 represents the lowest quality. Columns with the same letter are not statistically different at the 5% level by LSD.

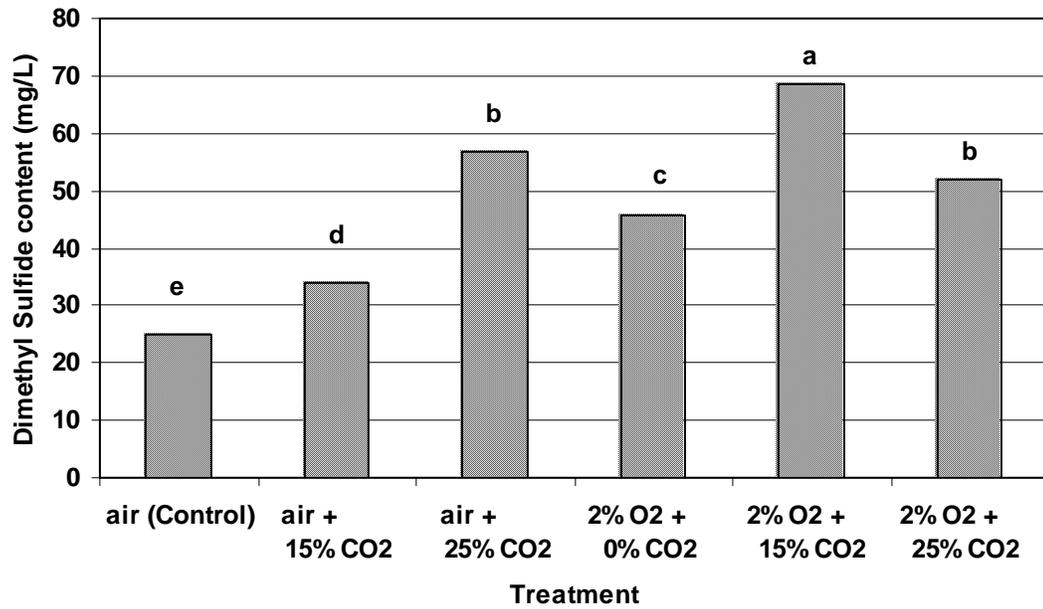


Fig. 3-4. Effect of controlled atmosphere storage on dimethyl sulfide content ($\text{mg}\cdot\text{L}^{-1}$) in sweetcorn kernels from cobs stored for 14 d in different gas compositions (air or 2% O_2 plus 0, 15, or 25% CO_2) at 5 °C. Columns with the same letter are not statistically different at the 5% level by LSD.

CHAPTER 4
PERFORATION MEDIATED MODIFIED ATMOSPHERE PACKAGING (PM-MAP)
OF SWEETCORN

Introduction

The beneficial effect of atmosphere modification during fresh fruit and vegetable storage is well documented (Kader et al., 1989; Kader, 1986b; Weichmann, 1986). Because of the limitation of CA storage to relatively large-scale systems, the modified atmosphere packaging (MAP) technique was developed to provide the optimal atmosphere, not for the entire storage facility, but for just the product, thus maintaining the desired atmosphere during almost all of the postharvest chain even during the retail display. Modified atmosphere packaging is an atmosphere control system that relies on the natural process of respiration of the product along with the gas permeability of the package holding the product. Due to respiration, there is a build up of CO₂ and a depletion of O₂ within the package and the package material helps to maintain these modified gas levels until the package reaches the steady state because of restricted gas permeability. The steady state levels of O₂ and CO₂ are a function of the mass of product, its respiration rate, the package permeability properties, the area of the package surface available for gas diffusion and temperature. In addition, the time to reach steady state conditions is largely a function of the void volume in the package. In the steady state, the O₂ diffusing into the package equals the O₂ being consumed by respiration, and the CO₂ diffusing out of the package equals the CO₂ being produced by respiration. Besides the benefits of modifying the O₂ and CO₂ levels in lowering respiration rate, MAP has the

additional benefits of water loss prevention, produce protection, and brand identification. (Kader et al., 1989)

The early work by Spalding et al. (1978) found that sweetcorn appearance and flavor were not significantly improved under modified storage conditions (CA and low pressure) and concluded that breeding cultivars that retain quality, in combination with prompt pre-cooling, offers more potential for success than modified atmospheres. In that work, sweetcorn cobs were held in 2 or 21% O₂ plus 0, 15, or 25% CO₂ atmospheres for 3 weeks at 1.7 °C. On the other hand, there are many reports stating the beneficial effects of using MAP for sweetcorn. Deak et al. (1987) demonstrate that using MAP (shrink wrap film with moisture permeability of 0.1 g·100 cm⁻²·24 h⁻¹ and O₂ permeability varying from 0.4 to 40 mL·100 cm⁻²·24 h⁻¹) at 10 °C eliminated water loss and maintained beneficial CO₂ and O₂ levels of ~14-18% O₂ plus ~4-5% CO₂ within the package. These effects together with lower storage temperature (10 °C versus 20 °C) markedly reduced postharvest deterioration and extended the shelf life. But MAP treatment increased microbial growth due to the water-saturated atmosphere. Similar results were obtained by Risse and McDonald (1990) who stored sweetcorn at 1, 4, or 10 °C for 26 d. either unwrapped or wrapped in stretch or shrink wrap. It was concluded that film wrapping maintained freshness and reduced moisture loss better than the lack of wrapping. Also these authors recommended stretch wrap over shrink wrap since stretch wrap generated higher CO₂ levels (4-9% versus 1-3%) and lower O₂ levels (14-19% versus 18-21%), which resulted in maintenance of higher total soluble solids.

Reduced O₂ and elevated CO₂ have been reported to reduce decay of sweetcorn and maintain husk chlorophyll levels (Aharoni et al., 1996; Schouten, 1993). Aharoni et al.

(1996) demonstrated that sweetcorn wrapped in either polyvinyl chloride (PVC) or polyolefin stretch films benefited from the reduction of water loss, but there was more pathological breakdown on the trimmed ends of the cobs in the case of the PVC film. Polyolefin stretch film has lower permeability rates for O₂ and CO₂ than PVC film and consequently generated higher CO₂ levels (~10% versus ~3%) and lower O₂ levels (~7% versus ~15%) in the packages and this resulted in a significant reduction in the decay incidence and water loss and a significantly better maintenance of the sweetcorn's general appearance (Aharoni et al., 1996). These levels of O₂ and CO₂ did not trigger anaerobic respiration, and ethanol levels in the packages were very low until the packages were transferred to 20 °C to simulate retail conditions. Aharoni et al. (1996) suggested that the increase in microbial growth reported by Deak et al. (1987) and Risse and McDonald (1990) was probably due to relatively low CO₂ levels within their packages, a consequence of the high ratio of the CO₂ versus O₂ permeability coefficients of plastic films as mentioned previously. Schouten (1993) demonstrated that using CA storage (2% O₂ plus 10% CO₂) retained higher sugar content than air storage and CA was more beneficial when used at a higher temperature of 5-6 °C compared with 1-2 °C. Similar results were obtained in the work of Rumpf et al. (1972), which clearly proved that the loss in sucrose content, the decisive factor in determining the taste quality of sweetcorn, may be delayed by both low temperature (0 °C versus 5 or 10 °C) and low O₂ content since 1% O₂ was the best treatment in retaining the sucrose levels after 11 d of storage at 5 °C followed by 2% O₂, then 4% O₂, and finally air storage. All the CA treatments in this experiment had 0% CO₂.

The development of MAP has faced several problems such as the lack of commodity respiration data under several temperatures and gas compositions, lack of permeability data for packaging materials at different temperatures and relative humidities, and also the lack of consistency in respiration data gathered for the same commodity (Kader, 1987; Kader et al., 1989).

In medium and high respiring commodities, like sweetcorn, using commonly available films such as low density polyethylene (LDPE), PVC, and polypropylene is not ideal due to their low gas transmission rates, which may lead to anaerobic respiration (Morales-Castro et al., 1994). Fonseca et al. (2000) summarized some of the limitations of using the flexible polymeric films that result from their structure and permeation characteristics such as 1) Films are not strong enough for large packages; 2) The film permeability characteristics change unpredictably when films are stretched or punctured; 3) Film permeability may be affected by water condensation; 4) Film permeability is too low for high respiring products; 5) Products that require high CO₂/O₂ concentrations may be exposed to anaerobiosis in film packages because of the high ratio of the film CO₂ and O₂ permeability coefficients. On the other hand, perforation-mediated modified atmosphere packaging (PM-MAP) is a potential technique for postharvest preservation of fresh horticultural commodities that overcomes several of the disadvantages associated with films. In this technique, instead of using the common polymeric films, a package is used in which the regulation of gas exchange is achieved by single or multiple perforations or tubes that perforate an impermeable package. (Emond and Chau, 1990a,b; Emond et al., 1992; Silva et al., 1999a).

For sweetcorn, the recommended atmosphere is 2% O₂ plus 10-20% CO₂ (Kader et al., 1989; Riad and Brecht, 2003; Schouten, 1993; Spalding, 1978) and, to get such an atmosphere, a package with a β value (the ratio between CO₂ and O₂ mass transfer coefficients) of around 1 is required. Using PM-MAP could be very beneficial in obtaining such a steady state atmosphere since the β value of a perforation is around 1, which is better than the common polymers used for film formulation. Most of such films have β values ranging from 2 up to 10 (Exama et al., 1993; Kader et al., 1989), which, for sweetcorn, would likely lower the package O₂ level and increase the CO₂ level to the critical levels that induce anaerobic respiration and accompanying increased ethanol, acetaldehyde, and off-flavor production.

Using the PM-MAP technique has many potential advantages (Fonseca et al., 1997) such as 1) The low values of mass transfer coefficients implying that high-respiring produce can be packed in this system; 2) MAP using perforations can be adapted easily to any impermeable container, including large bulk packages; polymeric films are not strong enough for packs much larger than those used for retail, but perforations can be applied to retail packages as well as shipping boxes because rigid materials can be used; rigid packages also can prevent mechanical damage to the product; 3) It is a flexible system due to the ability to change the gas transfer coefficients by selecting the adequate size and shape of the perforation; 4) Commodities requiring high CO₂ concentrations along with relatively high O₂ concentrations can be packed with this system.

The respiratory quotient (RQ) is the ratio between the respiration rate expressed in terms of CO₂ production and respiration expressed as O₂ consumption. The RQ has been observed to depend on both temperature and gas composition. It increases with a decrease

in O₂ level or an increase in CO₂ level and also with an increase in the temperature (Beaudry et al., 1992; Joles et al., 1994). Joles et al., (1994) reported that in red raspberry RQ was around 1 at 0 °C and increased to about 1.3 at 10 or 20 °C. The RQ is assumed to be around 0.7 when the respiratory substrate is lipid, 1.0 when it is sugar, and up to 1.3 when it is acid. Under stress conditions, when the O₂ level decreases or CO₂ increases beyond the critical level and anaerobic respiration takes place, the RQ usually is much greater than one. In grated carrots and mushrooms, the RQ reached high levels of up to 5-6 when anaerobic respiration started (Beit-Halachmy and Mannheim, 1992; Carlin et al., 1990; Mannapperuma et al., 1989).

The general objectives of this study were to examine the validity and the effectiveness of PM-MAP to create the previously predicted atmosphere and also to study the effect of this treatment on the visual and chemical quality of sweetcorn.

Materials and Methods

Plant Material

Fresh harvested sweetcorn (*Zea mays* L. *rugosa* var. Prime Time) cobs were harvested from a local farm in Florida (Hugh Branch Inc., Pahokee, Fla.) on 24 January 2000 pre-cooled, and delivered overnight by refrigerated truck to a local grocery chain's distribution facility in Jacksonville (Publix Distribution Center, Jacksonville, Fla.) on 25 January. The sweetcorn was shipped to Gainesville, Fla. on 26 January where it was then picked up and transferred to the postharvest lab (University of Florida, Gainesville, Fla.) and the experiment started on the same day. As soon as the sweetcorn arrived at the lab, the cobs were de-husked and stored at 5 °C until they were distributed among the PM-MAP treatments (within 6 h).

Perforation Mediated Modified Atmosphere Packaging Treatment

The PM-MAP was established using 3-L glass wire bail jars with glass lids (Village Kitchen, Redding, Calif.). Two holes were drilled in the glass lids; one of them was fitted with an appropriate size brass tube and the other fitted with a rubber serum stopper to facilitate gas sample withdrawal. The brass tube size was determined using a computer program (Chau, 2001) that depends on the equations of Fonseca et al. (2000). This program uses respiration rate, RQ, commodity weight, and tube diameter and length. In this program, tube design can be based on the desired O₂ or CO₂ concentrations. In this experiment, all the calculations were made based on the final desired CO₂ level in the containers; also, RQ = 1 was used in all these calculations. Brass tubes with the diameters and lengths shown in Table 4-1 were cut from commercial brass pipes. From previous work by the author, three gas compositions were chosen (15, 20, and 25% CO₂) and two storage temperatures (1 and 10 °C). The average respiration rate in the previous experiments was used to calculate the appropriate dimensions of the brass tubes for the PM-MAP.

Sweetcorn Storage

Three cobs of sweetcorn (about 500 g) were placed in each of three replicate containers per treatment and stored at 1 or 10 °C for 10 d. Kernel samples for sugar measurement were taken after storage by removing the kernels from the cob using a sharp stainless steel knife. Kernel samples (about 100 g) were placed in plastic freezer storage bags and stored at -20 °C until analysis.

Parameters

Gas composition

Oxygen and CO₂ treatment levels were measured each 12 h for 10 d using a PBI Dansensor Checkmate O₂ and CO₂ analyzer (Topac, Hingham, Mass.) with zirconium and infrared detectors, respectively. The unit is designed to measure package atmospheres and automatically withdraw samples through a tube fitted with a needle that is inserted in the package or through a septum in a jar lid in this experiment and gives a reading every 5 s.

Respiratory quotient

Respiratory quotient was calculated for each gas composition measurement using the following formula,

$$RQ = \text{CO}_{2 \text{ jar}} / (\text{O}_{2 \text{ air}} - \text{O}_{2 \text{ jar}})$$

Where:

RQ = Respiratory quotient

CO_{2 jar} = Carbon dioxide concentration in the jar

O_{2 air} = Oxygen concentration in the air outside the jar

O_{2 jar} = Oxygen concentration in the jar

Weight loss

Cob weight was recorded initially before starting the experiment and also after the storage period, and percentage of weight loss was calculated.

Sugar content

Total soluble sugars were measured in raw and cooked kernels using the phenol-sulfuric method described by Dubois et al. (1956). In this experiment, 15 g of sweetcorn kernels were blended in a commercial blender with 85 mL of 95% ethanol for 2-3 min then were heated for 20 min in an 85 °C water bath. Then the samples were stored overnight at -20 °C to precipitate ethanol-insoluble materials. The samples were then filtered through Whatman #2 filter paper in a Buchner funnel attached to a side-arm Erlenmeyer flask. A vacuum pump was connected to the side arm to expedite the extraction. The collected filtrates were volumetrically adjusted to 200 mL using 95% ethanol and 20 mL portions of the extracts were stored in scintillation vials at -20 °C until the measurements were performed.

To prepare samples for measurement of total soluble sugars, 0.5 mL of 5% phenol (w/w) was added to 0.5 mL of diluted (1:250) ethanol extract in test tubes, the mixture was vortexed, then 2.5 mL concentrated sulfuric acid was added and the mixture vortexed again. The test tubes were let to stand for 10 min then were placed in a 25 °C water bath for another 10 min to stop the reaction. The total soluble sugars were measured by reading the absorbance at 490 nm using a Shimadzu UV-1201 spectrophotometer (Shimadzu Scientific Instruments, Inc.; Tokyo, Japan). The absorbance values were compared to a standard curve of glucose.

Ethanol and acetaldehyde content

Ethanol and acetaldehyde were measured in the storage container headspace. Measurements were done using an HP 5890 Series II gas chromatograph equipped with flame ionization detector and a Hewlett Packard Model 3390A integrator (Hewlett Packard, Avondale, Pa.). A 3 mm by 2 m column of 5% Carbowax 20M on

80/120 Carbopack B (Supelco, Bellefonte, Pa.) was used and the carrier gas was N₂ (30 mL·min⁻¹). Oven, injector, and detector temperatures were 80, 110, and 300 °C, respectively.

A 1-mL sample of the container headspace gas was withdrawn using a 1.0 mL plastic syringe and injected on the gas chromatograph. Calibration was done before sample analysis using standards prepared from authentic compounds.

Statistical Analysis

The experimental design was a completely randomized design with three replicates consisting of three sweetcorn cobs in each of three jars per treatment. The data were analyzed using the Statistical Analysis System (SAS). Analysis of variance (ANOVA) was used to determine the effect of treatments on the dependent variables. Means were separated by the least significant difference (LSD) test at $P < 0.05$.

Results and Discussion

Using PM-MAP could be a helpful tool in storing sweetcorn. The high respiration rate of sweetcorn makes the use of a package with a low β value very beneficial in order to reduce the possibility of the sweetcorn switching to anaerobic respiration.

In this work, the use of PM-MAP to store sweetcorn cobs for 10 d was very successful. After 10 d of storage, the visual quality was very high in all treatments with no sign of microbial growth or deterioration even at the higher temperature (10 °C), which might be due to the fungistatic effect of high CO₂ (El-Goorani and Sommer, 1981). Similar results were obtained in a study by Aharoni et al. (1996) with sweetcorn MAP using polyolefin stretch film in which the high CO₂ and low O₂ levels resulted in a significant reduction in both decay and water loss and hence maintained significantly better appearance quality than that of unpackaged controls.

The weight loss was very slight in all treatments, which is a common observation for MAP treatment of sweetcorn. Many researchers have demonstrated that using film wrapping reduces water loss compared with unwrapped sweetcorn and furthermore, using films with lower water permeability further reduces water loss compared with films with high water permeability (Aharoni et al., 1996; Deak et al., 1987; Risse and McDonald 1990). In this work, weight loss ranged between 0.05 and 0.08% at 1 °C with no significant differences among the MAP treatments, while at 10 °C it ranged between 0.12 and 0.22% with significant differences among MAP treatments that corresponded to tube size (i.e., treatments utilizing smaller tubes lost less water). The higher weight loss at the higher temperature was also probably due to the larger brass tubes used for PM-MAP at 10 °C than at 1 °C. The low weight loss at both temperatures might be one of the factors that helped in maintaining the high quality of the sweetcorn with shiny kernels and no denting, both of which are affected by low humidity levels around the sweetcorn (Wiley et al., 1989).

The expected gas composition was successfully obtained in all treatments at 1 °C (Fig. 4-1) while at 10 °C the levels obtained were less than expected by about 3-5% CO₂. This might have been due to overestimation of the respiration rate while designing the tubes for PM-MAP, which led to steady state CO₂ levels around 10, 17, and 22% CO₂ instead of 15, 20, 25% CO₂, respectively (Fig. 4-2). Sweetcorn stored at 1 °C reached the expected CO₂ level in about 5 d with slightly more time required for the higher concentrations (Fig. 4-1). The sweetcorn stored at 10 °C was faster in reaching the steady state (about 2 d.) and this was expected due to higher CO₂ production at the higher temperature (Fig. 4-2).

There was a noticeable change in the RQ during storage that was most noticeable at 1 °C. There was a gradual increase in the RQ during the storage period and the RQ was generally higher at 10 °C (Fig. 4-3). Similar results were observed in blueberry and red raspberry, for which the RQ values varied between 1.0 and 1.3 and there was also an increase with increasing storage temperature. Also, when the blueberry and raspberry packages reached a very low steady state O₂, the RQ increased to ~4-5 (Beaudry et al., 1992; Joles et al., 1994). In this experiment, RQ values never exhibited such a sudden increase, which usually indicates the switching to anaerobic respiration, but rather ranged between 0.9 and 1.4.

Ethanol production was very low in all treatments, around 1-3 ppm, and there were no significant differences among the treatments. This is in agreement with Aharoni et al. (1986) and Rodov et al. (2000) who reported that no significant production of ethanol was detected when sweetcorn was stored in MAP at 1 or 2 °C although higher ethanol production (200-500 ppm) was detected when the MAP packages were later transferred to 20 °C.

Sugar content was higher in the sweetcorn stored at the lower temperature, but there were no significant differences among the treatments at 1 °C (Fig. 4-4). The only difference in sugar concentrations that was observed was in the sweetcorn stored at 10 °C with 15% CO₂, which had significantly lower sugar content than sweetcorn stored in 20 or 25% CO₂. This finding is in agreement with previous work with CA or MAP storage of sweetcorn in which it was reported that there were higher sugar concentrations in kernels stored in high CO₂ and/or low O₂ (Risse and McDonald, 1990; Rumpf et al., 1972; Schouten, 1993)

This work showed that using PM-MAP could be a very useful tool in sweetcorn storage at different storage temperatures. Perforation mediated modified atmosphere packaging was mostly successful in generating the desired and expected gas compositions and maintaining them during a 10-d. storage period. The PM-MAP system was also successful in maintaining sweetcorn quality for 10 d of storage. On the other hand, using active gas modification could help reduce the time required to reach equilibrium, especially at the lower temperature. More work needs to be done to collect improved respiration data for different temperatures and also to determine the switching point from aerobic to anaerobic respiration in sweetcorn, which will lead to a better understanding of possible O₂ and CO₂ levels that could be used for sweetcorn storage.

Table 4-1. Diameters and lengths of brass tubes used for creating PM-MAP with desired CO₂ levels at different temperatures.

Temperature (°C)	Desired CO ₂ (%)	Diameter (mm)	Length (mm)
1	15	4	27.1
	20	4	43.8
	25	4	63.7
10	15	8	19.4
	20	8	31.4
	25	8	45.7

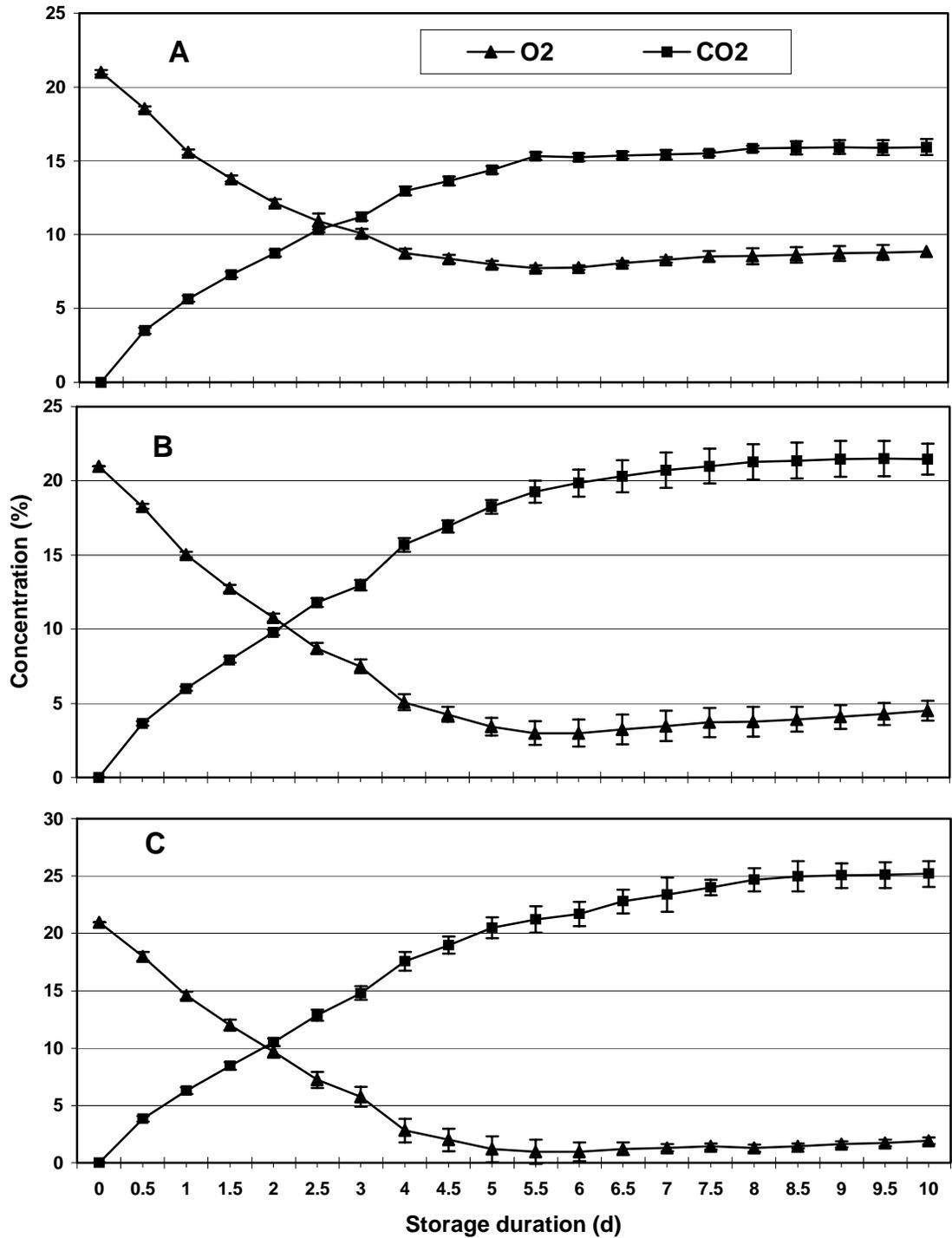


Fig. 4-1. Gas composition in perforation-mediated MAP originally designed to generate 15% CO₂ (A), 20% CO₂ (B) or 25% CO₂ (C) in sweetcorn packages stored for 10 d at 1 °C. Bars indicate ± 1 SD (n = 3).

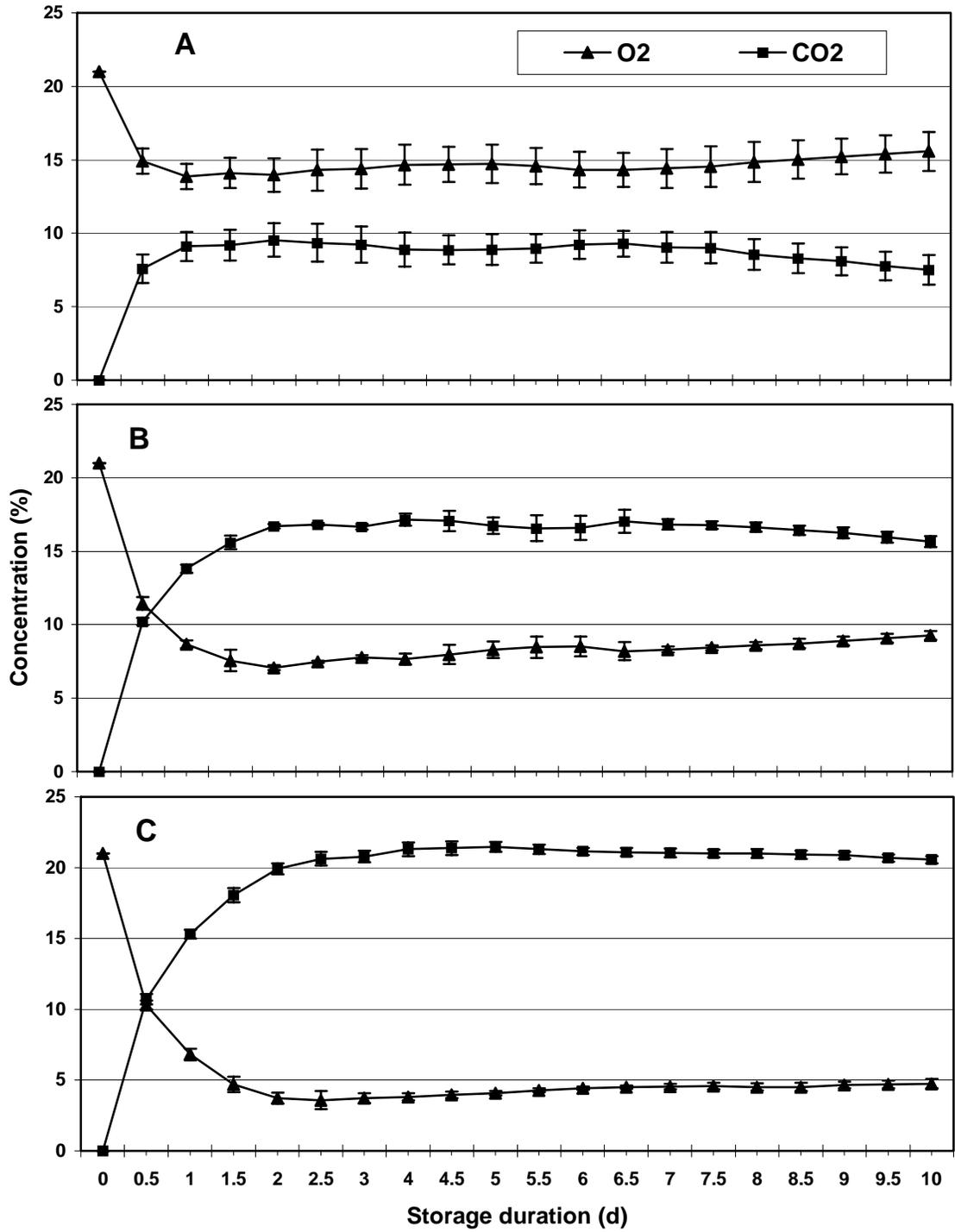


Fig. 4-2. Gas composition in perforation-mediated MAP originally designed to generate 15% CO₂ (A), 20% CO₂ (B) or 25% CO₂ (C) in sweetcorn packages stored for 10 d at 10 °C. Bars indicate ± 1 SD (n = 3).

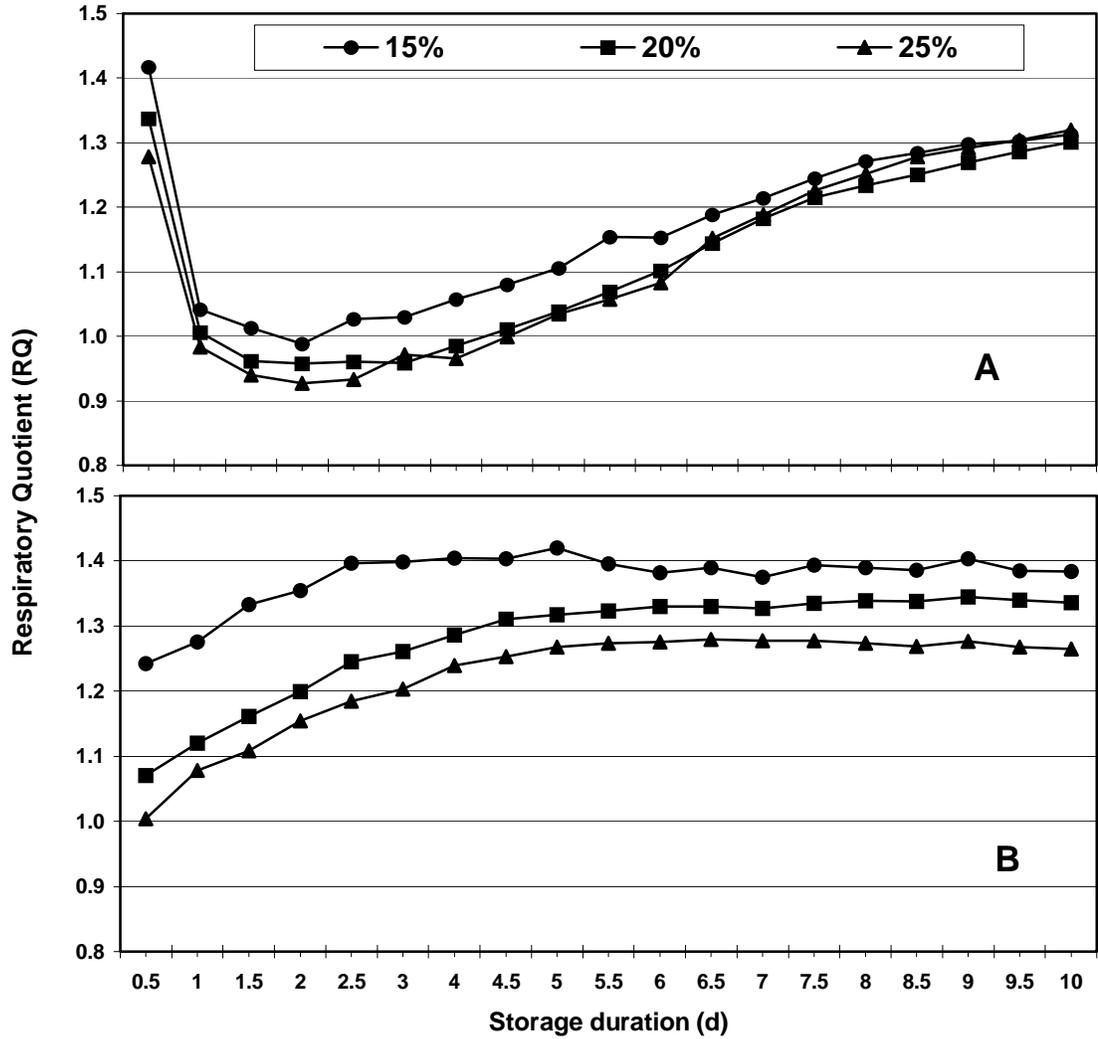


Fig. 4-3. Effect of different levels of CO₂ generated through PM-MAP on the respiratory quotient (RQ) of sweetcorn stored for 10 d at 1 °C (A) or 10 °C (B).

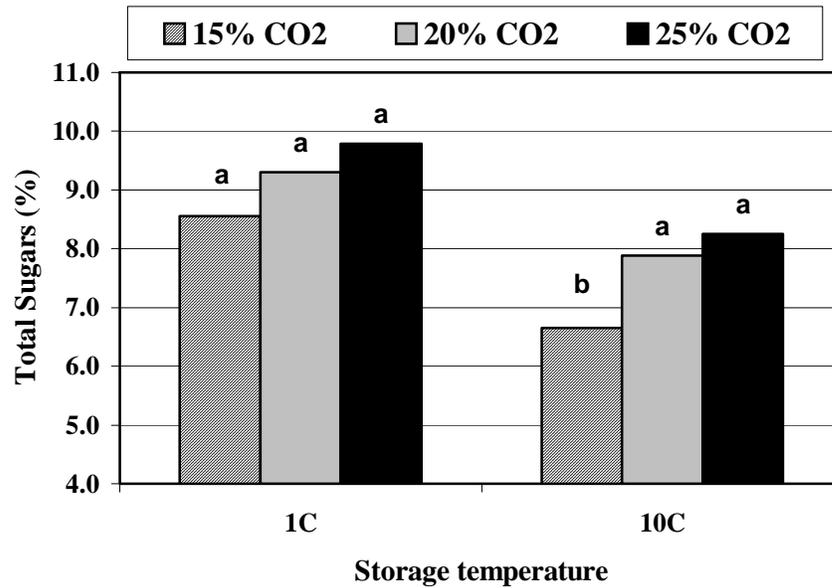


Fig. 4-4. Effect of temperature and different CO₂ levels built up through perforation-mediated MAP on total sugars (%) in sweetcorn stored for 10 d at 1 or 10 °C. Means with the same letter in each storage temperature are not significantly different at $P < 0.05$ by LSD.

CHAPTER 5
SELECTION OF MATURITY STAGE, STORAGE TEMPERATURE, AND
ATMOSPHERE FOR OPTIMUM POSTHARVEST QUALITY OF FRESH-CUT
SWEETCORN KERNELS

Introduction

Shelf life of perishable fruits and vegetables can be extended by a variety of techniques. The most widely used techniques are refrigeration and controlled atmosphere (CA) storage or modified atmosphere packaging (MAP; Chinnan, 1989; Kader et al., 1989). For sweetcorn, the consensus recommendation is to keep it as close to 0 °C as possible during transportation and storage (Brecht, 2002) and this may be of even more importance for fresh-cut sweetcorn. On the other hand, CA storage and MAP benefits with regard to extension of fruit and vegetable shelf life are well documented (Anzueto and Rizvi, 1985; Brecht, 1980; Nakashi et al., 1991; Zagory and Kader, 1988), including sweetcorn (Aharoni et al., 1996; Deak et al., 1987; Risse and McDonald, 1990; Schouten, 1993)

Sweetcorn shelf life is one of the shortest among fruits and vegetables. This is due to its high metabolic and respiration rates, which lead to severe sugar loss and rapid quality deterioration. Although sweetness is the most important quality characteristic in sweetcorn (Evensen and Boyer, 1986; Showalter and Miller, 1962), the importance of other flavor components in consumer acceptance is also well documented (Flora and Wiley, 1974; Wiley, 1985; Williams and Nelson, 1973). These other flavor components include texture and aroma.

With the increasing acceptability and demand for fresh-cut or ready-to-eat products, fresh-cut sweetcorn kernels could be a valuable new product. Previous work on fresh-cut sweetcorn showed that intact kernels (prepared by splitting the ears lengthwise and then pushing the kernels off the cob) were much better than cut kernels (which were prepared similarly to the canned or frozen sweetcorn by cutting the whole kernels from the cob) in maintaining higher sugar content and better flavor. Intact kernels showed little or no after cooking brown discoloration but were very difficult to prepare (Brecht, 1999).

Browning during cooking is a common occurrence during the preparation of many foods. One of its causes is the non-enzymatic browning reaction usually associated with thermal processing that is known as the Maillard reaction. In this reaction, brown pigments are generated from the reaction between free sugars and basic amino acids at elevated temperatures (Bayindirli et al., 1995; Gogus et al., 1998). Sweetcorn has a high background of sugars (Courter et al., 1988) and amino acids (Grunau and Swiader, 1991). Protein degradation in sweetcorn kernels may also occur during storage, increasing the concentration of free amino acids, which could increase the probability of occurrence of Maillard reaction. Unlike many other foods in which browning during cooking is a desirable occurrence, browning of fresh-cut sweetcorn kernels is a negative quality factor that would make the product unsalable.

The main objective of this work was to determine the effects of maturity stage, storage temperature, and atmosphere modification on the quality of fresh-cut sweetcorn in order to develop recommendations for commercial development of this as a fresh-cut product.

Materials and Methods

Plant Material

Fresh harvested sweetcorn (*Zea mays* L. rugosa var. Prime Time) cobs were harvested from a local farm in Florida (Wilkinson-Cooper Produce Inc., Belle Glade, Fla.) on 11 January 2002 pre-cooled, and delivered overnight by refrigerated truck to a local grocery chain's distribution facility in Jacksonville (Publix Distribution Center, Jacksonville, Fla.) on 12 January where it was picked up and transferred to the postharvest lab (University of Florida, Gainesville, Fla.) and the experiment started on the same day. As soon as the sweetcorn arrived at the lab, the cobs were de-husked and divided into two groups according to the maturity stage. The more mature sweetcorn had larger kernels and the kernels were more compact on the cob, while the less mature kernels were smaller and there were still some spaces between the kernel rows (Fig. 5-1).

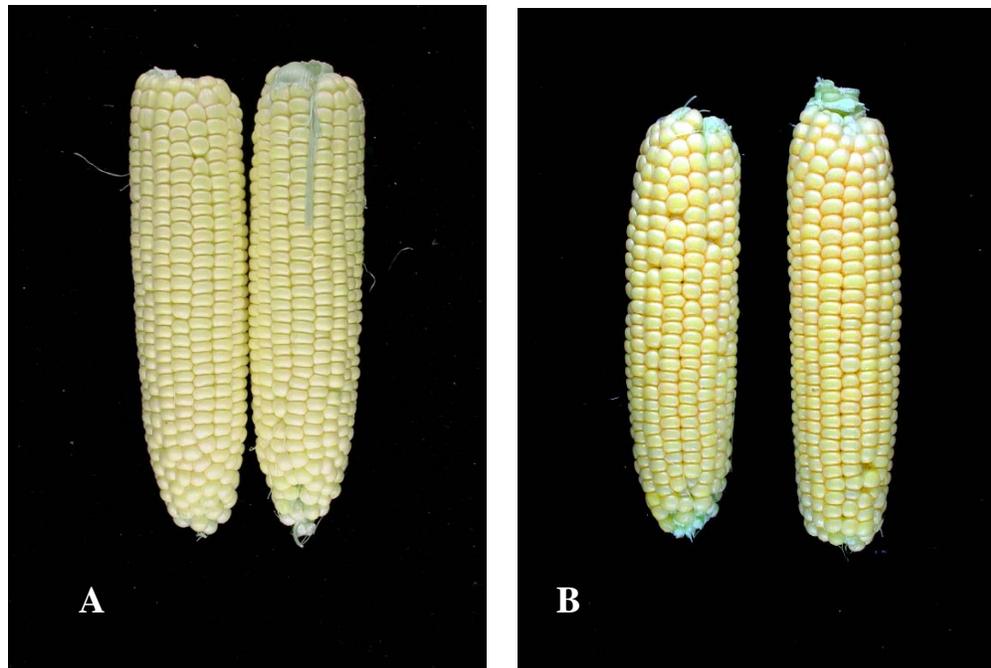


Fig. 5-1. The differences between the freshly harvested more mature (A) and less mature (B) sweetcorn cobs.

The cobs were held at 5 °C while the kernels were cut. The kernels were cut from the cobs using a sharp stainless steel knife. The kernels in the first row were popped out first using the edge of the knife and discarded, then the adjacent rows were cut from the cob near the kernel base and the cut kernels were collected. Special care was taken during cutting in order to minimize damage to the kernels. After cutting the kernels, they were dipped in chlorinated (1.34 mM NaOCl), 5 °C water at pH 7 for 1 min. for sanitation and kept covered at 5 °C until distributed among the treatments.

Controlled Atmosphere Treatment

Controlled atmosphere treatment was used to determine the effect of atmosphere modification on fresh-cut sweetcorn quality and the potential application of MAP technology for fresh-cut sweetcorn handling. The CA mixtures were established using a system of pressure regulators, manifolds and needle valve flowmeters to blend air, N₂, and CO₂ from pressurized cylinders. The CA was a flowing system with a flow rate set to maintain respiratory CO₂ accumulation under 0.5%. The air-flow was humidified by bubbling through water before introducing it to the containers. Three replicates from each treatment were prepared by putting 300 g of cut kernels in 1.2-L plastic containers (Tupperware; Orlando, Fla.) fitted with 2 plastic connections that attached to the plastic tubing and served as in and out ports for the gas flow.

Cut kernels were exposed to three gas compositions: Air, 2% O₂ plus 10% CO₂, or 2% O₂ plus 20% CO₂. Gas composition was monitored daily using a Servomex O₂ and CO₂ analyzer (Servomex, Norwood, Mass.).

Fresh-Cut Kernel Storage and Cooked Sample Preparation

The cut kernels were stored at 1 or 5 °C for 10 d with subsamples taken at 4 and 7 d. The other treatments (maturity stage and CA) were distributed randomly in each

storage room. After the storage period, the cut kernels were cooked by placing 50 g of kernels in 250 mL boiling water for 5 min. Both fresh and cooked samples were stored at -20 °C until analysis.

Parameters

Respiration rate

Carbon dioxide levels were measured daily using a Gow Mac series 580 gas chromatograph (Gow Mac Instruments Co., Bridgewater, N.J.) equipped with thermal conductivity detector (TCD) and a Hewlett Packard Model 3390A integrator (Hewlett and Packard Co. Avondale, Pa.). The respiration rate was measured using the static method in which the gas flow was blocked using metal clips and the first sample was withdrawn. The containers were then incubated for 2 h before taking the second sample and removing the metal clips. The difference in CO₂ concentration in the samples withdrawn before and after the incubation period was used to calculate the respiration rate. The gas samples were withdrawn from the container headspace using 1.0 mL BD plastic syringes and a 0.5-mL sample was injected on the gas chromatograph. Calibration was done before sample analysis using a certified standard mixture.

Sugar content

Total soluble sugars were measured in raw and cooked kernels using the phenol-sulfuric method described by Dubois et al. (1956). In this experiment, 15 g of sweetcorn kernels were blended in a commercial blender with 85 mL of 95% ethanol for 2-3 min then were heated for 20 min in an 85 °C water bath. Then the samples were stored overnight at -20 °C to precipitate ethanol-insoluble materials. The samples were then filtered through Whatman #2 filter paper in a Buchner funnel attached to a side-arm Erlenmeyer flask. A vacuum pump was connected to the side arm to expedite the

extraction. The collected filtrates were volumetrically adjusted to 200 mL using 95% ethanol and 20 mL portions of the extracts were stored in scintillation vials at -20 °C until the measurements were performed.

To prepare samples for measurement of total soluble sugars, 0.5 mL of 5% phenol (w/w) was added to 0.5 mL of diluted (1:250) ethanol extract in test tubes, the mixture was vortexed, then 2.5 mL concentrated sulfuric acid was added and the mixture vortexed again. The test tubes were let to stand for 10 min then were placed in a 25 °C water bath for another 10 min to stop the reaction. The total soluble sugars were measured by reading the absorbance at 490 nm using a Shimadzu UV-1201 spectrophotometer (Shimadzu Scientific Instruments, Inc.; Tokyo, Japan). The absorbance values were compared to a standard curve of glucose.

Free amino acids

The ethanol extracts prepared for soluble sugar measurements were used to measure free amino acids colorimetrically using the ninhydrin method described by Lee and Takahashi (1966). In this method, a 0.1-mL aliquot of the ethanol extract was added to 1.9 mL of reaction mixture [0.5 mL 1% ninhydrin solution (1 g ninhydrin in 100 mL 0.5 M citrate buffer) with 1.2 mL glycerol and 0.2 mL 0.5 M citrate buffer (0.5 M citric acid and 0.5 M sodium citrate)], mixed well, heated in a boiling water bath for 12 min, then cooled in a water bath at room temperature. Samples were mixed once more then measured at 570 nm on a Shimadzu UV-1201 spectrophotometer (Shimadzu Scientific Instruments, Inc.; Tokyo, Japan). Total free amino acid concentration was calculated from a standard curve of tryptophan (Sigma Chemical Co.; St. Louis, Mo.).

Statistical Analysis

The experimental design was a completely randomized design with three replicates. The data were analyzed using the Statistical Analysis System (SAS). Analysis of variance (ANOVA) was used to determine the effect of treatments on the dependent variables. Means were separated by the least significant difference (LSD) test at $P < 0.05$.

Results and Discussion

Fresh-cut sweetcorn is a potential product for the fresh-cut produce industry. Fresh-cut sweetcorn was stored up to 10 d without severe loss in visual quality even at 5 °C. Also, dipping the kernels in chlorinated water (1.34 mM NaOCl) for 1 min after cutting was apparently effective in controlling decay since there was no visual appearance of any microbial growth in these experiments.

Mature sweetcorn kernels had a respiration rate that was about 50% lower than the less mature kernels (Fig. 5-2). Also, the respiration rate of kernels stored at 5 °C was about 25% higher than those stored at 1 °C (Fig. 5-2), which is in agreement with previous work by Brecht (1999). Using CA with 2% O₂ plus 10% CO₂ significantly reduced the respiration rate by about 25%, but using 2% O₂ plus 20% CO₂ nearly doubled the respiration rate through most of the storage period (Fig. 5-2), which may have been due to initiation of anaerobic respiration. Despite the increase in the respiration rate when kernels were held in 2% O₂ plus 20% CO₂, this treatment did not seem to result in a significant difference in kernel taste compared with the air control (personal assessment by the author, no taste panel conducted).

Kernels stored at 1 °C had 28% higher sugar content at the end of the storage period than kernels stored at 5 °C (Fig. 5-3). Storing sweetcorn kernels in 2% O₂ plus 10% or 20% CO₂ maintained about 30% higher sugar content than air storage (Fig. 5-3).

On the other hand, less mature kernels had about 25% more sugar at the beginning of the experiment but levels decreased more rapidly than in the mature kernels such that there was no significant difference between the two maturities by the end of the experiment. This may have been due to the higher respiration rate of the less mature kernels, which led to a greater sugar loss. These results are in agreement with several research reports indicating that temperature control is the most important factor in the preservation fruits and vegetables generally and in sweetcorn specifically due to its high metabolic rate, which makes sweetcorn more prone to rapid deterioration (Brecht, 2002; Brecht and Sargent, 1988; Evensen and Boyer, 1986). It is also in agreement with work on the importance of gas modification (CA and/or MA) in maintaining quality in fruits and vegetables (Anzueto and Rizvi, 1985; Nakashi et al., 1991; Zagory and Kader, 1988) including sweetcorn cobs (Aharoni et al., 1996; Deak et al., 1987; Risse and McDonald, 1990; Schouten, 1993)

Visual quality was not greatly affected by any of the storage treatments (Fig. 5-5, 7, 9, 11, and 13). The main problem in all treatments was loss of flavor and the kernels becoming more watery with a bland taste which is in agreement with Kader (2002) that the post-cutting life based on flavor is shorter than that based on appearance. Fresh-cut sweetcorn stored in air at 5 °C did not show a severe loss in visual quality after storage for 10 d (Fig. 5-9), but there was severe browning of the kernels after cooking (Fig. 5-10) compared with the appearance of kernels cooked before storage (Fig. 5-6). While after cooking browning appeared in cooked kernels stored in air at 5 °C, it did not occur in cooked kernels after storage in air at 1 °C (Fig. 5-8), and did not appear in the kernels stored in CA at either 1 or 5 °C (Fig. 5-12 and 14). This browning also was more severe

in the kernels from the more mature cobs. More mature kernels and kernels stored at 5 °C or in air had higher content of free amino acids at the end of storage than less mature kernels and the kernels stored at 1 °C or in CA (Fig. 5-4), supporting the possibility of involvement of Maillard reaction in the after cooking browning. An increase in free amino acids during storage due to protein degradation, a symptom of deterioration and senescence, may have predisposed those kernels to undergo the browning reaction.

This experiment indicated clearly that temperature is the most important factor in maintaining fresh-cut sweetcorn kernel quality. All quality factors evaluated were better maintained during storage at 1 °C compared with 5 °C storage. Also, using a secondary preservation factor such as CA was beneficial in maintaining the quality of fresh-cut sweetcorn by maintaining sweetness and preventing after cooking brown discoloration. After cooking browning that occurred in 5 °C air-stored kernels was completely inhibited by storage in 2% O₂ plus 10% CO₂ at 5 °C. The tolerance of fresh-cut sweetcorn kernels to elevated CO₂ appears to be quite high as well since, in addition to preventing after cooking browning, exposure to 20% CO₂ for 10 d caused no negative quality changes. This suggests that using MAP could be a useful technique for commercial handling of fresh-cut sweetcorn. On the other hand, more detailed work is needed on the effect of these treatments on different aspects of the flavor and aroma of fresh-cut sweetcorn.

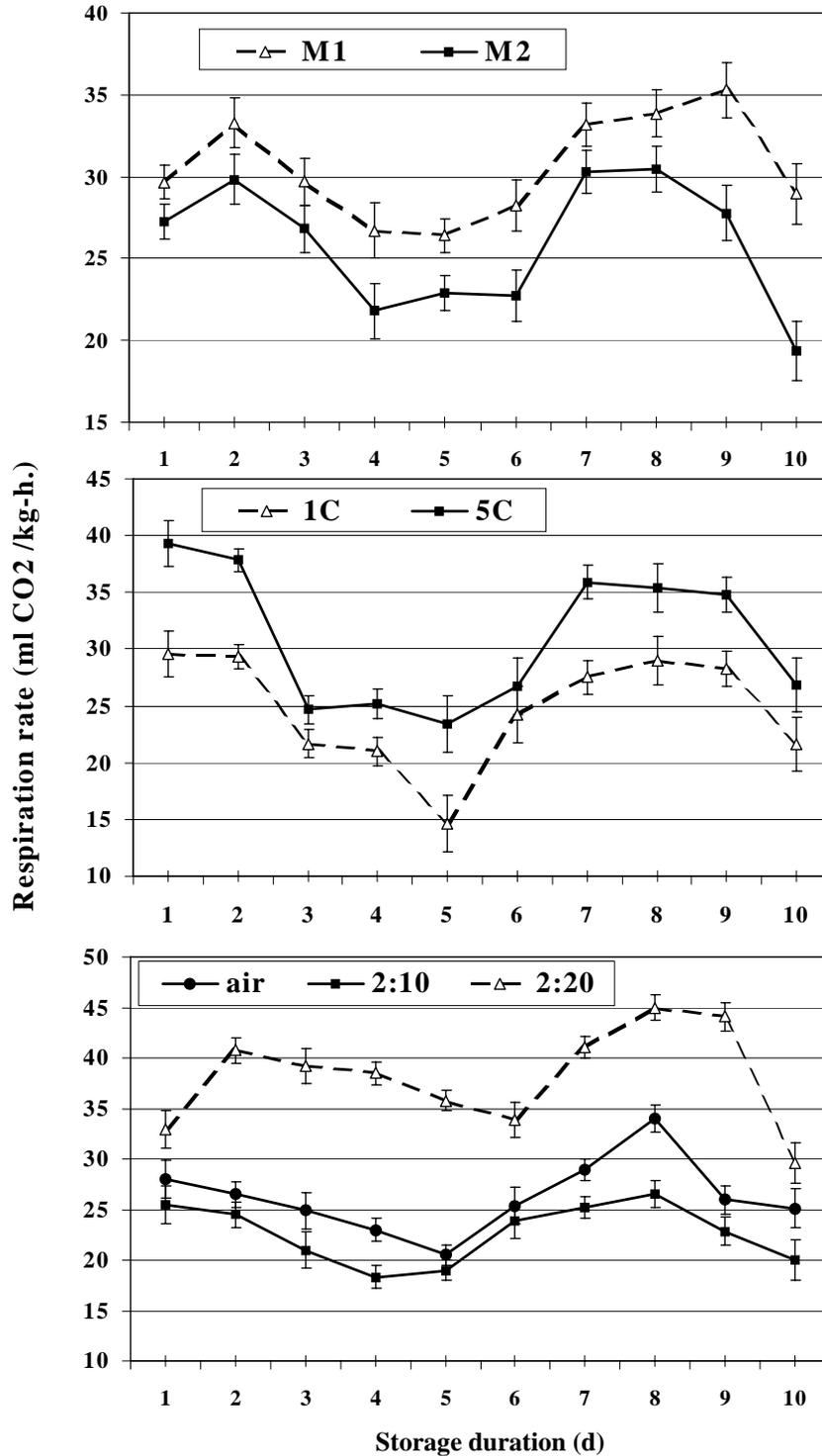


Fig. 5-2. Effect of maturity stage (M1, less mature and M2, more mature kernels), storage temperature (1 °C or 5 °C), and controlled atmosphere (air control, 2% O₂ plus 10 or 20% CO₂) on respiration rate of fresh-cut sweetcorn. Bars indicate the mean +SD (n=3).

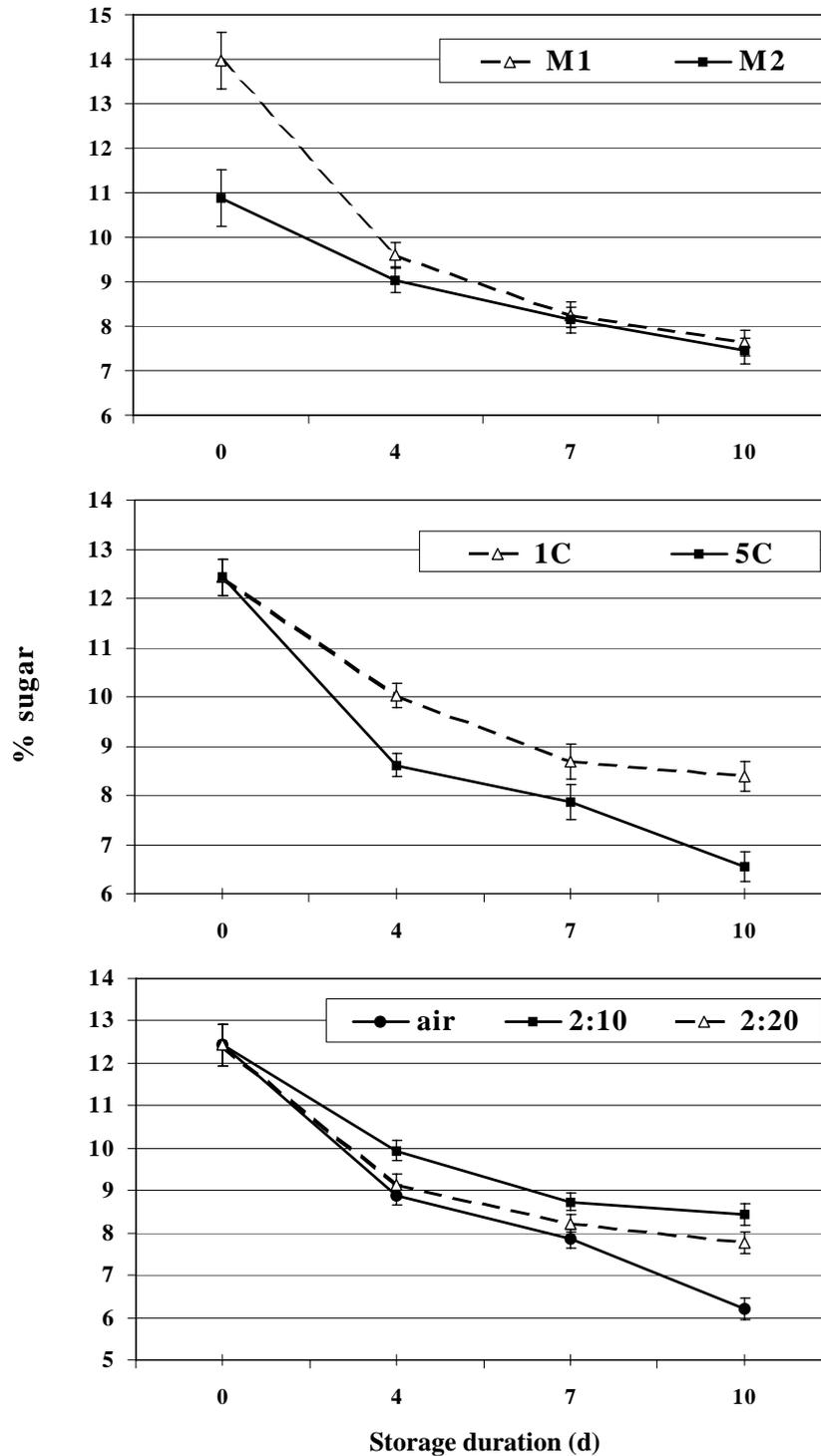


Fig. 5-3. Effect of maturity stage (M1, less mature and M2, more mature kernels), storage temperature (1 °C or 5 °C), and controlled atmosphere (air control, 2% O₂ plus 10 or 20% CO₂) on sugar content in fresh-cut sweetcorn. Bars indicate the mean +SD (n=3).

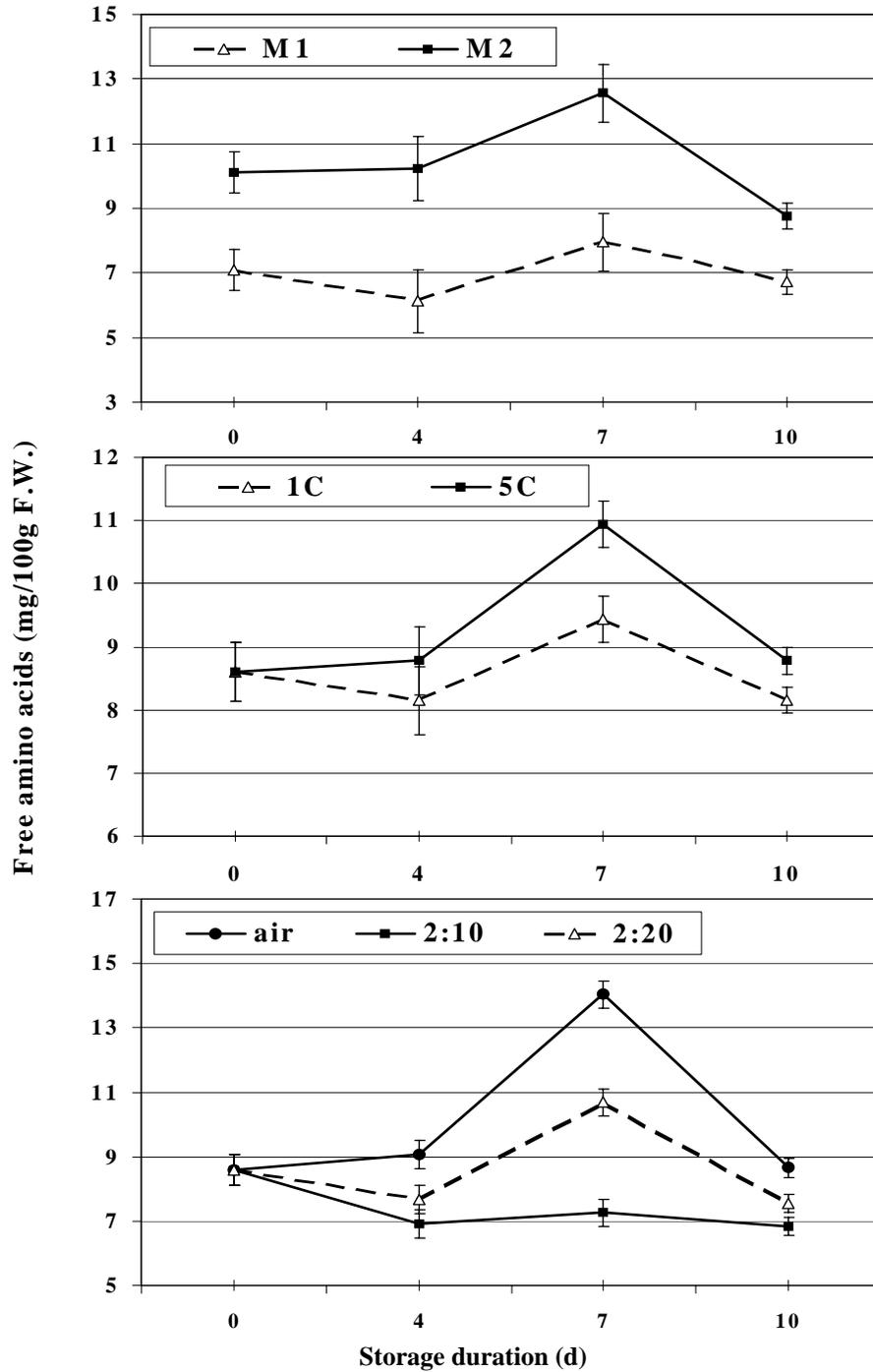


Fig. 5-4. Effect of maturity stage (M1, less mature and M2, more mature kernels), storage temperature (1 °C or 5 °C), and controlled atmosphere (air control, 2% O₂ plus 10 or 20% CO₂) on free amino acids in fresh-cut sweetcorn. Bars indicate the mean +SD (n=3).

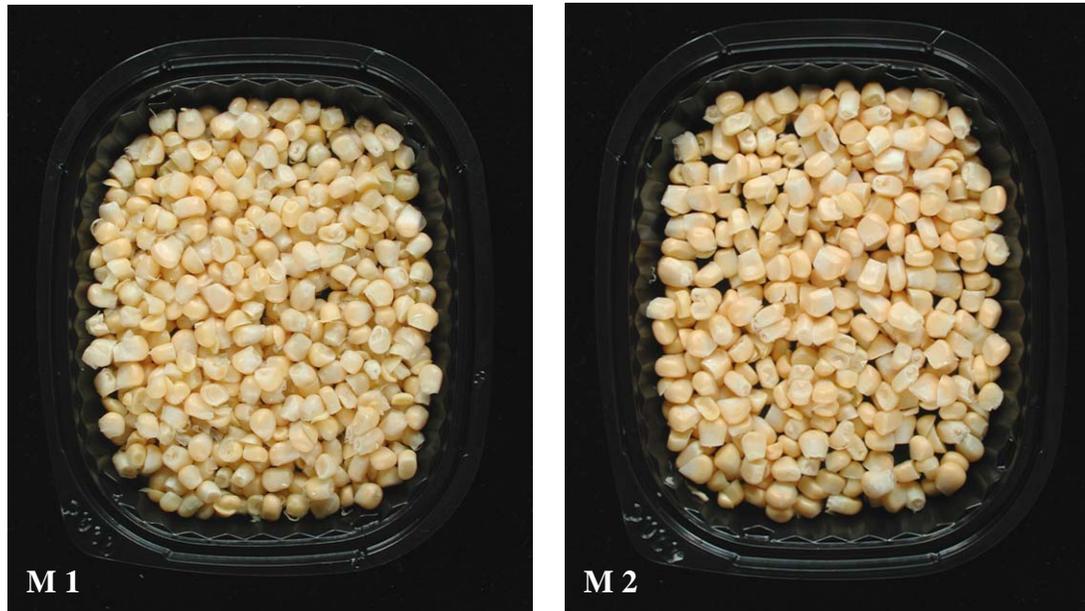


Fig. 5-5. Fresh-cut sweetcorn kernels at day 0 (just after preparation). M1 = kernels from less mature cobs while M2 = kernels from more mature cobs.

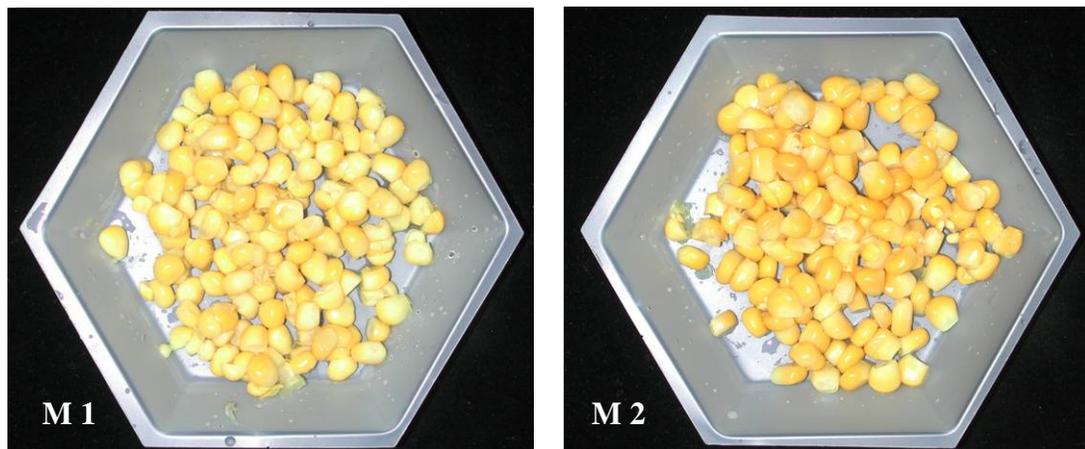


Fig. 5-6. Cooked fresh-cut sweetcorn kernels at day 0 (just after preparation). M1 = kernels from less mature cobs while M2 = kernels from more mature cobs.

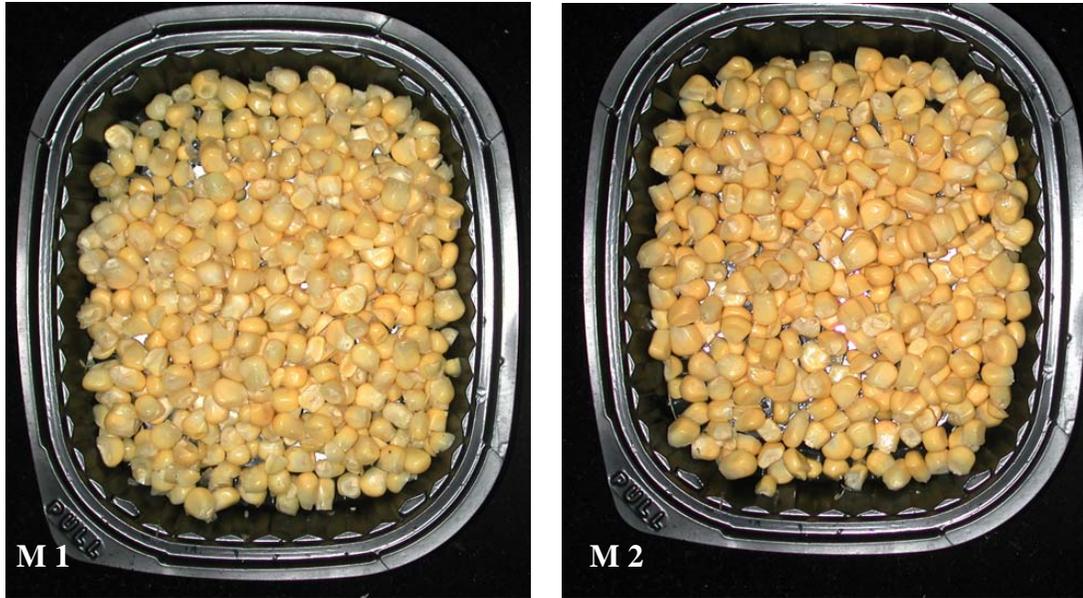


Fig. 5-7. Fresh-cut sweetcorn kernels after storage for 10 d at 1 °C in air. M1 = kernels from less mature cobs while M2 = kernels from more mature cobs.

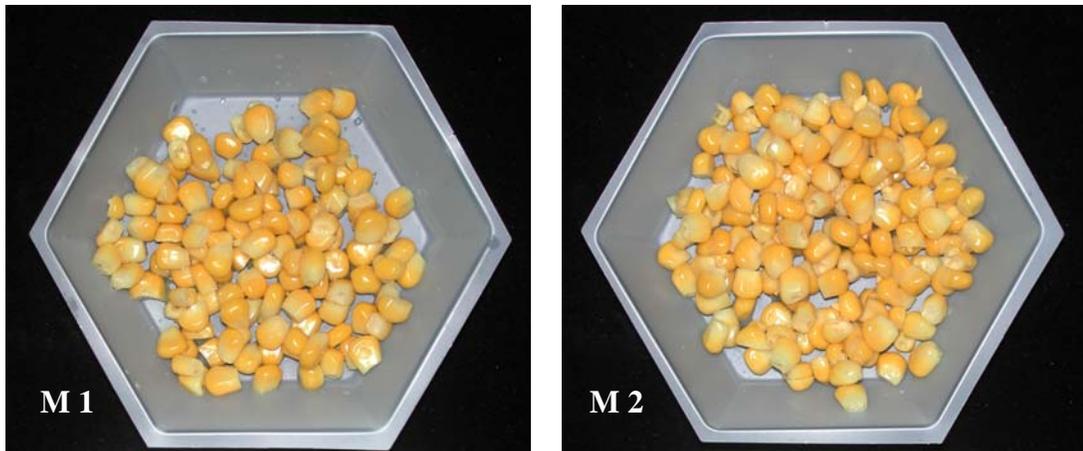


Fig. 5-8. Cooked fresh-cut sweetcorn kernels after storage for 10 d at 1 °C in air. M1 = kernels from less mature cobs while M2 = kernels from more mature cobs.

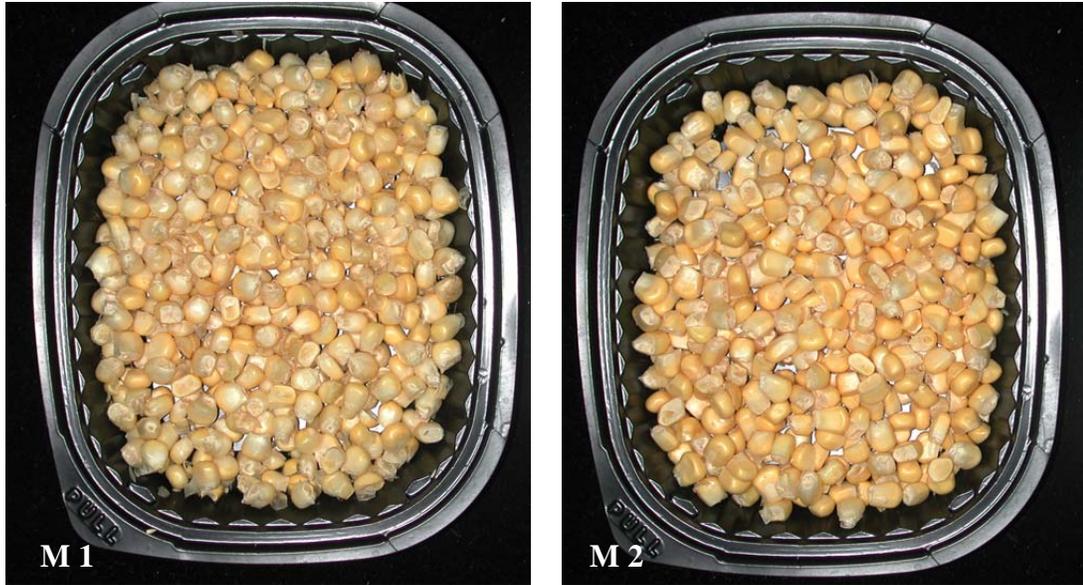


Fig. 5-9. Fresh-cut sweetcorn kernels after storage for 10 d at 5 °C in air. M1 = kernels from less mature cobs while M2 = kernels from more mature cobs.

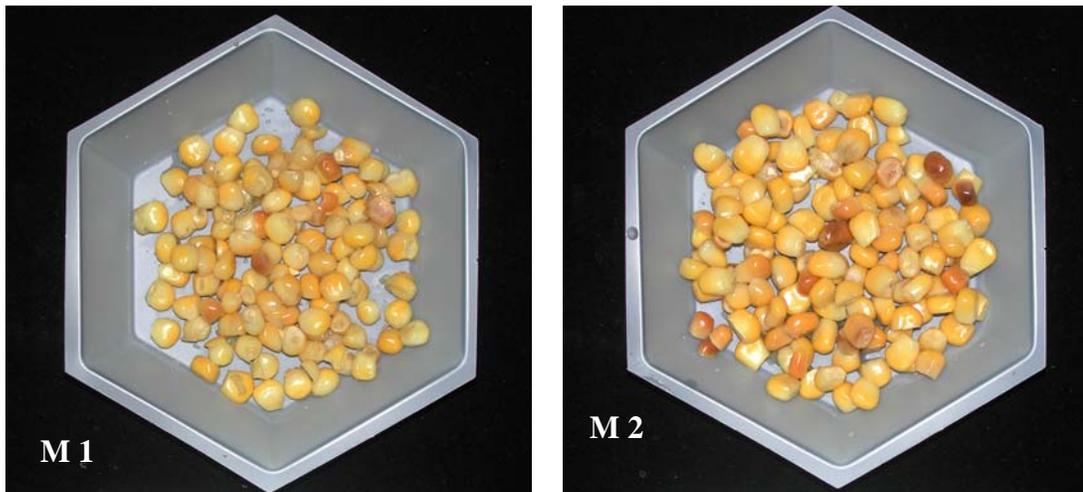


Fig. 5-10. Cooked fresh-cut sweetcorn kernels showing after cooking browning after storage for 10 d at 5 °C in air. M1 = kernels from less mature cobs while M2 = kernels from more mature cobs.

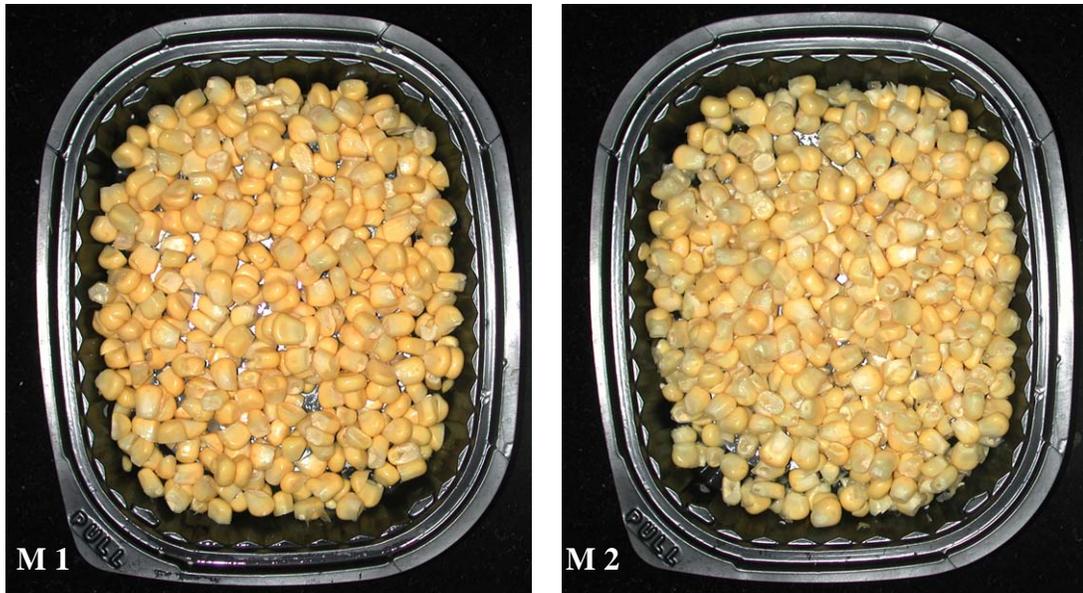


Fig. 5-11. Fresh-cut sweetcorn kernels after storage for 10 d at 1 °C in 2% O₂ plus 10% CO₂. M1 = kernels from less mature cobs while M2 = kernels from more mature cobs.

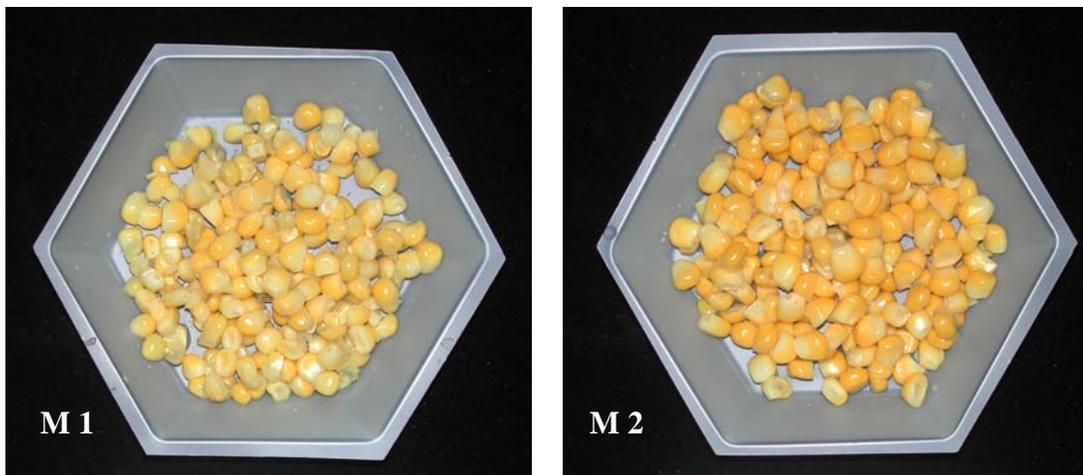


Fig. 5-12. Cooked fresh-cut sweetcorn kernels after storage for 10 d at 1 °C in 2% O₂ plus 10% CO₂. M1 = kernels from less mature cobs while M2 = kernels from more mature cobs.

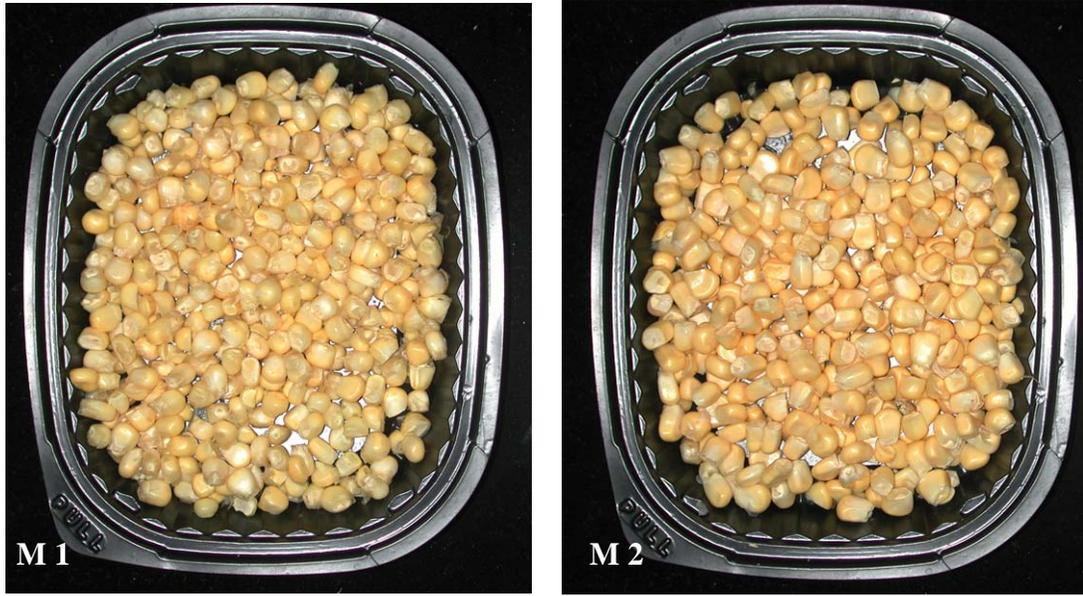


Fig. 5-13. Fresh-cut sweetcorn kernels after storage for 10 d at 5 °C in 2% O₂+10% CO₂. M1 = kernels from less mature cobs while M2 = kernels from more mature cobs.

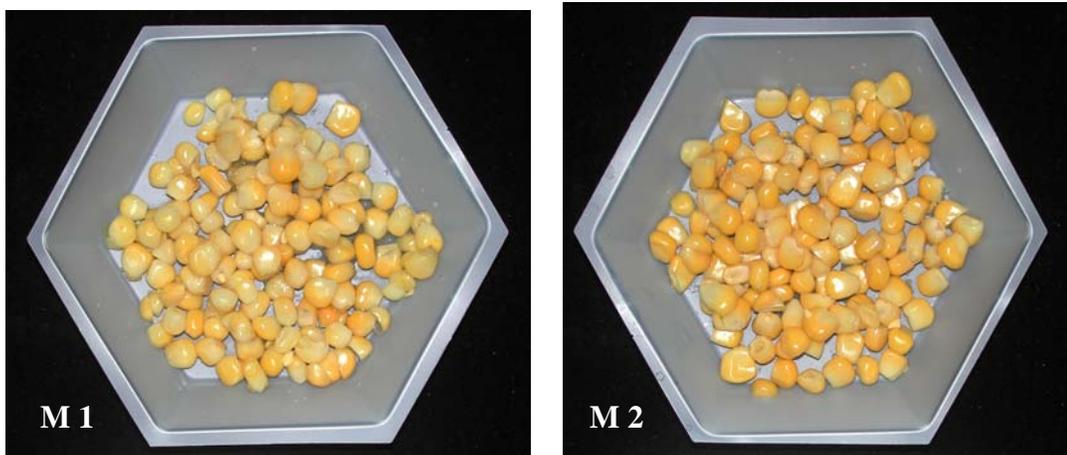


Fig. 5-14. Cooked fresh-cut sweetcorn kernels showing no after cooking browning after storage for 10 d at 5 °C in 2% O₂+10% CO₂. M1 = kernels from less mature cobs while M2 = kernels from more mature cobs.

CHAPTER 6
REDUCED OXYGEN AND ELEVATED CARBON DIOXIDE TO PREVENT
BROWNING OF FRESH-CUT SWEETCORN KERNELS AFTER COOKING

Introduction

Fresh-cut sweetcorn is a very perishable product since the kernels have a very high respiration rate that increases the rate of deterioration compared to intact sweetcorn. Previous work with fresh-cut sweetcorn showed that this product can be stored for 10 d in low temperature (0-1 °C) (Brecht, 1999; Riad and Brecht, 2001). Using atmosphere modification (2% O₂ plus 10% CO₂) enabled storage even at higher temperature (5 °C) without much loss in visual or chemical quality. There are two main problems associated with this product. The first is loss of flavor after preparation of the fresh-cut product, which is associated with reduced concentrations of soluble sugars and dimethyl sulfide, the main component of sweetcorn aroma. The second is browning that can occur after cooking when the fresh-cut kernels are stored in air at higher than optimum temperatures (Riad and Brecht, 2001). Blanchard et al. (1996) described a similar after cooking browning problem in air-stored diced onion, which was eliminated by storage in an atmosphere containing air plus 10% CO₂.

After-cooking browning as a result of the Maillard reaction (a non-enzymatic reaction between sugars and free amino acids usually associated with thermal processing) is common in the food processing industry. This reaction produces 5-hydroxymethylfurfural (HMF) (Bayindirli et al., 1995; Gogus et al., 1998). In the case of sweetcorn, the possibility of Maillard reaction would seem to be very high since

sweetcorn has high levels of free amino acids and sugars (Courter et al., 1988; Grunau and Swiader, 1991). The free amino acid content would be expected to increase in response to wounding and as the kernels senesce during storage due to protein degradation. Also, the brown color doesn't develop until the kernels are cooked. Another possibility for cause of this browning is autooxidation of phenolic compounds that causes polymerization of polyphenolic compounds (Talcott and Howard, 1999). It is well known that phenolic acids increase after wounding (Babic et al., 1993a,b; Howard and Griffin, 1993; Ramamurthy et al., 1992). Brecht (1999) noticed that the browning is severe in cut kernels but there is no browning in intact kernels, which also suggests that the cutting or injured tissues in the kernels might result in elevated levels of phenolics that may play a role in the after cooking browning.

Fresh-cut products may have high microbial loads (circa 10^5 to 10^7 CFU·g⁻¹) and these populations may increase during storage and reach high levels (e.g., 10^8 CFU·g⁻¹) in commercial soup mixes (Manzano et al., 1995). Using disinfectants such as chlorine can be very beneficial in reducing the microbial load on fresh-cut commodities. Adams et al. (1989) found that using 100 ppm free chlorine (pH 5) in washing lettuce leaves resulted in a 6.22 log reduction in the microbial population count. Generally using chlorine as a disinfectant in the washing water results in a 1-2 log reduction in the microbial population in fruit and vegetable (Cherry, 1999). A high microbial load can negatively affect the sensory quality of fresh cut products (Abbey et al., 1988; Manzano et al., 1995). Heard (2002) summarized the different symptoms of microbial spoilage in fresh-cut fruits and vegetables as follows:

- Soft rot, which is the result of maceration of the tissue caused by enzymatic degradation of the plant cell wall by pectinolytic enzymes.

- Off-odors and off-flavors production due to the activity of microbial enzymes and also due to the fermentative reactions that increase the levels of acids, alcohols, and CO₂.
- Loss of texture and wilting as a result of vascular infections.
- Production of brown discoloration since the polyphenol oxidase activity of the microflora may contribute to the brown discoloration.

This work was carried out to investigate the cause of after cooking browning of fresh-cut sweetcorn using a CA treatment previously shown to be optimal for reducing the losses of sugar and aroma in fresh-cut sweetcorn. The effect of CA storage on the concentrations of phenolics and HMF in fresh-cut sweetcorn was quantified, as well as its effect on microbial growth on the sweetcorn kernels.

Materials and Methods

Three experiments were done to study the after cooking browning in fresh-cut sweetcorn. In the first experiment, the effect of controlled atmosphere on the sugar content, the soluble phenolic content, and microbial load of fresh-cut sweetcorn kernels was measured and related to the after cooking browning. In the second experiment, microbes isolated from the first experiment were used to inoculate fresh-cut sweetcorn kernels to determine the effect of the microbial load on the after cooking browning. In the last experiment several attempts were made in an effort to isolate and identify the compound(s) responsible for the after cooking browning

Experiment I: CA Effect on Fresh-Cut Sweetcorn Kernels

Plant material

Fresh harvested sweetcorn (*Zea mays* L. rugosa var. Prime Time) cobs were harvested from a local farm in Florida (Hugh Branch Inc., Pahokee, Fla.) on 6 February 2001 precooled, and delivered overnight by refrigerated truck to a local grocery chain's

distribution facility in Jacksonville (Publix Distribution Center, Jacksonville, Fla.) on 7 February. The sweetcorn was shipped to Gainesville, Fla. on 8 February where it was then picked up and transferred to the postharvest lab (University of Florida, Gainesville, Fla.) and the experiment started on the same day.

Preparation of the fresh-cut sweetcorn

As soon as the sweetcorn arrived at the lab, the cobs were de-husked and the kernels were cut from the cobs using a sharp stainless steel knife. The kernels in the first row were popped out first using the edge of the knife and discarded, then the adjacent rows were cut from the cob near the kernel base and the cut kernels were collected. Special care was taken during cutting in order to minimize damage to the kernels. After cutting the kernels, they were dipped in chlorinated (2.7 mM NaOCl), 5 °C water at pH 7 for 2 min for sanitation and kept at 5 °C until distributed among the treatments.

Storage and treatments

Fresh-cut sweetcorn kernels were stored in either air or CA (2% O₂ plus 10% CO₂). This was the best atmosphere for fresh-cut sweetcorn as determined by Riad and Brecht (2001). The CA mixture was established using a system of pressure regulators, manifolds, and needle valve flowmeters to blend air, N₂, and CO₂ from pressurized cylinders. The CA was a flowing system with the flow rate set to maintain respiratory CO₂ accumulation under 0.5%. The gas flow was humidified by bubbling through water before introducing it to the containers. Four replicates of 300 g of sweetcorn kernels per treatment were stored at 5 °C for 10 d.

Sugar content

Total soluble sugars were measured in raw and cooked kernels using the phenol-sulfuric method described by Dubois et al. (1956). In this experiment, 15 g of sweetcorn kernels were blended in a commercial blender with 85 mL of 95% ethanol for 2-3 min then were heated for 20 min in an 85 °C water bath. Then the samples were stored overnight at -20 °C to precipitate ethanol-insoluble materials. The samples were then filtered through Whatman #2 filter paper in a Buchner funnel attached to a side-arm Erlenmeyer flask. A vacuum pump was connected to the side arm to expedite the extraction. The collected filtrates were volumetrically adjusted to 200 mL using 95% ethanol and 20 mL portions of the extracts were stored in scintillation vials at -20 °C until the measurements were performed.

To prepare samples for measurement of total soluble sugars, 0.5 mL of 5% phenol (w/w) was added to 0.5 mL of diluted (1:250) ethanol extract in test tubes, the mixture was vortexed, then 2.5 mL concentrated sulfuric acid was added and the mixture vortexed again. The test tubes were let to stand for 10 min then were placed in a 25 °C water bath for another 10 min to stop the reaction. The total soluble sugars were measured by reading the absorbance at 490 nm using a Shimadzu UV-1201 spectrophotometer (Shimadzu Scientific Instruments, Inc.; Tokyo, Japan). The absorbance values were compared to a standard curve of glucose.

Total Soluble phenolics

The Folin-Ciocalteu assay was used to measure total soluble phenolics in previously prepared ethanol extracts from raw and cooked kernels as gallic acid equivalents (Howard et al., 1996). In this experiment, 0.5 mL of the ethanol extract was added to a 20 mL test tube and 0.5 mL of 0.25N Folin-Ciocalteu phenol reagent

(Sigma-Aldrich Co. St. Louis, Mo.) was added, the tube then vortexed and left to stand for 3 min to allow reduction of phosphomolybdic acid by phenolic compounds. Next, 0.5 mL of 1N sodium carbonate was added and the samples left to stand for 7 min before addition of 3.5 mL DI-water. Finally, the samples were mixed and left to stand for 30 min before measuring. After 30 min, the tubes were then centrifuged for 5 min at 5040 g_n in order to remove the suspended starch molecules. The visible blue chromophores were then measured colorimetrically using a Shimadzu UV-1201 spectrophotometer (Shimadzu Scientific Instruments, Inc.; Tokyo, Japan) at 726nm.

Phenolic acids and 5-hydroxymethylfurfural (HMF)

Phenolic acids and HMF were extracted by blending a 5-g sample of kernels with 10 mL of methanol acidified with HCl (1000:1) for 1 min. The homogenate was filtered through Miracloth (Calbiochem, San Diego, Calif.). A 3-mL portion was then centrifuged at 2500 g_n for 5 min at 18 °C, filtered, and analyzed by HPLC for individual phenolic acids. Separation was conducted on a 100 × 4.6 mm Spherisorb ODS C18 column (Alltech Associates, Inc., Deerfield, Ill.) connected in series to a 150 × 3.9 mm Nova-Pak C18 column (Waters Corporation, Milford, Mass.), and peaks were monitored using a Waters 996 photodiode array detector at 280 nm. Spectral characteristics of phenolic acids were compared to those of external standards (Sigma Chemical Co., St. Louis, Mo.) for identification and quantification. A model Maillard reaction was performed by boiling glucose (1 $g \cdot L^{-1}$) with tryptophan (0.25 $g \cdot L^{-1}$) in acidified medium to produce HMF, which was analyzed as described above. The solution was prepared with a raised pH (1N NaOH was used to obtain pH 10) and, when development of the brown color occurred, the solution was neutralized (using 1N HCl) and then it was injected onto the HPLC.

Microbial analysis.

Total aerobic plate counts were performed for each treatment after 0, 4, 7, and 10 d of storage using Petrifilm plates (3M Company, St. Paul, Minn.). A 5-g sample of kernels from each treatment was placed into a 50-mL disposable sterile centrifuge tube containing 45 mL phosphate buffer solution (PBS), which was then placed in a rotary shaker for 20 min. Serial dilutions were plated on Petrifilm plates and incubated at 30 °C for 3 d. Aerobic microbial counts were reported as logarithm of colony forming units per gram (\log_{10} CFU·g⁻¹).

After cooking browning.

A 50-g sample of kernels was added to 300 mL of boiling water for 10 min. After cooking browning was evaluated using a reflectance colorimeter (Model CR200; Minolta Camera Co. Ltd., Japan). Color was measured using the CIE L*, a*, b* scale. The a* and b* values were converted to hue angle ($\tan^{-1} b^*/a^*$) and chroma ($((a^{*2}+b^{*2})^{1/2})$). Cooked samples (25 g) were blended with 25 g of water, the resulting slurry was poured into a Petri dish, and the color of the slurry was measured.

Statistical analysis

The experimental design was a completely randomized design with four replicates per treatment. The data were analyzed using the Statistical Analysis System (SAS). Analysis of variance (ANOVA) was used to determine the effect of treatments on the dependent variables. Means were separated by the least significant difference (LSD) test at $P < 0.05$.

Experiment II: Microbial Load Effect on the After Cooking Browning

Plant material

Fresh harvested sweetcorn (*Zea mays* L. *rugosa* var. Prime Time) cobs were harvested from a local farm in Florida (Hugh Branch Inc., Pahokee, Fla.) on 17 April 2001, precooled, and delivered overnight by refrigerated truck to a local grocery chain's distribution facility in Jacksonville (Publix Distribution Center, Jacksonville, Fla.) on 18 April where it was picked up and transferred to the postharvest lab (University of Florida, Gainesville, Fla.). The sweetcorn was kept at 5 °C and the experiment started on the following day, 19 April.

Preparation of the fresh-cut sweetcorn

Fresh-cut sweetcorn was prepared as described for experiment I.

Isolation of the microbes

From the last experiment (Experiment I) aerobic microbes were transferred from 3M Petri films to Petri dishes containing either nutrient agar (NA; to isolate bacteria) or acidified potato dextrose agar (APDA; to isolate yeasts and molds). The NA Petri dishes were incubated at 30 °C for 2 d and the APDA Petri dishes were incubated at room temperature (approximately 24 °C) for 3 d. The resulting colonies were then transferred to new plates with a total of three different yeasts and five different bacteria being isolated.

Preparation of the inoculum

On the day of inoculation, three plates per unique microbe isolate were washed with sterilized water and the rinsate placed on a magnetic stirrer to obtain a suspension of the microbes. Cell concentrations were measured using a spectrophotometer, and then

10 L of microbe suspension was prepared for each microbe and adjusted to contain 10^6 cells for the bacteria and 10^5 cells for the yeasts.

Inoculation process

Cheesecloth sacks were prepared to contain about 2.5 kg of fresh-cut sweetcorn. Two buckets (10 L) were filled with 5 L of each inoculum and a sweetcorn sack was dipped in each bucket for 5 min then it was removed and the excess inoculum was drained. About 75 g of kernels from each sack were placed in four, 125-mL glass flasks with sponge plugs per microbe suspension.

Controlled atmosphere treatment, storage, and sampling

The glass flasks were placed in two sealed metal chambers with Plexiglas front covers in a 5 °C storage room for 10 d. The chambers were ventilated with either air (control) or a CA of 2% O₂ plus 10% CO₂ prepared as described in Chapter 3.

After cooking browning measurement

After cooking browning was evaluated in the cooked fresh-cut sweetcorn kernels using the Horsfall-Barratt scale (Horsfall and Barratt, 1945) in which the percentage of the kernels in a sample affected by the browning were scored using a scale from 1 to 10. The score is based on 50% as a mid-point and the grades differ by a factor of two in either direction as follows:

1=0, 2= 6, 3= 12, 4 = 25, 5=50, 6= 75, 7= 87, 8= 94, 9=97, and 10= 100

The Horsfall-Barratt scale was originally developed for evaluating disease incidence in field plots. Because the severity of after cooking browning increased as the incidence of brown kernels increased, higher scores necessarily imply both increased incidence and severity of browning.

Experiment III: Attempts to Identify the Browning Reaction Source

Fresh-cut sweetcorn kernels were stored for 10 d in air at 5 °C to induce after cooking browning. After storage, the kernels were crushed in a mortar and pestle then pressed using cheesecloth to separate the kernel juice from the cell wall materials and each portion placed in a 20 mL test tube with sealed plastic cap, then each of those two components were placed in a boiling water bath for 5 min.

In the next step, the kernel juice was centrifuged and the juice solids were precipitated using acetone. The resulting supernatant was collected in a test tube and the pellet was re-dissolved in water in another test tube. Both tubes were placed in a boiling water bath for 5 min. The extracts containing the dark pigment resulting from boiling the re-dissolved pellets was added to crushed raw kernels for 1 min then the kernels washed with either water or ethanol to remove the pigment.

The last attempt was to run this unidentified brown pigment on the HPLC using the same protocol that was used to measure phenolic compounds concentration.

The unidentified brown pigment was measured in both extracts using a Waters Alliance 2690 HPLC (Waters Corp., Milford, Mass.) equipped with a dual column system using a Supelcosil LC-18 column, 250 x 4.6 mm, and a Waters Spherisorb C-18 ODS (5 µm) column, 4.6 x 250 mm. Polyphenolic compounds were detected using a Waters 996 photodiode array (PDA) detector. Individual compounds were separated with gradient mobile phases of acidified water (98:2, water: acetic acid) and (68:30:2, water: acetonitrile: acetic acid) at a flow rate of 0.8 mL·min⁻¹ with PDA detection at 280 nm. (Talcott et al., 2000).

Results and Discussion

Experiment I

Fresh-cut sweetcorn is a very perishable product with a high respiration rate, which makes storage at low temperatures, as close to 0 °C as possible, critical to ensure quality retention (Riad and Brecht, 2001). In this work, the effect of gas modification as a secondary method of quality preservation was used at a relatively high storage temperature (5 °C). The sugar content was significantly higher in the kernels from CA storage than from air storage. Also, the sugar content was significantly higher in raw kernels than in cooked kernels (Fig. 6-1), which might be due to leaching of soluble solids from the cut kernel surfaces during boiling. After 10 d at 5 °C, the sugar content on a FW basis was about 9% in CA storage and about 6.5% in air storage, both of which are very acceptable levels for sugars in sweetcorn.

The main problem in this product is the after cooking browning, which causes the appearance of a dark brown pigment in the kernels that appears to be attached to the cell wall materials. As a result, the pigment responsible for the after cooking browning was not soluble in water or ethanol. The after cooking browning occurred mainly in kernels stored in air (Fig. 6-5, 6, and 8). The browning first occurred after 7 d of storage in air and was severe after 10 d (Fig. 6-6, and 8). This after cooking browning was controlled by using CA storage with 2% O₂ plus 10% CO₂ even after 10 d of storage at 5 °C (Fig.6-7, and 9). The L value or lightness of the sweetcorn slurry did not change during the storage period, however, the a* value (as a measure of redness) was significantly higher in the air storage after 7 and 10 d of storage (with the start of the browning), and the b* value (as a measure of yellowness) was also higher in the air storage after 7 and 10 d as an indication of a color change toward brown color in the air-stored treatment

compared to the light yellow color of the CA-stored treatment (Table 6-1). There was no significant difference in hue angle between treatments until after 10 d of storage, when the hue of air-stored kernels dropped sharply, while the chroma value was significantly higher in air storage after both 7 and 10 d.

In the process of studying the cause of the after cooking browning of fresh-cut sweetcorn kernels, Riad and Brecht (2001) suggested that this browning could be due to a Maillard reaction since higher concentrations of free amino acids were found in the treatments with more severe browning, and very high soluble sugar concentrations were maintained in those treatments. In this work, no HMF, the intermediate of the Maillard reaction, was found in any of the sweetcorn samples before or after cooking (Fig. 6-2), which indicates that the Maillard reaction is not responsible for the after cooking browning in fresh-cut sweetcorn. However, HMF was successfully measured in a reaction model consisting of boiled glucose plus tryptophan solution.

There were no differences found in the phenolic profiles of extracts from air- or CA-stored sweetcorn kernels (Fig. 6-2) and there also was no trend or significant difference in their total soluble phenolics contents (Fig. 6-3), but there was a significant reduction in the levels of soluble phenolics noticed after cooking (Fig. 6-3). This could mean that soluble phenolic compounds in fresh kernels were converted to insoluble compounds during cooking or the soluble phenolics might have leached out in the boiling water and been diluted. More work needs to be done to identify the individual polyphenolic compounds that might be responsible for the after cooking browning via autooxidation, which has been shown to cause formation of brown color during thermal processing in other food products such as carrot puree (Talcott et al., 2000).

The initial microbial count was about $\log 2 \text{ CFU}\cdot\text{g}^{-1}$ and increased only slightly in either air or CA storage during the first 4 d of storage at 5 °C (Fig. 6-4). There was a >2-log increase in microbial count in air-stored sweetcorn kernels between days 4 and 7 of storage, and a further almost 1-log increase between days 7 and 10 (to $\log 5.5 \text{ CFU}\cdot\text{g}^{-1}$) (Fig. 6-4), which corresponds to the appearance and worsening severity, respectively, of after cooking browning in those kernels. Controlled atmosphere significantly reduced the increase in total aerobic microbial count during storage (Fig. 6-4), but there was still about a 2-log increase in microbial count in the CA-stored kernels between days 4 and 10 of storage. It is interesting to consider the possibility that the higher microbial load on the air-stored kernels might play a role in the after cooking browning. It may be possible that the browning is related to a direct response of the sweetcorn tissue to the microorganisms, or the browning may alternatively be associated with some product of microbial enzyme activity.

In conclusion, fresh-cut sweetcorn kernels can be successfully stored for 10 d in CA with little loss of visual or sensory quality. After cooking browning can be totally controlled by storage in 2% O₂ plus 10% CO₂, even at a higher than recommended temperature (5 °C versus 0-1 °C). Although high levels of free amino acids and sugars are found in sweetcorn kernels that develop after cooking browning, the Maillard reaction is not the cause of the after cooking browning since no HMF was detected in the browned kernels. More work needs to be done regarding the identification of different phenolic-protein or phenolic-amino acid complexes that might play a role in the after cooking browning. Also, more work is needed to determine the relationship, if any, of the microbial load with the after cooking browning.

Experiment II

In this experiment, the microbial load on inoculated fresh-cut sweetcorn reached 10^8 within 2 d of storage at 5 °C, but did not increase more than that during the rest of the storage period. After cooking browning started earlier in the air controls than in the CA-stored treatments and, after 4-5 d, all the air control kernels turned brown after cooking. The CA storage significantly reduced the microbial load, but it was not effective in reducing the after cooking browning. This is in contrast to the obvious effectiveness of CA in reducing after cooking browning in non-inoculated sweetcorn kernels and may have been due the initial high microbial load already present at the beginning of storage in this experiment due to inoculation. There was no specific effect of any of the isolated microbes used to inoculate the sweetcorn kernels since all the inoculated treatments had severe browning compared to the non-inoculated controls. The appearance of after cooking browning was advanced by 1-2 d for some microbes, but all the microbe treatments had 100% browning after about 4-5 d.

These results indicate that after cooking browning is not caused directly by the presence of a specific microorganism. The microbe load could be responsible for after cooking browning in an indirect way such as by increasing the kernel deterioration rate. Alternatively, microbial activity may act on the sweetcorn substrate to release or induce the formation of a browning precursor, either alone or through reaction with one or more other compounds. This possible precursor could then result in the browning when the kernels are heated.

Experiment III

In this experiment, boiling the separated kernel juice and cell wall materials did not cause browning in the kernel residue while the juice started to brown within 1-2 min. When the

kernel juice was centrifuged to remove suspended material and the juice dissolved solids were precipitated using acetone, the resulting supernatant did not brown, but when the acetone-insoluble pellet was re-dissolved in water and the solution was boiled, the solution quickly turned brown within seconds (the reaction started after 20 s and the solution was very dark brown to nearly black after 60 s).

When the solution with dark pigment that was obtained from boiling the re-dissolved pellet of acetone-insoluble material was added to crushed raw kernels it stained the tissue with a brown color that was not subsequently washable. This supports the idea that the brown compound is a water-soluble brown pigment that is developed during boiling and then reacts with (i.e., binds to) cell wall material and becomes insoluble. This could be the reason why we were unable to isolate this pigment from cooked sweetcorn kernels using water, ethanol, or acetone.

When this unidentified brown pigment was run on HPLC it was found that it is a very water-soluble compound(s). It was eluted very quickly, before any known authentic compounds we have, and it was absorbed in the entire UV spectrum (Fig. 6-10) as a result we were unable to identify it.

Table 6-1. Effect of atmosphere modification and storage period on the color parameters of cooked fresh-cut sweetcorn kernels stored in air or controlled atmosphere.

Parameter	Treatment	Storage duration (d)			
		0	4	7	10
L*	air	61.46	62.54 a*	64.44 a	62.03 a
	CA ¹		63.38 a	62.84 a	64.50 a
a*	air	-2.25	-1.93 a	-1.71 a	2.37 a
	CA		-2.13 a	-1.95 b	-1.71 b
b*	air	19.7	20.97 a	23.04 a	24.39 a
	CA		20.62a	20.94 b	21.15 b
Hue angle	air	96.52	95.26 a	94.24 a	84.45 b
	CA		95.90 a	95.32 a	94.62 a
Chroma	air	19.83	21.06 a	23.10 a	24.50 a
	CA		20.73 a	21.03 b	21.22 b

* Values in the same column within the same color parameter followed by the same letter are not significantly different at $p < 0.05$ by LSD; $n = 4$.

¹ CA = controlled atmosphere storage with 2% O₂ plus 10% CO₂.

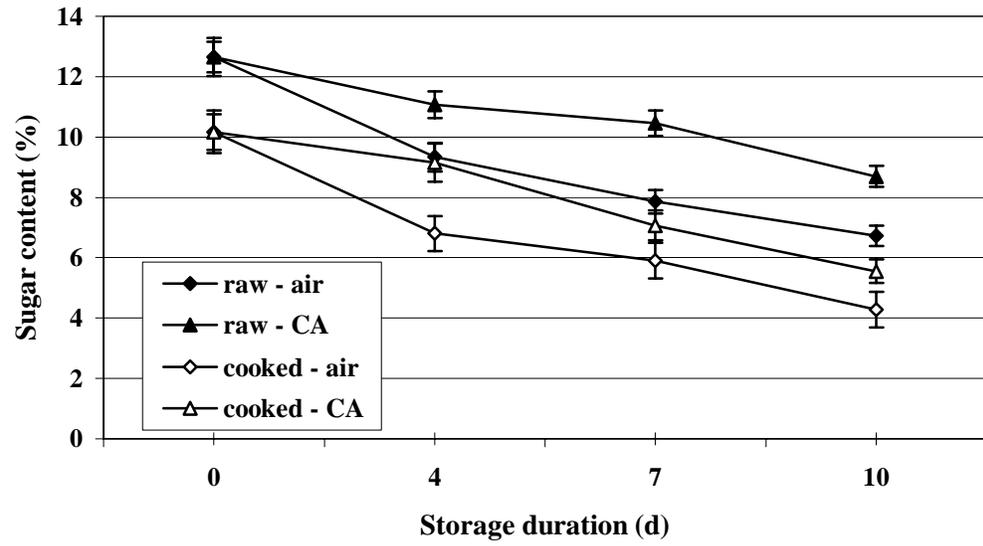


Fig. 6-1. Effect of controlled atmosphere storage on the sugar content (%) of raw and cooked fresh-cut sweetcorn kernels stored in air or CA (2% O₂ plus 10% CO₂) for 10 d at 5 °C. Bars indicate the mean \pm SD; n = 4.

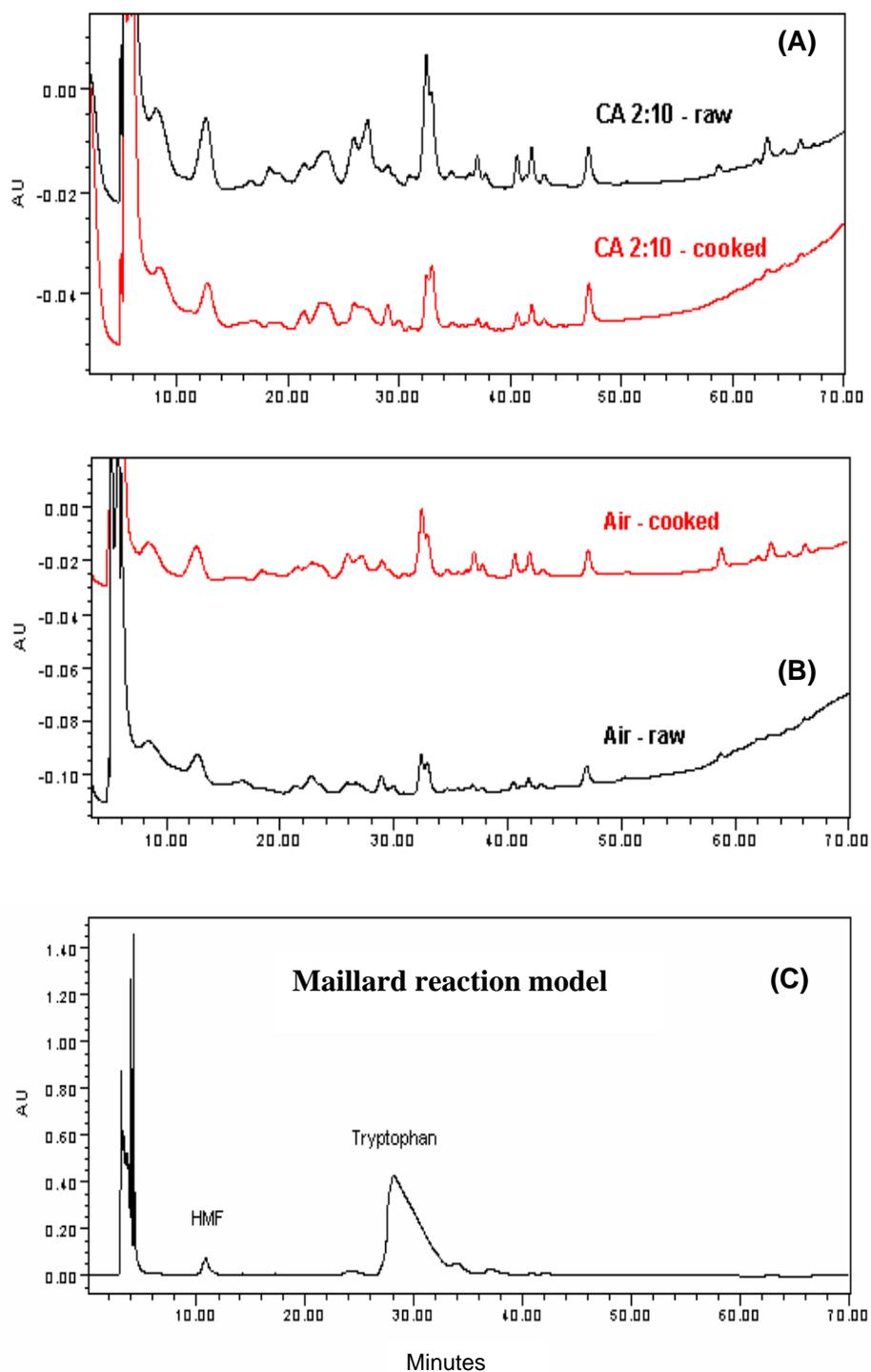


Fig. 6-2. Typical phenolic acids profiles of fresh-cut sweetcorn kernels stored at 5 °C for 10 d (A) Kernels stored in air, raw and cooked, (B) kernels stored in 2% O₂ plus 10% CO₂, raw and cooked, (C) glucose + amino acid (tryptophan) as a model for HMF.

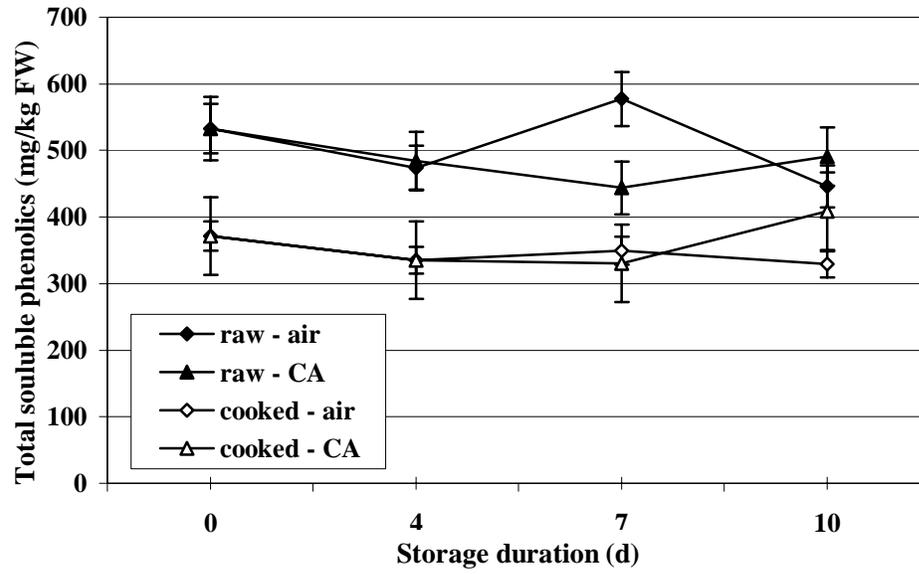


Fig. 6-3. Total soluble phenolics (mg/kg FW) in raw and cooked fresh-cut sweetcorn kernels stored in air or CA (2% O₂+10% CO₂) for 10 d at 5 °C. Bars indicate the mean \pm SD; n = 4.

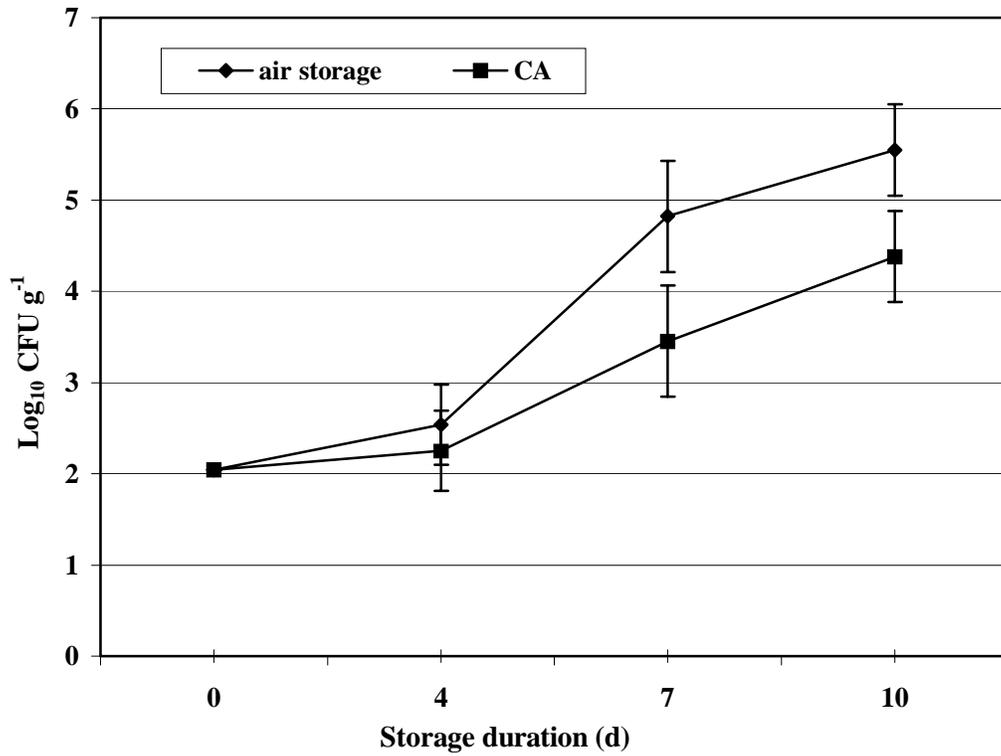


Fig. 6-4. Total aerobic microbial count (\log_{10} CFU·g⁻¹) of fresh-cut sweetcorn kernels stored in air or CA (2% O₂+10% CO₂) for 10 d at 5 °C. Bars indicate the mean \pm SD; n = 4.

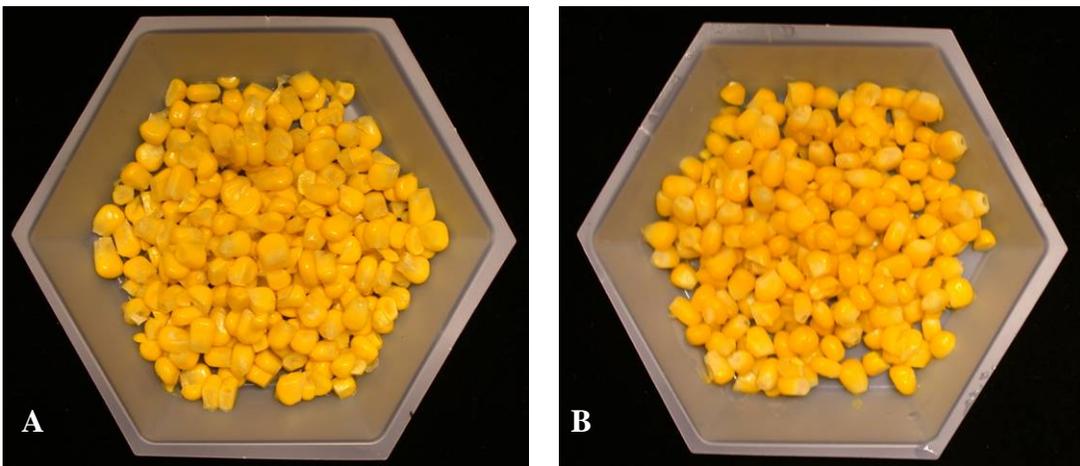


Fig. 6-5. Raw (A) and cooked (B) fresh-cut sweetcorn kernels at day 0 (just after preparation).

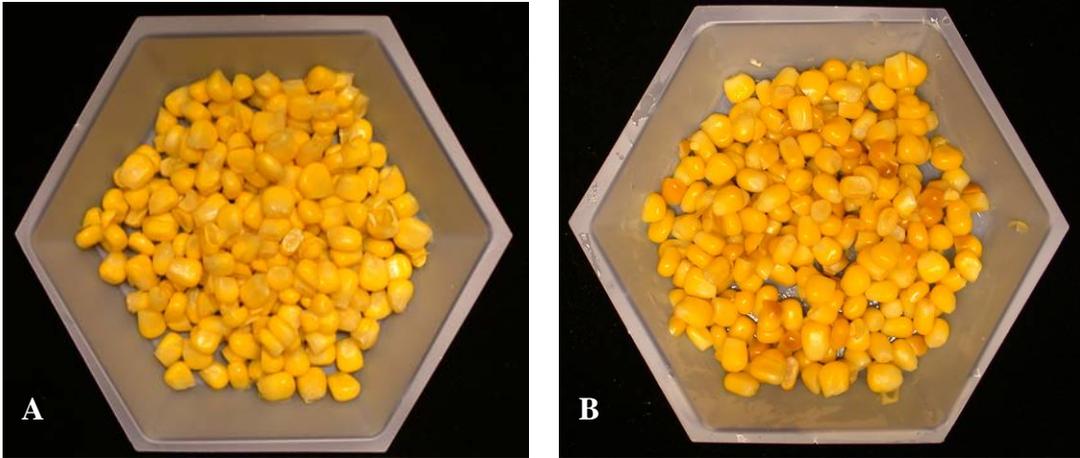


Fig. 6-6. Raw (A) and cooked (B) fresh-cut sweetcorn kernels stored in air for 7 d at 5 °C.

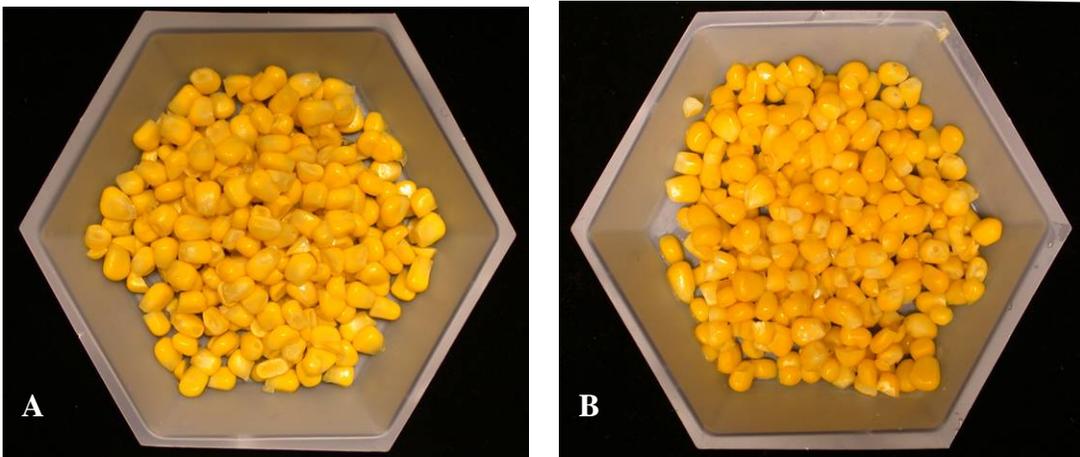


Fig. 6-7. Raw (A) and cooked (B) fresh-cut sweetcorn kernels stored in CA (2% O₂+10% CO₂) for 7 d at 5 °C.

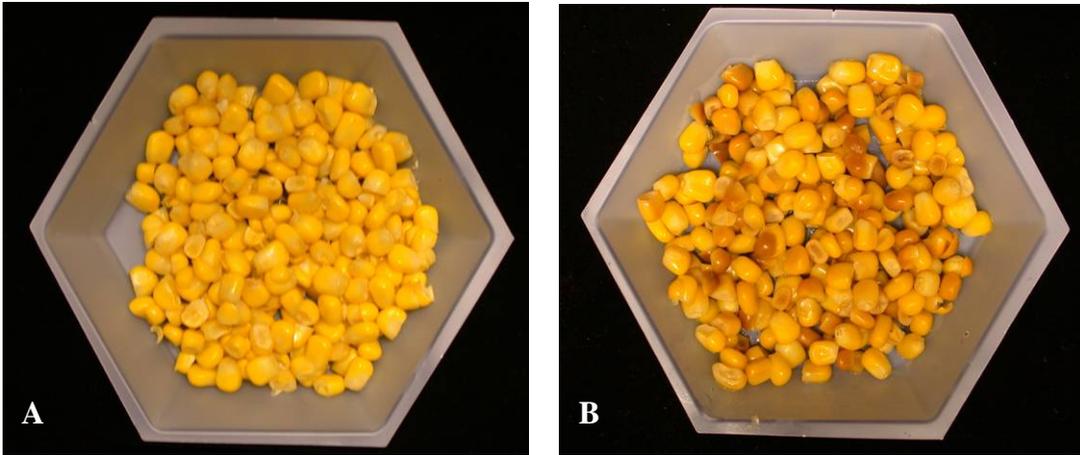


Fig. 6-8. Raw (A) and cooked (B) fresh-cut sweetcorn kernels stored in air for 10 d at 5 °C.

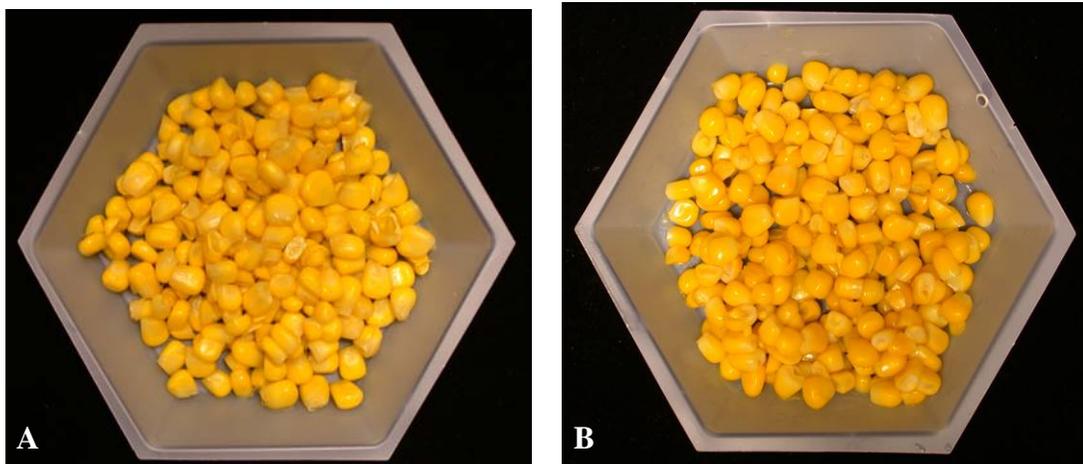


Fig. 6-9. Raw (A) and cooked (B) fresh-cut sweetcorn kernels stored in CA (2% O₂+10% CO₂) for 10 d at 5 °C.

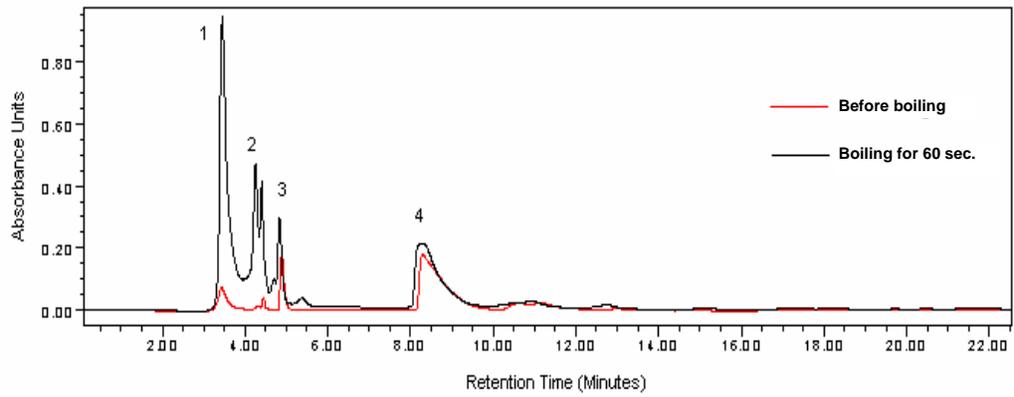


Fig. 6-10. HPLC chromatogram of the acetone-insoluble fraction of fresh-cut sweetcorn kernel juice (after storing the kernels for 10 d at 5 °C in air). Unidentified brown pigments (peaks 1, 2, and 3) increased dramatically upon boiling and are probably responsible for after-cooking browning. Peak 4 was identified as galacturonic acid, which is not affected by boiling.

CHAPTER 7
PHENOLICS PROFILE AND ANTIOXIDANT CAPACITY IN FRESH-CUT
SWEETCORN DURING STORAGE

Introduction

The polyphenolic content of fruits and vegetables is an important contributor to color quality and sensory properties (Macheix et al., 1990). Interest in these compounds has increased in recent years because of their possible health benefits, and these benefits are mainly attributed to their antioxidant characteristics. Epidemiological studies strongly suggest a protective effect of dietary antioxidants against many chronic diseases and suggest that increased consumption of fruits and vegetables may be related to a reduction of the risk of cardiovascular disease and certain types of cancer (Block and Langseth, 1994; Kohlmeier et al., 1995; Steinmetz and Potter, 1996). Also, dietary antioxidants other than vitamins (i.e., polyphenolic compounds) have been shown to be a major dietary factor associated with such protective effects (Hertog et al., 1992; 1994; 1997). Gil et al. (2002) found that the contributions of phenolic compounds to antioxidant activity were much greater than those of vitamin C and carotenoids in 25 cultivars of nectarines, peaches, and plums. Despite these facts, there are no published reports on the polyphenolic composition and antioxidant capacity of sweetcorn.

The benefit of using CA/MA in fruit and vegetable storage is well established (Chinnan, 1989; El-Gorani and Sommer, 1981; Kader, 1987; Kader et al., 1989; Shewfelt, 1986; Zagory and Kader, 1988) but there is little available work on the effect of such storage treatments on the antioxidant capacity of fruits and vegetables. Cranberry

fruit stored in 21% O₂ plus 30% CO₂ had lower antioxidant activity than air-stored fruit after 2 months of storage at 3 °C (Gunes et al., 2002). Storage of blueberries in a high O₂ atmosphere for 35 d at 5 °C was beneficial in maintaining significantly higher antioxidant capacity compared to air storage, with 80-100% O₂ found to be the best treatment (Zheng et al., 2003).

The use of CA was found to have a beneficial effect on both sweetcorn cobs or fresh-cut sweetcorn kernels in reducing respiration and hence maintaining higher sugar content, the most important characteristic in sweetcorn quality (Riad and Brecht, 2003; Riad et al., 2002), but the most important benefit of using CA for fresh-cut sweetcorn kernels is the prevention of after cooking browning, which is the major limiting factor for air storage (Riad and Brecht, 2001; Riad et al., 2003). There is no published work on the effect of atmosphere modification on the antioxidant capacity of sweetcorn or fresh-cut sweetcorn kernels.

Phytonutrients with antioxidant capacity can be found in free, soluble forms and also in insoluble, bound forms (Adom and Liu, 2002). Most of the insoluble, bound antioxidants are bound to cell wall materials (Bunzel et al., 2001; Sosulski et al., 1982). About 74% and 69% of the total phenolics present in rice and corn, respectively, are in the insoluble, bound forms, with ferulic acid being the major phenolic compound present (Adom and Liu, 2002).

A number of methods have been developed to measure the reducing efficiency or antioxidant capacity of dietary antioxidants either as pure compounds or in food extracts. These methods focus on different mechanisms of the antioxidant defense system, i.e., scavenging of oxygen and hydroxyl radicals, reduction of lipid peroxy radicals,

inhibition of lipid peroxidation, or chelation of metal ions. The ORAC (oxygen radical absorbance capacity) assay (Cao et al., 1995) depends on free radical damage to a fluorescent probe to effect a change in its fluorescence intensity. The change of fluorescence intensity is an index of the degree of free radical damage. In the presence of an antioxidant, the inhibition of free radical damage, which is reflected in the protection against the change in probe fluorescence, enables quantification of the antioxidant capacity present. The ORAC assay is a common method to measure antioxidant capacity of food products and it was used in this study.

The objectives of this study were to determine the polyphenolic composition and antioxidant capacity of fresh-cut sweetcorn kernels in both the free fraction (water soluble fraction) and in the bound fraction (hydrolyzed fraction of the water insoluble compounds) during storage in either air or CA.

Materials and Methods

Plant Material

Fresh harvested sweetcorn (*Zea mays* L. rugosa var. Prime Time) cobs were harvested from a local farm in Florida on 25 March 2002, precooled, and delivered overnight by refrigerated truck to a local grocery chain's refrigerated distribution facility in Jacksonville (Publix distribution center, Jacksonville, Fla.) on 24 March where it was picked up in the morning and transferred within 2 h to the postharvest lab by air-conditioned car (University of Florida, Gainesville, Fla.) and the experiment started at the same day. The cobs were de-husked and de-silked, and held at 5 °C while the kernels were cut. The kernels were cut from the cobs using a sharp stainless steel knife. The kernels in the first row were popped out first using the edge of the knife and discarded, then the adjacent rows were cut from the cob near the kernel base and the cut kernels

were collected. Special care was taken during cutting in order to minimize damage to the kernels. After cutting the kernels, they were dipped in chlorinated (1.34 mM NaOCl), 5 °C water at pH 7 for 2 min. for sanitation and kept at 5 °C until distributed among the treatments.

Controlled Atmosphere Treatment

Controlled atmosphere was used to determine the effect of atmosphere modification on fresh-cut sweetcorn antioxidant and phenolic content. The CA mixtures were established using a system of pressure regulators, manifolds, and needle valve flowmeters to blend air, N₂, and CO₂ from pressurized cylinders. The CA was a flowing system with the flow rates set to maintain respiratory CO₂ accumulation under 0.5%. The air flow was humidified by bubbling through water before introducing it to the containers. Cut kernels were exposed to two different gas compositions: Air (control) and 2% O₂ plus 10% CO₂, which had been previously determined to be the best gas composition for maintaining fresh-cut sweetcorn quality and for preventing after cooking browning in the cooked sweetcorn kernels (Riad and Brecht, 2001; Riad et al., 2003). Gas composition was monitored daily using a Servomex O₂ and CO₂ analyzer (Servomex; Norwood, Mass.).

Fresh-Cut Kernel Storage and Sample Preparation

The cut kernels were stored at 5 °C for 10 d with subsamples taken after 4 and 7 d. Three replicate samples per treatment were prepared by putting ~300 g of cut kernels in each of three, 1.2-L plastic containers (Tupperware; Orlando, Fla.). The containers were each fitted with two plastic connections that were attached to plastic tubing and served as the inlet and outlet for the gas flow. After the storage period, the samples were stored frozen at -20 °C until analysis.

Extraction of Phenolic Compounds

Extraction of free phenolic compounds

Free phenolic compounds in fresh-cut sweetcorn kernels were extracted by blending 10 g of kernels in 50 mL acetone for 10 min. After centrifugation at 2500 g_n for 10 min, the supernatant was removed, and the extraction was repeated on the precipitate. Supernatants were pooled, evaporated at 45 °C to about 10 mL, and reconstituted with water to a final volume of 10 mL (Eberhardt et al., 2000).

Extraction of bound phenolic compounds

The precipitates from free phenolic extraction (i.e., bound phenolics) were digested at room temperature with 10 mL 2M NaOH per sample for 1 h with shaking. The mixture was neutralized with an appropriate amount of 2M HCl. The final solution was extracted several times with acetone. The supernatant was then was evaporated to dryness. Phenolic compounds were reconstituted in 10 mL of water with the help of a sonic water bath (Sosulski et al., 1982).

Parameters

Measurement of polyphenolic compound concentrations

Free and bound polyphenolic compounds in raw fresh-cut sweetcorn kernels were separated using a Waters Alliance 2690 HPLC (Waters Corp., Milford, Mass.) equipped with a dual column system using a Supelcosil LC-18 column, 250 x 4.6 mm, and a Waters Spherisorb C-18 ODS (5 μ m) column, 4.6 x 250 mm. Polyphenolic compounds were detected using a Waters 996 photodiode array (PDA) detector. Individual polyphenolic compounds were separated with gradient mobile phases of acidified water (98:2, water: acetic acid) and (68:30:2, water:acetonitrile:acetic acid) (Talcott et al., 2000) at a flow rate of 0.8 mL \cdot min⁻¹ with PDA detection at 280 nm. Ultraviolet spectral

properties (200-400 nm) and retention time of each compound were compared to that of authentic standards obtained (Sigma Chemical Co., St. Louis, Mo.).

Total phenolics

Total phenolics concentrations were measured in both the free and bound fractions using the Folin-Ciocalteu assay. A 100- μ L aliquot of each extract was mixed with 100 μ L Folin reagent (Sigma Chemical Co., St. Louis, Mo.) for 3 min to allow reduction of phosphomolybdic acid by phenolic compounds. Then 100 μ L of 1N Na₂CO₃ was added as an alkali to form chromophores with a visible blue color. After 7 min, the test solutions were brought up to 1 mL total volume with water and read spectrophotometrically using a Beckman DU 640 Spectrophotometer (Beckman Coulter, Inc., Fullerton, Calif.) at 726 nm after 30 min. Total soluble phenolics were measured as gallic acid equivalents (Howard et al., 1996).

Quantification of antioxidant capacity

Antioxidant capacity was measured in both the free and bound phenolic fractions using the ORAC assay described by Cao et al. (1995) and modified by Ou et al. (2002) with the use of fluorescein as the fluorescent probe. Peroxyl radicals were generated by 2,2-azobis (2-amidinopropane) dihydrochloride, and fluorescence loss was monitored using a 96-well Molecular Devices fmax fluorescent microplate reader (485 nm excitation and 538 nm emission; Molecular Devices Corp., Sunnyvale, Calif.) with fluorescent readings taken every 2 min for 70 min at 37 °C and the fluorescent decay curves were calculated (Talcott et al., 2003). A 40-fold dilution of each extract (free and bound phenolic extracts) in a pH 7.2 ORAC phosphate buffer (61.6:38.9 v/v, 0.75 M K₂HPO₄ and 0.75M NaH₂PO₄) was used and compared to a standard curve representing 0, 6.25, 12.5, 25, and 50 μ M of α -Trolox, a water soluble form of vitamin E (Sigma

Chemical Co., St. Louis, Mo.). Measurements were done by adding 20 μL of the diluted sample or the Trolox to 160 μL of working β -phycoerythrin (β -phycoerythrin stock solution was prepared by dissolving 1 mg in 5.9 mL phosphate buffer and the working β -phycoerythrin was prepared by adding 300 μL of the stock to 13.4 mL of phosphate buffer) and the initial relative fluorescence were measured, then 20 μL of 2,2-azobis (2-amidinopropane) dihydrochloride were added and the relative fluorescence (RF) was measured every 2 min for 70 min. The fluorescent decay curves were calculated in relation to Trolox and the data were expressed in Trolox equivalents.

Statistical Analysis

The experimental design was a completely randomized design with three replicates. The data were analyzed using the Statistical Analysis System (SAS). Analysis of variance (ANOVA) was used to determine the effect of treatments on the dependent variables. Means were separated by the least significant difference (LSD) test at $P < 0.05$.

Results and Discussion

The increased popularity and demand for fresh-cut produce coincides with an increased consumer interest in the nutritional value of fresh produce, especially its antioxidant content. The high antioxidant content of fruits and vegetables adds another health benefit for the consumer since there are many epidemiological studies showing that polyphenolic compounds may be among the most important compounds in nutrition. Most phenolic compounds have a relatively high antioxidant capacity - recent work has shown that the contribution of polyphenolics to the total antioxidant capacity of fruits and vegetables is greater than the more well-known antioxidants like vitamin C and carotenoids (Hertog et al., 1992; 1994; 1997).

The pectin galacturonic acid was the first of the acetone insoluble compounds in the sweetcorn extracts to elute (RT ~6.4 min). The CA-stored kernels usually had higher concentrations of galacturonic acid and its concentration increased with increased storage time (Fig. 7-8), which may have been due to the degradation of the cell walls. Phenolic compounds that were found included *p*-coumaric acid, *p*-OH-benzoic acid, and ferulic acid; also, several unidentified phenolic compounds were present in low concentrations (Fig. 7-1 to 7-7). Ferulic acid was the most prominent phenolic compound in the bound fraction (Fig. 7-1, 2, 4, and 6), which is in agreement with work done on field corn, for which ferulic acid was reported to be the major phenolic compound and its concentration in the bound fraction was found to be 1000-fold its concentration in the free fraction (Adom and Liu, 2002)

The antioxidant capacity was much higher in the bound phenolic fraction than in the free fraction (Fig. 7-9), and this is in agreement with the work of Adom and Liu (2002), who reported that most of the antioxidant capacity in four grains (rice, corn, wheat, and oat) was in the bound fraction. The fact that most of the antioxidant capacity in sweetcorn kernels are in the bound form does not reduce its importance since there are many epidemiological studies showing that bound phytochemicals can survive stomach and intestinal digestion to reach the colon where the colonic microflora have the ability to digest them and release the health beneficial compounds (Adam and Liu, 2002).

Controlled atmosphere treatment did not have much effect on the sweetcorn kernel antioxidant capacity during the first 7 d of storage (Fig. 7-9), but it significantly reduced antioxidant capacity by 10 d of storage. Similar research has shown that CA prevented an antioxidant capacity increase in cranberry fruit compared to air storage (Gunes et al.,

2002). This reduction in the antioxidant capacity in CA compared with air storage could be due to the effect of CA in delaying deterioration and maintaining cell wall integrity, hence preventing the release of many compounds that have antioxidant activity. Similar results were reported by Dewanto et al. (2002) who found an increase in the antioxidant capacity in heat-processed tomatoes due to the disruption of cell membranes and the release of lycopene from the cell matrix and insoluble fiber during thermal processing.

On the other hand, total phenolics content in the fresh-cut sweetcorn kernels followed a pattern mirroring that of the antioxidant capacity. There was not much change in free or bound phenolic content during the first week of storage in either air or CA, except for a significant increase in the free phenolic fraction after 10 d for air-stored kernels. This increase in free phenolics might have been due to the release of cell wall bound phenolics as a result of deterioration in the air storage treatment. Also, after 10 d of storage at 5 °C, the CA-stored kernels had significantly lower levels of total phenolics in both the free and bound fractions.

In conclusion, fresh-cut sweetcorn kernels could serve as a good dietary source for polyphenolics and antioxidants, however, fresh-cut sweetcorn antioxidant capacity declines during storage. Since CA storage is essential to maintain the quality of this highly perishable commodity, it is not recommended to use air storage instead of CA despite the greater loss of antioxidant capacity late in CA storage, because of the many other benefits of atmosphere modification for quality preservation.

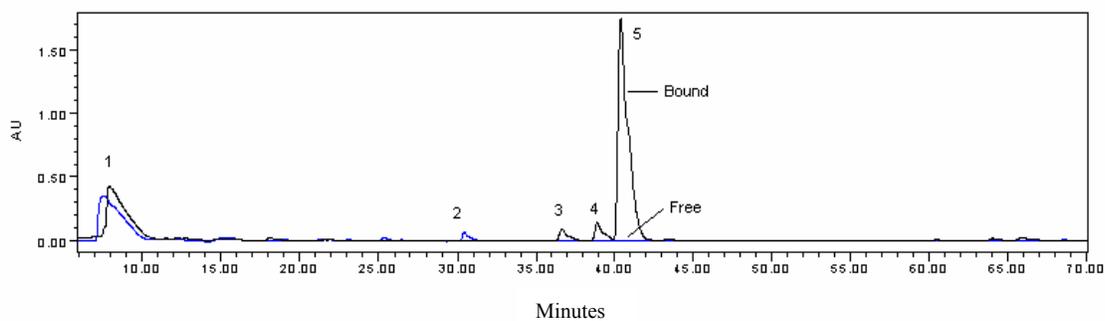


Fig. 7-1. HPLC chromatograms of free and bound polyphenolic compounds in sweetcorn extract on day 0 (before the storage treatment). Identification of compounds was made by comparing retention times and/or spectral properties to authentic standards. Tentative peak identifications include 1) galacturonic acid, 2) unknown, 3) *p*-coumaric acid, 4) unknown, 5) ferulic acid.

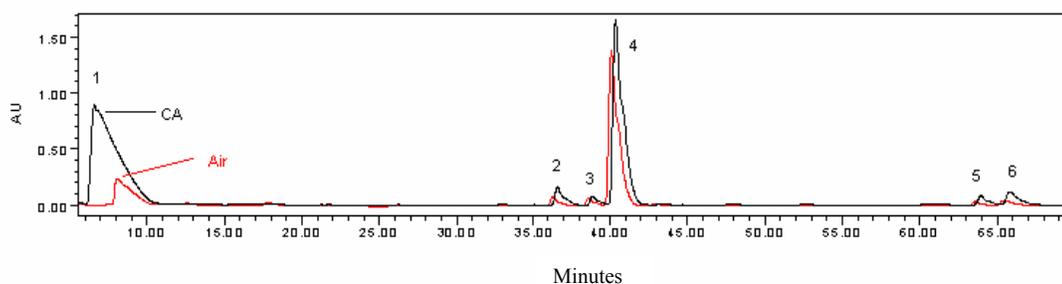


Fig. 7-2. HPLC chromatograms of bound polyphenolic compounds in the sweetcorn extract after 4 d of storage at 5 °C in air (control) or CA. Identification of compounds was made by comparing retention times and/or spectral properties to authentic standards. Tentative peak identifications include 1) galacturonic acid, 2) *p*-coumaric acid, 3) unknown, 4) ferulic acid, 5) unknown, and 6) ferulic acid polymer.

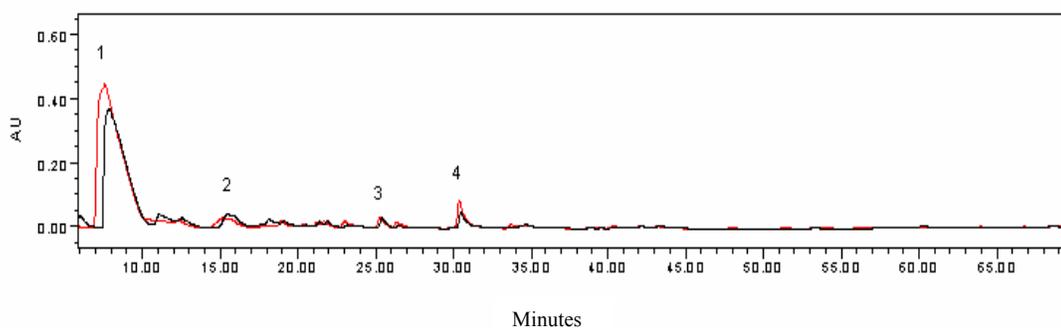


Fig. 7-3. HPLC chromatograms of free polyphenolic compounds in the sweetcorn extract after 4 d of storage in air (control) or CA. Identification of compounds was made by comparing retention times and/or spectral properties to authentic standards. Tentative peak identifications include 1) galacturonic acid, 2) *p*-OH-benzoic acid, 3) tryptophan and 6) tryptophan-containing protein.

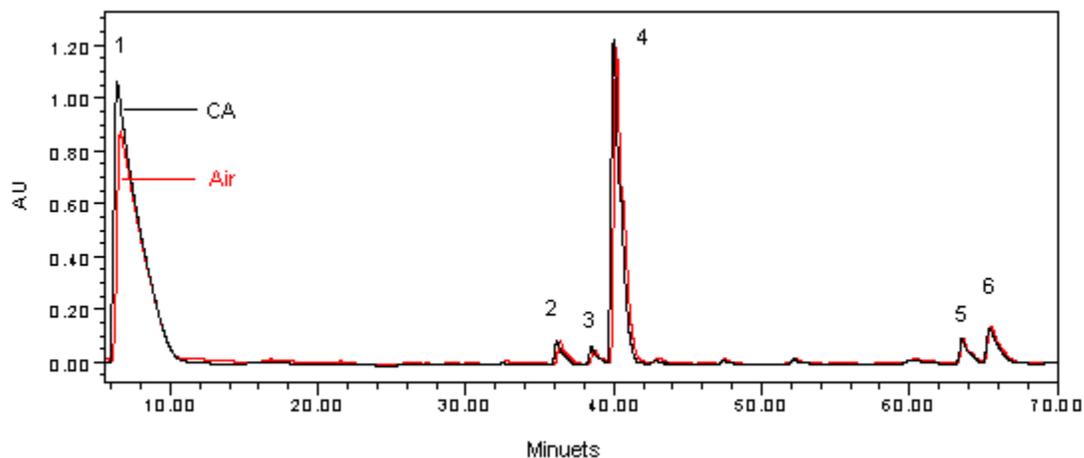


Fig. 7-4. HPLC chromatograms of bound polyphenolic compounds in the sweetcorn extract after 7 d of storage in air (control) or CA. Identification of compounds was made by comparing retention times and/or spectral properties to authentic standards. Tentative peak identifications include 1) galacturonic acid, 2) *p*-coumaric acid, 3) unknown, 4) ferulic acid, 5) unknown, and 6) ferulic acid polymer.

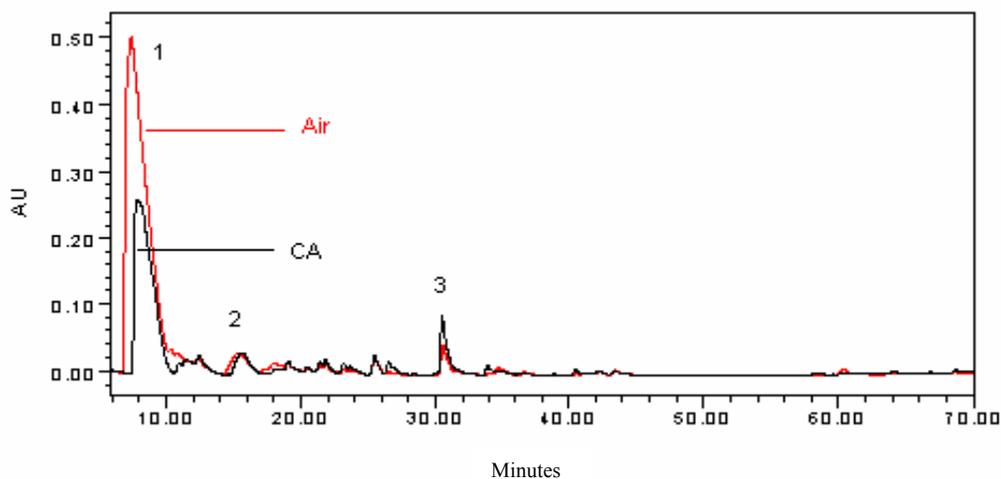


Fig. 7-5. HPLC chromatograms of free polyphenolic compounds in the sweetcorn extract after 7 d of storage in air (control) or CA. Identification of compounds was made by comparing retention times and/or spectral properties to authentic standards. Tentative peak identifications include 1) galacturonic acid, 2) *p*-OH-benzoic acid and, 3) tryptophan-containing protein.

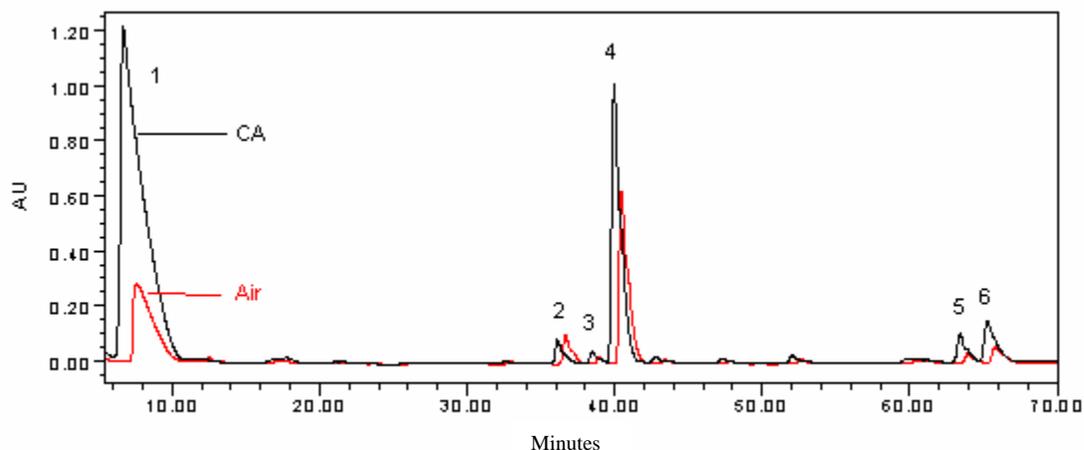


Fig. 7-6. HPLC chromatograms of bound polyphenolic compounds in the sweetcorn extract after 10 d of storage in air (control) or CA. Identification of compounds was made by comparing retention times and/or spectral properties to authentic standards. Tentative peak identifications include 1) galacturonic acid, 2) *p*-coumaric acid, 3) unknown, 4) ferulic acid, 5) unknown, and 6) ferulic acid polymer.

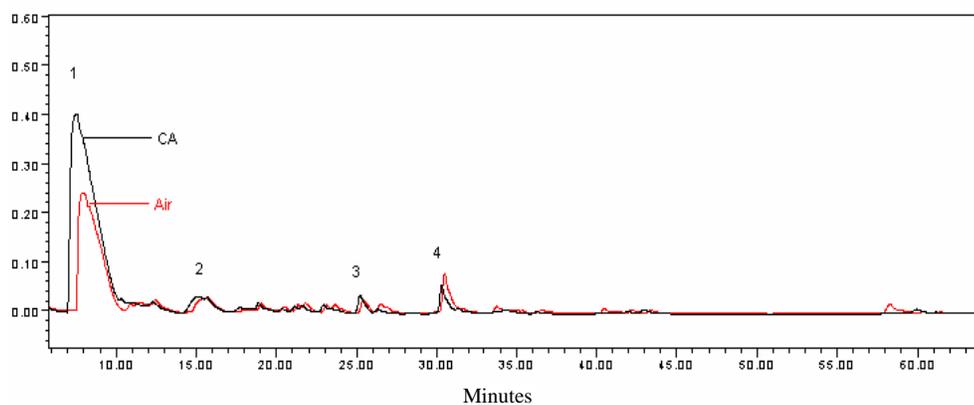


Fig. 7-7. HPLC chromatograms of free polyphenolic compounds in the sweetcorn extract after 10 d of storage in air (control) or CA. Identification of compounds was made by comparing retention times and/or spectral properties to authentic standards. Tentative peak identifications include 1) galacturonic acid, 2) *p*-OH-benzoic acid, 3) tryptophan and 6) tryptophan- or gallic acid-containing protein.

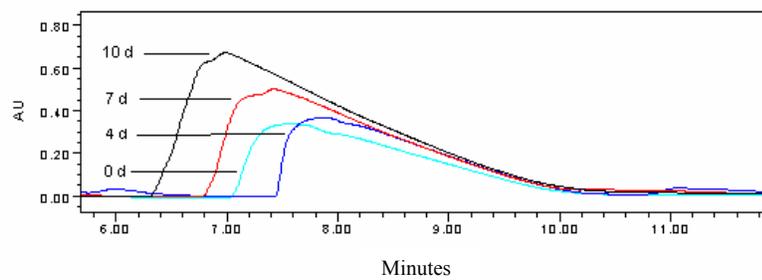


Fig. 7-8. HPLC chromatogram of galacturonic acid in the free phenolic sweetcorn extract during 10 d of storage in CA.

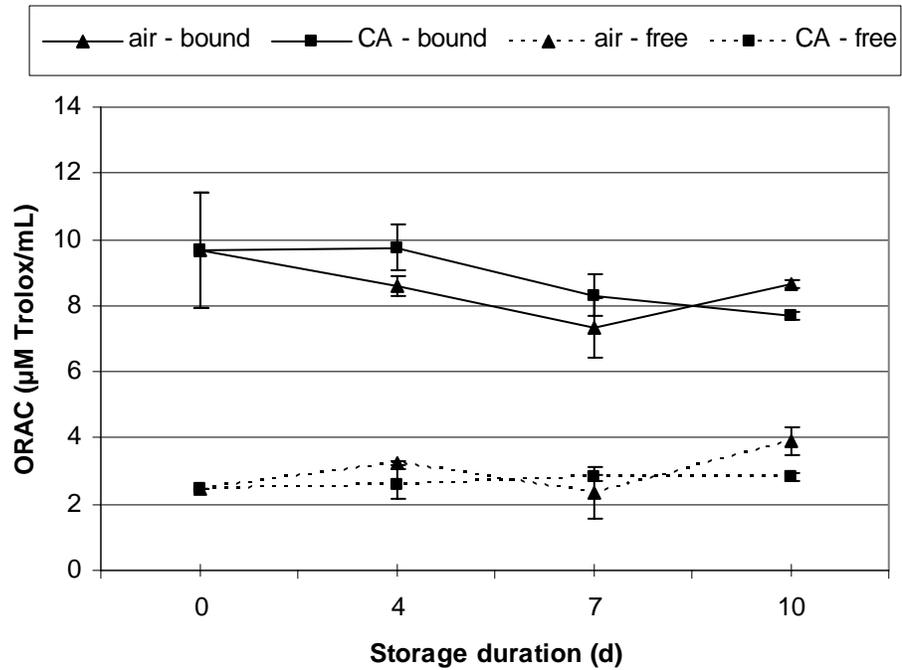


Fig. 7-9. Antioxidant capacity (Trolox equivalents; $\mu\text{M}\cdot\text{mL}^{-1}$) of free and bound phenolic fractions obtained from fresh-cut sweetcorn kernels as affected by storage in air or CA (2% O_2 plus 10% CO_2) for 10 d at 5 °C. Bars indicate the mean +SD.

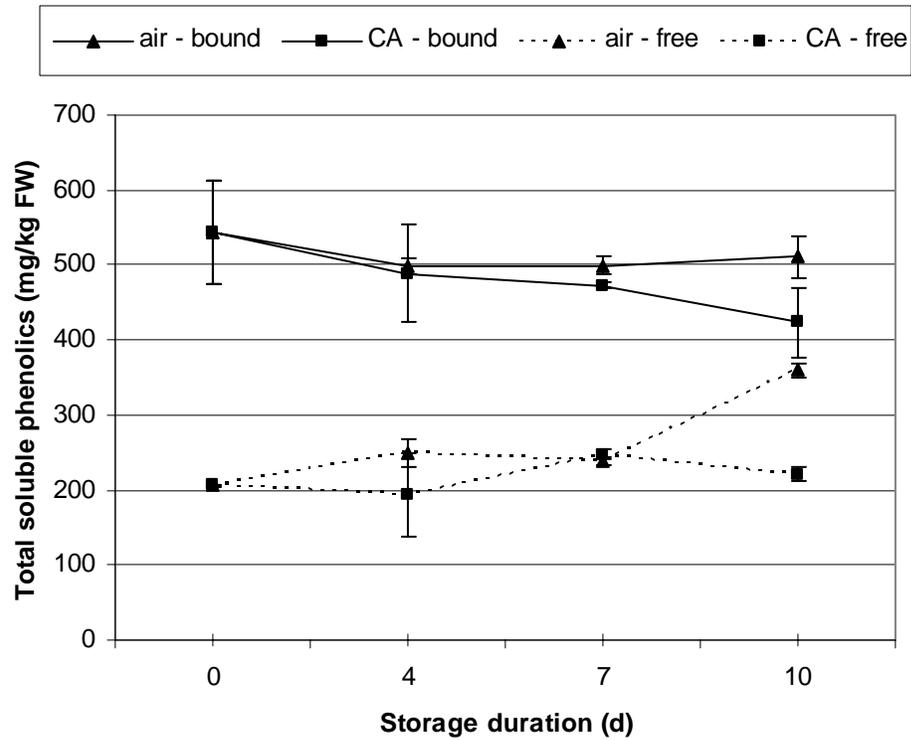


Fig. 7-10. Total soluble phenolics ($\text{mg}\cdot\text{kg}^{-1}$ FW) from free and bound fractions obtained from fresh-cut sweetcorn kernels as affected by storage in air or CA (2% O_2 plus 10% CO_2) for 10 d at 5 °C. Bars indicate the mean +SD.

CHAPTER 8 SUMMARY AND CONCLUSION

Sweetcorn Cob Storage

Due to the perishable nature of sweetcorn and as a result of its very active metabolism and high respiration rate, sweetcorn is greatly affected by control of the primary and secondary factors used to optimize preservation. The primary factor temperature control was proven again in this work to be the most important factor in sweetcorn storage. Furthermore, this work confirms the previous recommendations on sweetcorn storage, i.e., storing the cobs as close to 0-1 °C as possible. On the other hand, using secondary factors to optimize preservation such as atmosphere modification either by using controlled atmosphere or modified atmosphere (CA or MA) was also very beneficial in sweetcorn cob storage.

The main benefit of CA was to significantly reduce respiration rate over that of air storage and the results suggest that sweetcorn is more sensitive to reduced O₂ plus elevated CO₂ together than to O₂ or CO₂ alone since there was greater reduction in the respiration rate when using both of them. As a result of respiration rate reduction in CA treatments, higher sugar levels were maintained, which is the most important quality characteristic for sweetcorn consumers. Also, treatments resulting in greater reduction in respiration had higher sugar levels at the end of the storage duration.

Controlled atmosphere treatments also had a positive impact on the visual quality of stored sweetcorn since elevated CO₂ significantly reduced loss of greenness and maintained the fresh look of the husks. The CA also improved silk appearance and kernel

appearance over the air control, but it had no effect on kernel denting. Storage in 2% O₂ plus 15% CO₂ resulted in the best score in all visual quality parameters compared with storage in air, 2% O₂, or 2% O₂ plus 20% CO₂.

Another benefit of using CA was to maintain significantly higher levels of dimethyl sulfide (DMS), which is the main characteristic volatile component in sweetcorn and is responsible for the characteristic “corny” smell of cooked sweetcorn. Storing sweetcorn in 2% O₂ plus 15% CO₂ resulted in the highest DMS content after 14 d of storage at 5 °C.

In conclusion, CA storage was beneficial in maintaining most sweetcorn quality parameters during 14 d of storage at 5 °C. Storage in 2% O₂ plus 15% CO₂ gave the best result in terms of quality maintenance since it preserved the highest sugar level, reduced deterioration in sweetcorn visual quality, and maintained the highest DMS content and subsequently better flavor and aroma.

On the other hand using perforation mediated modified atmosphere packaging (PM-MAP) was another beneficial tool in storing sweetcorn. The desired gas composition was successfully obtained in all treatments at 1 °C while at 10 °C the obtained levels were less than expected by about 3-5% CO₂ and this might have been due to overestimation of respiration rate while designing the tubes for PM-MAP, which led to having steady state CO₂ levels around 10, 17, and 22% CO₂ instead of 15, 20, and 25% CO₂, respectively.

Sweetcorn stored at 1 °C reached the expected CO₂ level in about 5 d with slightly more time required for the higher concentrations. The sweetcorn stored at 10 °C was faster in reaching the steady state (about 2 d) and this was expected due to the higher CO₂ production rate at the higher temperature.

After 10 d of storage in PM-MAP, sugar content was higher in the sweetcorn stored at the lower temperature, but there were no significant differences among the treatments at 1 °C. The only difference in sugar concentrations that was observed was in the sweetcorn stored at 10 °C with 15% CO₂, which had significantly lower sugar content than sweetcorn stored in 20 or 25% CO₂.

Also, PM-MAP was beneficial in maintaining the visual quality of all treatments with no sign of microbial growth or deterioration even at the higher temperature (10 °C) and the weight loss was very slight in all treatment as a result of the high relative humidity surrounding the cobs. There was no significant production of ethanol or acetaldehyde even with high levels of CO₂ and the low levels of O₂, which indicate that the sweetcorn cobs did not switch to anaerobic respiration.

This work showed that using PM-MAP could be a very useful tool in sweetcorn storage at different storage temperatures. PM-MAP was mostly successful in generating the desired and expected gas compositions and maintaining them during a 10-d storage period while maintaining high sweetcorn quality.

Fresh-Cut Sweetcorn Kernel Storage

This work was carried out in an attempt to introduce fresh-cut sweetcorn kernels as a potential product for the fresh-cut produce industry. Fresh-cut sweetcorn is a very perishable commodity - even more perishable than sweetcorn cobs - and requires special care during preparation and storage to ensure maintaining quality.

Fresh-cut sweetcorn was successfully stored up to 10 d without severe loss in visual quality even at 5 °C. Also, dipping the kernels after cutting in chlorinated water was effective in controlling decay since there was no visual appearance of any microbial growth in these experiments.

Maturity stage of the kernels also affected the quality mainly through its effect on the respiration rate. More mature sweetcorn kernels had a lower respiration rate than the less mature kernels. The respiration rate of kernels was also affected by temperature and gas composition. Kernels stored at 5 °C had a higher respiration rate than those stored at 1 °C, and using CA with 2% O₂ plus 10% CO₂ significantly reduced the respiration rate.

As a result of respiration reduction, kernels stored at 1 °C had higher sugar content at the end of the storage period than kernels stored at 5 °C. Also, storing sweetcorn kernels in 2% O₂ plus 10% or 20% CO₂ maintained higher sugar content than air storage.

Fresh-cut sweetcorn kernels could serve as a good dietary source for polyphenolics and antioxidants, which might have several health benefits for the consumer and could help in reducing the risk of cancer and many chronic diseases.

Several phenolic compounds were found in fresh-cut sweetcorn including *p*-coumaric acid, *p*-OH-benzoic acid, and ferulic acid, as well as several unidentified phenolic compounds that were present in low concentrations. The concentration of phenolic compounds was much higher in the bound fraction than the free fraction, but this probably does not impact the nutritional benefits because it has been shown for other food crops that bound phenolics are as nutritionally available as soluble phenolics. The antioxidant capacity followed a similar pattern and was also much higher in the bound phenolic fraction than in the free fraction.

Controlled atmosphere treatment did not effect the antioxidant capacity during the first 7 d of storage, but it reduced antioxidant capacity by day 10 compared with air storage. Despite these results, CA storage is essential to maintain the quality of this

highly perishable commodity and it is not recommended to use air storage instead of CA because of the many other benefits of atmosphere modification for quality preservation.

This work indicated clearly that temperature is the most important factor in maintaining fresh-cut sweetcorn kernel quality. All quality factors evaluated were better maintained during storage at 1 °C compared with 5 °C storage. Also, using a secondary preservation factor such as CA was beneficial in maintaining the quality of fresh-cut sweetcorn

The main problem with fresh-cut sweetcorn was severe browning of kernels after cooking. This browning also was more severe in the kernels from the more mature cobs. Both low temperature and CA were effective in controlling the after cooking browning since it appeared only in cooked kernels stored in air at 5 °C and did not appear in the kernels stored in CA (2% O₂ plus 10% CO₂) at either 1 or 5 °C, or in samples stored in air at 1 °C.

Higher content of free amino acids were found in treatments with more severe browning (more mature kernels and kernels stored at 5 °C or in air) than in treatments with less or no browning (less mature kernels and kernels stored at 1 °C or in CA), which supported the possibility of involvement of Maillard reaction in the after cooking browning. Nevertheless, it was concluded that Maillard reaction is not responsible for the after cooking browning since no 5-hydroxymethylfurfural (the intermediate of the Maillard reaction) was found in any of the sweetcorn samples before or after cooking, which excludes Maillard reaction as a cause for the after cooking browning in fresh-cut sweetcorn

On the other hand, there were no differences found in the phenolic profiles of the sweetcorn kernels from the different maturity, temperature, and atmosphere treatments, and there also was no trend or significant difference in the total soluble phenolics contents, which reduces the possibility of the phenolic acids as a cause of the after cooking browning.

When the microbial load on the fresh-cut sweetcorn kernels were studied, it was found that the microbial load increase with storage duration and this increase was reduced using CA storage. Since the treatments that had more browning also had a higher microbial load, it was thought that the after cooking browning might be caused by microbes, but this was proven to be not totally true. When fresh-cut kernels were inoculated separately with several microbes (five bacteria and three yeasts), the after cooking browning started earlier and was more severe as a result of the increased microbial load, but this effect was not specific to any of the isolated microbes used to inoculate the sweetcorn kernels. These results indicate that after cooking browning is not caused directly by the presence of a specific microorganism, but microbial load could be responsible for after cooking browning in an indirect way, such as by increasing the kernel deterioration rate.

Separation of kernel juices from the water insoluble cellular materials allowed formation of the brown pigment to occur in a soluble form in order to measure it. The pigment was found to be associated with the acetone insoluble fraction of the sweetcorn kernel juice. The attempt to identify the brown pigment compound(s) failed since this pigment was very water soluble and it eluted very fast from the HPLC - before any of the authentic compounds we had available.

In conclusion, fresh-cut sweetcorn kernels can be successfully stored for 10 d in CA with little loss of visual or sensory quality, especially at the lower temperature of 1 °C. Controlled atmosphere was more critical at the higher temperature (5 °C), since after cooking browning was totally controlled by storage in 2% O₂ plus 10% CO₂.

After Cooking Browning, What Is It and What Is It Not?

Several preliminary experiments have been conducted in an effort to identify the cause of the after cooking browning that occurs in stored fresh-cut sweetcorn kernels. In this section, a summary of this work and some of the results obtained are presented, followed by some of the available information about after cooking browning. This information is presented in the hope that it may serve as the basis for future work on the same project.

After cooking browning, what is it?

Here is the information we know about the after cooking browning in fresh-cut sweetcorn:

1. The browning was never observed to develop on fresh kernels during storage. It occurs only upon exposure to heat, such as during heating in boiling water.
2. After cooking browning occurs only in stored, wounded kernels; it does not occur in freshly cut (i.e., not stored) kernels; neither does it occur in uninjured kernels, whether they are stored attached to the cob or removed from the cob.
3. Development of the disorder is slowed down by low storage temperature. There was no after cooking browning in kernels stored at 1 °C for 10 d. It requires storage of cut kernels for at least 4 d at 5 °C before the after cooking browning will start to show up.
4. Development of the disorder is oxygen dependant – when kernel samples were stored without air for 10 d at 5 °C the kernels did not brown, but if the samples were exposed to air after that for just 1 d, the kernels did brown. This may be the reason that the after cooking browning was reduced by CA treatment. This also excludes aerobic microbes as a cause of browning since storing kernels in sealed bags without air for 10 d promoted excessive anaerobic microbial growth.

5. The brown pigment precursor occurs in the juice of stored sweetcorn kernels and is precipitated by acetone. The browning reaction could be enzymatic or the brown pigment may be proteinaceous, since acetone precipitates are common ways to crudely concentrate enzymes for further purification.
6. The browning precursors (the pellets that were obtained after precipitation of sweetcorn juice with acetone) are soluble in water and methanol (not 100%), and insoluble in acetone, ethanol, hexane, and ethyl acetate. No other solvent was identified that solubilizes the brown pigment precursors. Also, very small amounts of acetone insoluble solids from stored sweetcorn kernels create a lot of brown pigment.
7. The browning reaction requires high temperature, i.e. around 100 °C - the browning didn't appear in kernels heated for 10 min in a water bath at 70 °C and the severity of the browning increased with increasing the water temperature to boiling.
8. Once the brown pigment was created during boiling it was not soluble and it couldn't be solubilized using any solvent (water, ethanol, acetone, ethyl acetate, etc.) that was tried.
9. The less the water used for boiling, the faster and darker the browning develops. It is a very fast reaction - when kernel juice precipitate is boiled, it starts to brown after about 20 s.
10. This reaction is also slowed down by adding a high concentration of reducing agents (5% ascorbic acid or 1-3% SO₂) to the boiling water.
11. The extract does contain some carbohydrates (glucose, sucrose, soluble starch, soluble pectin, and/or galacturonic acid; their role in the browning was not identified). It also has some polyphenolics.

After cooking browning, what is it not?

The following are some of the things that could be excluded as the cause of the after cooking browning:

1. The browning is not associated with cell wall bound compounds since isolated cell wall material did not brown when heated.
2. It is not native starch (non-gelatinized) or starch-related since most of the native starch was excluded by the centrifugation of the kernel juice.
3. Most probably it is not a Maillard reaction (a non-enzymatic reaction associated with thermal processing of food products) despite the fact that the substrates of the Maillard reaction are present in sweetcorn – there are amino acids present, especially tryptophan, and also soluble carbohydrates are present in the precipitate (tested positive in the phenol-sulfuric acid assay although it was not quantified).

The reason for ruling out the Maillard reaction is that there was no hydroxymethyl furfural (HMF) found, which is the characteristic product of Maillard reactions. Also, when the acetone insoluble material of sweetcorn juice was re-dissolved in a small amount of water, it could turn brown at room temperature without heating, which also rules out a Maillard reaction.

4. Most probably the browning is not phenolic related since all the cell wall bound phenolics were removed and also most of the soluble phenolics. There is a very low concentration of unidentified polyphenolics present in the extract, but it is doubtful it has an effect.
5. It is not a microbial reaction, or at least not directly, since the browning was not caused by a specific microbe, but as a general rule the higher the microbial load, the more browning appears, which might indicate an indirect effect of microbes.

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BIOGRAPHICAL SKETCH

Gamal Riad was born on October 20, 1970, in Cairo, Egypt. He is son of Samir Riad, a retired teacher; and Hanaa Attia, an accountant; and he has only one brother, Ahmed.

Gamal has been working as an assistant researcher in the National Research Center, Giza, Egypt since 1993. He received his primary and secondary education in Cairo, Egypt; and Sebha, Libya. He graduated from Elkoba High School, Cairo, Egypt in 1986. He attended the College of Agriculture at Ain Shams University, Cairo, Egypt where he was awarded a BS degree in Horticultural Science in 1991.

In 1991, he entered a graduate program (in the Horticultural Sciences Department) at Ain Shams University, where he was awarded an MS degree in 1996 and enrolled in a PhD program in the same university from 1997 to 1999 until he traveled to the United States. He got an assistantship toward his PhD degree in 2000 at the University of Florida. Upon completing his PhD degree, he will return to the National Research Center as a researcher.