Modelling quality of fresh-cut tomato based on stage of maturity and storage conditions

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To Hlinho, without which help this work
would not have been accomplished

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CHAPTER 1

INTRODUCTION

1. Fresh-cut fruit and vegetables

Fresh-cut fruit and vegetables, initially called minimally processed or lightly processed products, are those that have been trimmed and /or peeled and/or cut into 100% usable product that is bagged or pre-packaged and kept at refrigerated storage (IFPA, 2005). The operations result in damage that shortens the shelf life in relation to the product from which they were obtained (Watada *et al*., 1996). In this aspect, it is opposite to most processing systems in which preservation is enhanced by the processing treatments.

Processors of fresh-cut fruit face numerous difficulties not commonly encountered during fresh-cut processing of vegetable products. Especially with those fruit that undergo ripening the results in maintaining quality and extending the shelf life are far from satisfactory (Beaulieu and Gorny, 2001).

The fresh-cut vegetable industry was first developed to supply hotels, restaurants, catering services, and other institutions. For the food service industry and restaurants it presents a series of advantages including a reduction in the need of manpower for food preparation, reduced need to have special systems to handle waste and the possibility to deliver in short notice specific forms of fresh-cut products (Watada *et al*., 1996). More recently, it has expanded considerably to include food retailers as a response to consumer's demand for fresh, healthy and convenient foods (Wiley, 1994; Watada *et al*., 1996).

2. Quality of fresh-cut fruits

The understanding of the processes that results in quality degradation after processing is essential to develop technologies to extend shelf life and to maintain quality during processing and distribution (Wiley, 1994; Ahvenainen, 1996).

The physiology of minimally processed fruits and vegetables is basically the physiology of wounded tissues (Brecht, 1995). This behaviour includes increased respiration rate with consequent increase in the degradation of sugars, proteins and lipids (Brecht, 1995; Watada and Qi, 1999) and higher ethylene production (Abe and Yoshimura, 1993; Brecht, 1995). The loss of cellular integrity destroys compartmentation of enzymes and substrates leading to browning reactions, membrane lipid degradation, and formation of secondary metabolites that alter the flavor and the color of the product (Aubert *et al*., 1993; Babic *et al*., 1993; Brecht, 1995; Lopez-Galvez *et al*., 1996). Reduction of the nutritional value, due to oxidative damage, was reported to some products (Barth and Hong, 1996; Li and Barth, 1998). The exposition of internal tissues increases the water loss, leading to changes in color and firmness and causing desiccation of the cut surface (Brecht, 1995).

In the following sections the quality aspects (properties) that will be object of investigation in the present thesis will be discussed in more detail. Evidence that the quality of a product can be

based on the behaviour of the intrinsic properties of a product has been reported by Tijskens (2000).

2.1. Texture

2.1.1. Definitions

The term texture covers a wide range of attributes that includes the structural and mechanical properties of a food and their sensory perception in the hand or in the mouth (Abbott, 2004). Research on fruit texture is mainly concerned with the mechanical properties of the tissue which led to the widespread use of the term firmness. Horticulturists use firmness to describe the mechanical properties of the fruit tissue particularly when measured as the force required to push a cylinder probe to a predetermined depth into the fruit flesh (Harker *et al*., 1997).

2.1.2. Importance of firmness as an attribute of quality

The firmness of fresh-cut products is considered an important quality attribute (Beaulieu and Gorny, 2001). Much effort has been done to evaluate the effect of different treatments in the extent of softening after processing. These include atmosphere composition (Gil *et al*., 2002; Gorny *et al*., 2002; Soliva-Fortuny *et al*., 2002a; Soliva-Fortuny *et al*., 2003); chemical treatments before (Perera *et al*., 2003; Bai *et al*., 2004) and after processing (Artes *et al*., 1999; Luna-Guzman and Barrett, 2000; Gorny *et al*., 2002; Saftner *et al*., 2003); storage temperature (Gil *et al*., 2002; Aguayo *et al*., 2004); magnitude of wounding (Portela and Cantwell, 2001; Aguayo *et al*., 2004) among others.

Changes in texture are also dependent on intrinsic characteristics of the intact product especially cultivar (Aguayo *et al*., 2004) and stage of maturity (Beaulieu *et al*., 2004; Soliva-Fortuny *et al*., 2004; Soliva-Fortuny *et al*., 2002c). The effect of stage of maturity in softening and in other quality changes that happens in fresh-cut products will be discussed in more detail in a following section.

2.1.3. Enhancement of softening by cutting

Firmness of fresh-cut fruit can be affected by cell softening enzymes present in fruit tissue and by decreased turgor due to water loss (Beaulieu and Gorny, 2001). Although the importance of both is recognized, only the first has been the focus of research on the effects of minimal processing on texture. In spite of the efforts to understand the biochemical basis of texture loss in fresh-cut fruit a conclusive model is not available. The results obtained for specific products are described as follows.

Sliced kiwifruit undergoes a pronounced decrease in firmness within 2 days storage. It was suggested that the mechanism of hydrolysis of cell wall components after slicing differ from those involved in the normal maturation of kiwi in which solubilization of propectins is predominant. Cutting induced the enzymatic breakdown of uronic acid polymers, demethylation of the pectin water soluble fraction and rupture of Ca bridges and had no effect on polygalacturonase (PG) activity (Varoquaux *et al*., 1990).

Papaya is another fruit that shows a rapid decrease in firmness after cutting. (Karakurt and Huber, 2003) suggested that a wound-induced increase in enzymes targeting cell walls and membranes contributes to the rapid deterioration of fresh-cut papaya compared with intact fruits stored under the same conditions. The activities of PG, alpha- and beta-galactosidases (alpha and beta-gal), lipoxygenase and phospholipase D increased within 24 h in fresh-cut fruit, and remained significantly higher compared with levels in intact fruit throughout the storage period.

However, this does not seem to be a general mechanism, since different results were observed in tomato fruit. The expression of PG, beta-gal and pectinesterase (PE) genes all increased within the first 6 h after wounding of tomato fruits, but in most cases, returned to pre-wounding levels after 24 h. The activities of these three enzymes were also examined in intact (control) and wounded tomato fruit tissues 24 and 48 h from wounding. In early breaker fruits, the increase in activities of PG and beta - gal associated with normal ripening were both retarded resulting in wounded fruits having reduced activities compared to the controls; whilst PE activity actually declined 24 h after wounding. In ripe tomato fruits, PG and PE activities both decreased 48 h after wounding, but beta -gal was unaffected (Thanh Tu and Tucker, 2003).

2.1.4. Instrumental measurement of firmness of fresh-cut fruits

Firmness of fresh-cut fruits (fleshy tissue) is most commonly measured using puncture tests (Bolin and Huxsoll, 1989; Varoquaux *et al*., 1990; Abe and Watada, 1991; Hong and Gross, 1998; Luna-Guzman and Barrett, 2000; Gorny *et al*., 2002; Karakurt and Huber, 2003). Compression tests (Abe and Watada, 1991; Artes *et al*., 1999; Beaulieu *et al*., 2004) and shear tests (Rosen and Kramer, 1989) are also reported for few products.

The following definitions are given by (Lu and Abbott, 2004). Puncture test measures the force required for a probe to penetrate into a sample for a pre-specified depth. The test involves both compression and shearing; it is an empirical technique and is somewhat imitative of the biting of a food item in the mouth. Compression tests are often conducted under uniaxial loading between two plates and the sample is allowed to expand freely in the other two directions. Shear often refers to the action of applying force to cut a sample into two separate pieces.

There are four basic values that can be obtained from these kind of tests: force (load), deformation (distance, displacement, penetration), slope (ratio of force to deformation), and area under the curve. Force and deformation values are more commonly used in applications dealing with fruits and vegetables than stress and strain values and are sufficient, provided that the contact area and the distance the probe travels are constant and sample dimensions are similar from sample to sample (Abbott, 2004). In most horticultural texture tests, the deformation is fixed and the force value is reported. In a few horticultural tests, a known force is applied to the product and the deformation after a specified time is reported (Abbott, 2004).

A typical force-deformation curve for fruit and vegetables consists of three parts (Lu and Abbott, 2004). During the first phase of deformation, the relation between force (or stress) and

deformation (or strain) is linear and elastic. Beyond the first phase (elastic limit), the force/deformation relationship becomes increasingly non-linear as the loads increase until reach a point (bioyield point) where a drop or no increase in force takes place with an increase in deformation. At this point, the cells star to rupture or move in relation to each other, what causes the decrease in slope. Beyond the bioyield point, the third phase of deformation starts; the force/deformation relationship becomes irregular and jagged, with numerous peaks and valleys until complete breakdown of the specimen. Beyond rupture, the force may again increase, level off, or decrease as deformation increases. At the maximum deformation point specified by the user, the probe is withdrawn and the force diminishes until contact is lost.

2.2. Optical Properties

2.2.1. Definitions

The optical properties of food products includes both chromatic (colour) and geometric attributes (gloss, shape, shininess, haze and translucency) .

Colour is a characteristic of light and light is that aspect of radiant energy, which a human observer is aware of through the visual sensations arising from the stimulation of the retina of the eye. Light is radiant energy in the visual range of the spectrum, which occurs in simple terms, within the limits of 700 and 400 nm (Arthey, 1975). Differential absorption of all wavelengths results in the sensation of colour. The degree to which the retina of the eye is stimulated will depend not only on the light available but also on the nature of the reflective surface (Arthey, 1975).

Colour can be identified by three-dimensional coordinate systems but geometric attributes which are associated with the spatial distribution of light by the object can not be uniquely defined by any organized coordinate arrangement (Hunter and Harold, 1987).

2.2.2. Changes in optical properties of fruit tissue induced by cutting

Minimal processing operations allows enzymes and substrates to come in contact, mainly at the surface of the product, bringing about enzymatic reactions related to colour deterioration (Dorantes-Alvarez and Chiralt, 2000). Besides that, other operations like immersion of the fruits and vegetables in solutions with chemical and acids, thermal treatments, and exposure of cut product to air and light can alter the compounds responsible for colour (Dorantes-Alvarez and Chiralt, 2000).

Degradation and oxidation of pigments like chlorophylls and carotenoids are likely to occur as a consequence of wounding (Heaton and Marangoni, 1996; Jamie and Saltveit, 2002) in addition to those changes in colour due to ripening (Artes *et al*., 1999). The production of wound-related compounds results in browning of cut surfaces (Bolin and Huxsoll, 1989; Rocha and Morais, 2003). Changes in optical properties, not related to changes in pigment composition and concentration, are also an important component of the final appearance of the product. Loss of water causes loss of sheen and gloss at the cut surface (Gorny *et al*., 2000) and a whitening or dehydrated surface develops

in cut carrot (Barry Ryan and O' Beirne, 1998), shredded green papaya (Techavuthiporn *et al*., 2003) and sliced tomato (Artes *et al*., 1999). Cutting induces the development of translucency or water-soaking in fleshy tissues (Bai *et al*., 2001; Portela and Cantwell, 2001).

2.2.3. Instrumental measurement of colour and geometric attributes

For more detailed information about the methods and instruments available for colour and geometric attributes evaluation the readers are addressed to (Hunter and Harold, 1987; Hutchings, 1994; MacDougall, 2002).

Instruments used to measure geometric attributes include goniophotometers, diffuse-reflection meters, specular-reflection meters, direction-contrast meters and haze meters. A detailed description of their characteristics and use is given in (Hunter and Harold, 1987).

The two main classes of instruments for colour evaluation are spectrophotometers and colorimeters (MacDougall, 2002). Spectrophotometers provides wavelength by wavelength spectral analysis of the reflecting and or transmitting properties of objects without interpretation by a human (HunterLab, 1995). Every colour is characterized by a reflectance curve. Colorimeters provide measurements that correlate with human eye-brain perception. Data is directly read and provided as tristimulus values (XYZ, Lab, RGB, etc.). Lab tristimulus values are most commonly used to express colour of plant and food material, where L indicates lightness with values ranging from black = 0 to white = 100, a indicates chromaticity on a green (-) to red (+) axis, and b chromaticity on a blue (-) to yellow (+) axis (McGuire, 1992).

Both spectrophometers and colorimeters were designed for flat, opaque, uniformly coloured materials such as paints, plastics and textiles. Because of that, these instruments are not appropriate for inhomogeneous products with irregular shape and surface characteristics and non-uniform colour. The recently developed technology of video image analysis (VIA) offers a methodology for specification of uneven coloration as much as the specification of other attributes besides colour, that contribute to the total appearance (MacDougall, 2002; Du and Sun, 2004).

The use of VIA to couple colour measurements with variation in appearance is likely to increase and an overview of its use in food quality evaluation is provided by (Du and Sun, 2004). In machine vision, colour images are typically quantified by red, green, and blue (RGB) values. The RGB intensity values represent integrated responses over RGB spectral bands measured through colour filters. The RGB response is affected by the specific configuration of a colour vision system, including factors related to the intensity and the spectral distribution of illumination, the lens and lens aperture, properties of RGB colour filters, the image sensor response, and the digitizer. As a result RGB values of an object using a specific system configuration should not be assumed to represent an absolute colour quantity and are difficult to compare using different systems configurations (Chang and Reid, 1996). Provided that the RGB signals from VIA systems and digital cameras can be translated into meaningful colour data then a wider and more adaptable approach to food colour appearance control might be possible. VIA systems could become a more direct way of relating measurements to visual

assessment and effectual quality assurance. Such systems in conjunction with standard colour measurements could possibly provide direct quality links by computer from the supplier to the processor to the supermarket .

2.3. Antioxidant properties

Oxidative stress, which releases free oxygen radicals in the human body, has been implicated in a number of disorders including cardiovascular malfunctions, cataracts, cancer, rheumatism and many other auto-immune diseases besides ageing. To counteract the effect of the free radicals, the body is equipped with a defence system which includes various enzymes and high and low molecular weight antioxidants. Antioxidants protect the cell against the potentially harmful effects of processes or reactions that can cause oxidative stress (Kaur and Kapoor, 2001). Antioxidants can delay or inhibit the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (Aruoma, 1994; Kaur and Kapoor, 2001).

The protective effect of a diet rich in fresh fruits and vegetables against chronic diseases is well documented and it is at least partially ascribed to the action of antioxidant compounds such as carotenoids, ascorbic acid, tocopheros, polyphenols and others (Klein and Kurilich, 2000; Prior and Cao, 2000; Wargovich, 2000; Grassmann *et al*., 2002). The joint FAO/WHO Expert consultation on diet, nutrition and prevention of chronic diseases, recommended the intake of a minimum of 400g of fruits and vegetables per day (excluding potatoes and other starch tubers) for the prevention of chronic diseases (FAO/WHO, 2005).

In urbanised societies, a series of barriers can result in less than optimal level of consumption of fruits and vegetables (FAO/WHO, 2005). These include, among others, lack of time for preparation and cooking as urbanization increases and more women work outside the home and rapid increase of fast-food culture. Fresh-cut fruits and vegetables are presented as an alternative for healthy and convenient foods. However, the impact of minimal processing on the amount and composition of nutrients and other bio-active components is not well known as discussed in the next section.

2.3.1. Impact of processing in the composition and amount of bioactive compounds

There is a general perception that processed foods are of lower nutritional value and that preservation methods cause a depletion of naturally occurring anti-oxidants in food. However food processing and preservation procedures can promote no change, increase or decrease the overall antioxidants properties of food depending on the considered product and procedure (Nicoli *et al*., 1999).

Most of the available literature on the effect of processing on dietary antioxidants from plant foods is related to operations such as canning, freezing, heating and blanching (Klein and Kurilich, 2000). Published data on nutrient content and nutrient retention of minimally processed foods are generally sparse and are needed (Lindley, 1998)

Operations involved in the preparation of fresh-cut products are expected to induce a rapid enzymatic depletion of natural antioxidants as a response to injury. Retention of ascorbic acid is dependent on the integrity of the tissue (Davey *et al*., 2000). Carotenoids are unstable when exposed to oxygen or light (Shi and Maguer, 2000), all of which may occur when cells are disrupted, and the internal tissue is exposed by cutting. Wounding also promotes the production of wound ethylene (Brecht, 1995), which hastens senescence, including the oxidation of fatty acids by lipoxygenase, during which carotenoids may be degraded by co-oxidation (Biacs and Daood, 2000). On the other hand, minimal processing induces phenolic compound biosynthesis (Kang and Saltveit, 2002; Del Caro *et al*., 2004) what can result in an increase in antioxidant activity.

2.3.2. Evaluation of anti-oxidant activity in fruits and vegetables

Evaluation of the impact of processing on the amount and composition of antioxidant compounds of fruits and vegetables can be done through the assessment of individual components known to be important antioxidants in each particular product (Takeoka *et al*., 2001; Piga *et al*., 2003). This procedure can be cumbersome when many compounds are of interest, each one of them evaluate by a different method. Besides that, evaluating individual components does not take into consideration important interactions between different antioxidants and does not consider other compounds, whose antioxidant activity is not known. Besides evaluating individual compounds there is interest in evaluating the total antioxidant activity. Total antioxidant evaluation is useful in obtaining a global picture of relative antioxidant activities in different body fluids, foods, and drinks, and how they change (e.g. in disease or after food processing or storage) as much as reveal synergistic interactions of antioxidants (Halliwell, 2002).

There are numerous antioxidants assays to measure antioxidant activity but there are no approved, standardized methods (Frankel and Meyer, 2000). The advantages and drawbacks of the most used assays has been the subject of numerous reviews (Arnao, 2000; Frankel and Meyer, 2000; Halliwell, 2002; Aruoma, 2003; Pellegrini *et al*., 2003; Roginsky and Lissi, 2005).

In short, the most widely used methods for measuring antioxidant activity are those that involve the generation of radical species *in vitro*, followed by its removal from the medium by the antioxidant present in the sample. Several protocols are described which differ in relation to the radical-generating system, methods for oxidation and end point observations. These include DPPH assay (Jiménez-Escrig *et al*., 2000), ABTS assay (Cano *et al*., 2000), TRAP assay (Pellegrini *et al*., 2003), ORAC assay (Cao *et al*., 1993) and others. These methods are sensitive and relatively fast and easy to perform but they do not taken into account the complexity of antioxidant actions. They neglect important compositional and interfacial phenomena concerning charge and solubility of multiple components in real food or biological systems that strongly affect antioxidant performance (Frankel and Meyer, 2000).

More complex methods involve testing antioxidants in foods and biological systems where a lipid or lipoprotein substrate is oxidised under standard conditions and the inhibition of oxidation

after addition of the antioxidant is measured (Chang *et al*., 2000; Frankel and Meyer, 2000; Sluis *et al*., 2000). Although closer to the situation experienced *in vivo*, these methods also presents some drawbacks related to partitioning properties of the antioxidants between lipid and aqueous phase, oxidation conditions and physical state of the oxidisable substrate (Frankel and Meyer, 2000).

3. Factors that affect the changes in quality after processing

Many factors can influence the intensity of the wound response in fresh-cut fleshy tissues. Characteristics of the raw material such as cultivars (Varoquaux *et al*., 1996); stage of physiological maturity (Gorny *et al*., 2000); chemical composition (Varoquaux *et al*., 1996) and enzyme activity especially those related with phenolic metabolism (Babic *et al*., 1993) are important factors. The extent of cutting operations can have a great impact of keeping quality (Abe and Chachin, 1995; Ahvenainen, 1996). Other factors includes temperature (Watada *et al*., 1996); atmosphere composition especially O_2, CO_2 and C_2H_4 concentrations (Watada *et al*., 1996); water vapor pressure (Watada *et al*., 1996); extent and composition of microbial contamination (Nguyen-the and Carlin, 1994; Watada *et al*., 1996).

In the present study, the effect of temperature and stage of maturity of the fruit at harvest will be the object of investigation and a more detailed discussion about these two factors follows.

3.1. Effect of storage temperature on the quality of fresh-cut fruits

3.1.1. Rate of quality deterioration versus temperature

Most of the metabolic reactions that happen in fresh-cut products and result in changes in quality are enzyme catalysed and as such very much dependent on temperature (Wiley, 1994). Low temperature reduces respiration, inhibits microbial growth, and retards metabolic activity, ripening and senescence (Wiley, 1994; Wang, 1999; Able *et al*., 2005).

Because the operations involved in the preparation of fresh-cut products results in acceleration of the metabolism, the use of low temperatures in the whole chain becomes then an absolute necessity for these products and it should start as soon as possible after harvesting. The maintenance of low temperature is also imperative to preserve the microbiological safety of this products and because of that, the recommended temperature for fresh-cut product is $\leq 5^{o}C$.

3.1.2. Chilling injury in fresh-cut products

The need to store fresh-cut products at low temperature raised the concern that products of tropical origin would suffer chilling injury. However, a significant number of fresh-cut fruits are not as chilling injury sensitive as the corresponding intact fruit before processing (Beaulieu and Gorny, 2001). Possible reasons for that include: the fresh-cut product is kept under storage for a shorter time, maybe not enough to allow the appearance of the symptoms of chilling injury; it is not removed to room temperature before consumption, what can be a necessary condition for the development of the

symptoms (King and Ludford, 1983); some process not occurring in intact but on cut products can interfere with the development of chilling injury (e.g. the production of ethylene or the increase in CO_2 production in response to wounding) (Hong and Gross, 2002); tissues normally damaged by storage at chilling temperatures are the inedible outer rind or skin portions, which is removed and discarded (Beaulieu and Gorny, 2001).

In a study with 15 different commodities it was conclude that chilling sensitive fresh-cut products should be held at chilling temperature since the injury from chilling will be of less consequence than the deterioration that results at non-chilling temperature (Watada *et al*., 1996). Similar results were obtained by other authors. Keeping honeydew and muskmelon cubes at 4ºC was better than at higher temperatures (O'Connor-Shaw *et al*., 1994). The amount of chilling injury at 4ºC caused less damage than the natural deterioration at higher temperatures. Sliced zucchini stored at 0ºC showed severe to extreme chilling injury after 17 days. At 5ºC half of the samples presented moderate to severe chilling plus natural deterioration after 16 days. At 10ºC, 90% of the samples presented moderate to severe browning and decay due to natural deterioration after 12 days. The best temperature was considered to be 5ºC even with some chilling injury, since at 0ºC the severity of chilling was too high and at 10ºC natural deterioration was more severe than chilling at 5ºC (Izumi and Watada, 1995).

3.2. Effect of ripening stage on the quality of fresh-cut fruits

The ripeness stage at processing influences both the shelf-life an the eating quality of fresh-cut fruits (Gorny *et al*., 2000; Beaulieu and Gorny, 2001; Soliva-Fortuny *et al*., 2004; Soliva-Fortuny *et al*., 2002c). pointed out that minor physiological differences in initial fruit quality appear to translate to substantial differences in the quality of cut product.

Ripeness stage at processing was shown to be important in many processes that results in changes in quality of fresh-cut products as respiration rate (Soliva-Fortuny *et al*., 2004), ethylene production (Soliva-Fortuny *et al*., 2002b; Soliva-Fortuny *et al*., 2004), apperance (Soliva-Fortuny *et al*., 2004), colour (Soliva-Fortuny *et al*., 2002c) and texture changes (Soliva-Fortuny *et al*., 2002b; Soliva-Fortuny *et al*., 2004). Harvest date is also accompanied by a decline in the non-enzymatic and enzymatic systems responsible for catabolism of active oxygen species in pear fruits (Lentheric *et al*., 1999) but the implications of these changes in the quality and shelf-life of fresh-cut products is not elucidated.

In general, when selecting less mature fruits for processing an extension in shelf life can be achieved due to firmness retention and less changes in appearance, but the amount and composition of volatiles and consequently the aroma/flavour can be not satisfactory (Gorny *et al*., 2000; Beaulieu and Lea, 2003; Beaulieu *et al*., 2004). The limiting factor determining the extension of shelf life is also dependent on the fruit stage of maturity.

Apple fruits processed at an earlier ripeness stage preserved their commercial quality for longer in terms of colour and firmness (Soliva-Fortuny *et al*., 2002c). Mango cubes obtained from

fruits processed at firm-ripe stage had a longer shelf life (11 days) than that of cubes obtained from fruits at soft-ripe stage (7 days) (Beaulieu and Lea, 2003). The main critical factor in reducing shelf-life of soft-ripe cubes was edge or tissue damage, resulting in poor texture and mushy tissue, followed by aroma loss and general discoloration. Firm-ripe cubes became unmarketable due to lack of aroma and desiccation. Honeydew cubes obtained from fruits at immature to mature threshold level (8.8% soluble solids (SS)) failed to retain the characteristic taste and aroma. Cubes obtained from fruits at 13% SS had good taste and aroma but deteriorated more rapidly than cubes obtained from fruits with 13% SS (Watada and Qi, 1999).

4. Modelling dynamic changes

When the knowledge of particular quality attributes is available, major efforts should be done to combine all the different factors that influence those attributes in a mathematical and simulation model. The combination of the initially determined quality with a quality change model would make it possible to calculate how actual product quality will change during storage and transport under defined conditions. This information, as the product moves through the supply chain, is a basic requirement for the introduction of quality-based information and communication technology in horticultural supply chains.

This approach was previously used by (Tijskens and Evelo, 1994)to study the change in colour of intact tomato fruit after harvest. The tomato fruit colour expressed as a* value, was described by a logistic function where the effects of temperature during storage and stage of maturity of the fruit at harvest were both incorporated, and the colour development at every maturity stage could be described by a same function. This approach was further developed by (Schouten *et al*., 1997) to describe changes in the colour of cucumber after harvest. In both cases, the concept of biological shift factor was taken into consideration. This means that the actual time (days after harvest) at a certain stage and temperature was converted to a standardized time at 20^{o}C (days to mature to a particular stage). By applying the biological shift factor over the mean of harvested batches it was possible to express the stage of maturity at harvest as the time (in days) necessary for the product in the plant to attain a particular stage of maturity. The advantage of thinking in biological age is that it does not matter how long it takes in calendar time to reach a certain stage. Once the produce reaches that stage, it will be identical (at least highly comparable) to all other individuals at that stage, no matter how that stage is reached. For purposes of modelling and understanding product behaviour, this is a very powerful and useful concept. Temperature (during growth or storage), light, fertilisation etc. do no longer reflect on the product behaviour or state, only on the calendar time necessary to reach that state. This concept also allows to link pre- and postharvest phases, which is essential for understanding the sometimes erratic behaviour during postharvest stage and handling (Heuvelink *et al*., 2004).

5. Outline of this thesis

The aim of this thesis is to model the deterioration of fresh cut tomato as a function of maturity development of the fruit at harvest combined with cutting operation and storage temperature. The main processes resulting in deterioration will be determined, measured and described mathematically in order to end up with a model describing how actual product quality changes, after processing and during storage, under defined conditions of temperature.

Chapter 1 gives an overview of the present knowledge about quality of fresh-cut fruit and vegetables, the main processes that result in quality degradation and the main factors that influence the rate of quality changes. Special emphasis is given to the changes in appearance, texture and content of bioactive compounds and how these changes are influenced by the stage of maturity of the fruit at harvest and by storage temperature.

Chapter 2 describes the changes in firmness of sliced tomatoes during refrigerated storage. The firmness decay of tomato slices is described by a first order reaction model that incorporates the stage of ripening of the fruit at harvest and both the temperature and time of storage. Successive ripening stages are analysed in sequence, as belonging to a same curve through the use of the biological shift concept.

In **Chapter 3** the methodology of video image analysis is used to asses the changes in optical properties of cut tomato. The same model described in Chapter 2 is used here to analyse the changes in RGB (Red, Green, Blue) colour aspects of fresh-cut tomato, incorporating the stage of ripening of the fruit at harvest, the storage temperature and the storage time. It is also demonstrated that data on different physical aspects (in this case colour and firmness) can be incorporated in one global analysis.

Chapters 4 and **5** bring an extension of the work described in Chapter 3 where the video image analysis is used to measure both changes in colour and the development of translucency in sliced tomato during refrigerated storage. In **Chapter 4** the effect of ripening stage is addressed and in **Chapter 5** the effect of storage temperature is addressed. In both chapters the data are analysed using univariate and multivariate statistical tests, in order to identify the most discriminant variables (colour aspects) for colour and translucency measurement. The potential to use video image analysis to measure translucency is evaluated and the need to transform RGB into Lab values in order to properly express changes in optical properties in fresh-cut tomato is examined.

In **Chapter 6** the changes in optical properties in the pericarp of sliced tomato are assessed using the Kubelka-Munk analysis. The effect of both stage of maturity of the fruit at harvest and storage temperature on the development of translucency is discussed. A verbal model to explain the development of translucency as a consequence of cutting is presented.

Chapter 7 brings an extension of the model presented in Chapter 3, where all the information presented in Chapters 4 to 6 is taken into consideration. In this extended model two separate processes are considered; one of colour change due to the production or degradation of pigments (ripening),

and one of physical change from opacity to translucency development due to flooding of intercellular spaces. Both processes are reflected in changes in the RGB values as detailed.

In **Chapter 8** kinetic modelling is used to describe the changes in lycopene concentration of fresh-cut tomato during refrigerated storage. The effect of stage of maturity at harvest and the temperature dependence of the process are discussed.

In **Chapter 9** the changes in total antioxidant activity of fresh-cut tomato during refrigerated storage are studied using two different methodologies; a radical scavenging assay and a lipid peroxidation inhibition assay. The advantages and drawbacks of each method are discussed. The information obtained from univariate analysis of variance is used in developing a mathematical model that describes the changes in antioxidant activity with a simple exponential model (first order kinetics) developing towards an asymptotic end value.

Chapter 10 discuss collectively the main results obtained in the previous chapters. It is discussed which quality attributes most change during storage, how they are influenced by the stage of maturity of the fruits at harvest and by storage temperature and how the use of dynamic quality models can help in the interpretation of the obtained results. The potential, advantages and drawbacks of the techniques used to measure quality are presented. Suggestions for future research are given in light of the obtained results.

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CHAPTER 2

EFFECTS OF STORAGE TEMPERATURE AND FRUIT MATURITY ON FIRMNESS OF FRESH CUT TOMATOES

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Abstract

Tomato fruits (cultivar Belissimo) were harvested at three different stages of maturity, sliced and stored at at 2, 5, 8, 12 and 16 °C. Firmness was measured as the force necessary to cause a deformation of 3 mm, in the outer and the radial pericarp, daily or every two days, depending on the combination stage of maturity and temperature. For constructing a model, firmness was considered to be built up by a variable part (e.g. pectin based firmness) that changes according to a first order reaction mechanism and a fixed part (e.g. cellulose or structure based firmness) that is invariable under the circumstances under study.

The firmness of the tomato slices decreased exponentially during storage, with a reference rate constant of 0.0975 $\pm$ 0.0183 J/mol/K for the outer pericarp and 0.0712 $\pm$ 0.0328 J/mol/K for the radial pericarp and energy of activation of 87.5 kJ/mol and 94.8 kJ/mol respectively for outer and radial pericarp. The parameters of the model were estimated using multiple variate non-linear regression analysis with time, temperature and stage of maturity of the fruit at harvest simultaneously as explaining variables. To combine the information on firmness behaviour during the preharvest and the postharvest period at different temperatures, it was assumed that the process of firmness decay during maturation was the same whether the fruits ripened at the plant or off-vine. So, the initial firmness at the postharvest period depends on the time the tomatoes were allowed to ripen at the plant (stage of maturity). The statistical analysis on the mean values of firmness provided a percentage variance accounted for of 92% and 77% for outer and radial pericarp respectively. Using a fundamental approach to build the reported model and using all available data and information in their entirety made it possible to describe and simulate the firmness behaviour of tomato slices as a function of the stage of maturity and the applied storage temperature.

Keywords: Lycopersicum esculentum; Minimally processed; Texture.

1.Introduction

The shelf life of many fresh-cut leafy and root products has been extended successfully, but for fruit that continue to ripen after harvest the results are far from satisfactory (Beaulieu and Gorny, 2001). A similar situation is encountered with fruit vegetables such as tomatoes. Although tomato is a natural ingredient in many salads, its use in ready-to-eat salads is still very restricted due to its short shelf life when minimally processed. The use of fresh-cut tomato by fast-food restaurants, food service institutions, and cafeterias is also limited by the many technical problems in maintaining its quality and microbiological safety during storage (Hong & Gross, 2003).

Accelerated loss of texture is considered one of the main factors that limit the shelf-life of fresh-cut tissue (King Jr. and Bolin, 1989; Beaulieu and Gorny, 2001), as reported for many different products such as papaya (Karakurt and Huber, 2003), kiwi (Varoquaux *et al*., 1990), strawberry (Rosen and Kramer, 1989) and pear (Soliva-Fortuny *et al*., 2002; Rosen and Kramer, 1989).

The texture breakdown of minimally processed tissue is expected to occur as a response to a wound-induced increase in enzymes targeting cell walls and membranes (Huber *et al*., 2001). The activities of polygalacturonase, alpha and beta-galactosidase, lipoxygenase and phospholipase D increased within 24 hours in fresh-cut papaya (Karakurt and Huber, 2003) compared with intact fruit. Further evidence was given by microscopic observations of fresh-cut pear tissue. A total flooding of intercellular gaps by cell juice from cells with decreased membrane integrity was observed, which in

turn would cause the decompartmentalisation of texture-related enzymes and their substrates (Soliva-Fortuny *et al*., 2002).

In a review about pectin degradation in wounded fruits, Huber *et al*. (2001) pointed out the possibility of the participation of radical oxygen species in pectin depolymerization. The radical-mediated polysaccharide degradation was shown to be relevant during many developmental processes including maturation. If these radical-based mechanisms are more operational in wounded tissues, then they could be of importance in the deterioration and softening of fresh-cut tissue.

Decreased turgor due to water loss has also been suggested as a cause of tissue softening (Beaulieu and Gorny, 2001) in fresh-cut fruit.

For fresh-cut tomato it is not established which attributes are limiting factors to product quality. In normally maturing tomato, the softening of the pericarp flesh is the major cause for loss in texture. Texture is therefore considered an appropriate index for the maturation process and for the associated tomato quality (Frenkel and Jen, 1989). For climacteric fruit, initial fruit firmness is considered to be a good indicator of fruit maturity for determining the shelf life of cut products (Beaulieu and Gorny, 2001). It was therefore reasonable to assume that the initial firmness could also be used both as index of maturation and of potential shelf life for fresh-cut tomatoes. Indeed the assessment of firmness has been included in the quality evaluation of fresh-cut tomato by many authors (Hong and Gross, 1998; Artes *et al*., 1999; Gil *et al*., 2001; Wu and Abbott, 2002).

The rate of softening after processing depends on many factors related to the product and to the processing and the storage conditions (Beaulieu and Gorny, 2001). The stage of maturity of the fruit at harvest and at cutting is of particular importance, since both affect post-cutting quality and shelf-life. Equally important is the temperature at which the processed product is stored, since most of the reactions in the product are bio-chemical and hence temperature dependent. Although storage at low temperature is a common practice to retard softening of fruits and vegetables, accelerated softening of tomato can occur at low temperature due to chilling injury (Jackman *et al*., 1992).

Usually only one stage of maturity (except for Wu and Abbott (2002)) and only one or a few storage temperatures were studied in the cited reports. It is therefore difficult to interpret whether the reported behaviour is specific to that particular condition or is true for tomato cut tissue in general. The aim of the present report was to study how the firmness of tomato slices changes after cutting and how these changes are affected by the stage of maturity of the fruit at harvest and by temperature during storage. A model was developed to describe the behaviour of firmness of tomato slices, comprising effects of storage time and temperature, and stage of maturity at harvest.

2. Materials and Methods

2.1. Harvesting and processing

Tomatoes (*Lycopersicum esculentum* cv. Belissimo) grown in the same greenhouse in Made

Legend of symbols

Name	Dimension	Meaning
Ea	kJ/mol	activation energy
F	N	maximal force at 3 mm compression
K	day^{-1}	rate constant of firmness decay
R	J/mol/K	universal gas constant (8.314 J/mol/K)
T	day	time
T	K (^{o}C)	temperature
Subscripts		
0		initial
1		of stage I
2		of stage II
3		of stage III
C		correction for measuring at storage temperature
Fix		invariable part
G		during growth
Ref		at reference temperature (= 10° C)

(The Netherlands) were harvested in a single day in May 2003 in three colour stages, named here as I, II and III corresponding to the following grades of the tomato colour scale (kleur stadia-tomaten) from The Greenery (http://www.the greenery.com):

I = grade 3 (equivalent to breaker stage)

II = grade 5 and 6 (equivalent to pink stage)

III = grade 9 (equivalent to red stage)

In the same day of harvest, the fruits were washed in tap water, immersed for 60 s in sodium hypochlorite solution (1mg / l – pH 6.8) and then rinsed in tap water. The next day, the fruits were sliced in 7 mm thick transversal slices. The first slice from the stem end was discarded while the following three were stacked in a plastic petri dish (diameter 90 mm and height 25 mm) in the same relative position they had in the fruit. The rest of the fruit (blossom end) was also discarded. For each combination of colour stage x day of evaluation x temperature of storage, five replicates, corresponding to one petri dish with 3 slices of a same fruit, were used. The sliced tomatoes were stored at 2 ± 0.5 °C, 5 ± 0.6 °C, 8 ± 1.5 °C, 12 ± 0.3 °C and 16 ± 0.5 °C.. Temperature data were recorded by an eight channel thermocouple with a personal computer interface.

2.2. Firmness Measurement

Four measurements were made on each slice, two in the outer pericarp and two in the radial pericarp, applying the force in the axial direction. Care was taken to standardise the measurements in the radial pericarp at half radius and in the outer pericarp between two junctions of outer and radial pericarp. The force necessary to cause a deformation of 3 mm with a speed of 0.02 mm/s was recorded using a Zwick Universal Testing Machine, with a 3.5mm diameter flat faced cylindrical probe. After slicing, but before cooling, five replicates from each maturity stage were analysed (time=zero). During storage, the slices were analysed daily or every two days depending on the combination stage of maturity and temperature, in a total of six evaluations (Table 1). Only the central slice in the stack was used in the analyses. The firmness measurement was performed immediately after removing the slice from the storage chamber (at storage temperature), except for time 0 as described.

Table 1 - Sampling frequency in days for firmness measurements of fresh-cut tomato slices.

Grade	2 °C	5 °C	8 °C	12 °C	16 °C
I	2	2	2	1	1
II	2	2	2	1	1
III	1	1	1	1	1

2.3. Model development

The decrease in firmness of fruits and vegetables after harvest has been described by first order reaction mechanism by Tijskens (1979),Van Dijk and Tijskens (2000), Tijskens *et al*. (2002). The firmness was considered to be built up by a variable part (e.g. pectin originating firmness) that changes according to a first order mechanism and a fixed part (e.g. cellulose or structure originated firmness) that is invariable for the circumstances under study.

This results in the basic first order model:

$$F = (F_0 - F_{fix}) \cdot e^{-k \cdot t} + F_{fix} \qquad \text{Eq. 1}$$

Where:

F= firmness at time t after harvest (in Newton)

F_0= initial firmness at harvest (in Newton)

F_{fix}= invariable part of firmness (in Newton)

k= reaction rate constant at temperature t

t= time (in days), counting from the moment of harvest

In the current experiment, tomatoes were harvested from the same greenhouse at the same growing conditions but at different stages of maturity. Assuming that the same mechanism is active during both the growth and the postharvest period, the initial firmness (F_0) measured immediately after slicing the fruits, depends on the time the tomatoes were allowed to ripen in the plant (t_g in Eq. 2).

$$F_0 = (F_{g,0} - F_{fix}) \cdot e^{-k_g \cdot t_g} + F_{fix} \qquad \text{Eq. 2}$$

Where:

$F_{g,0}$= the firmness (in Newton) of tomato at stage I when both t_g and t = 0

k_g = reaction rate constant at growth temperature

t_g= time of growth (days) arbitrarily counting from reaching state I

Combining both equations, results in a model description were the initial firmness depends on the maturity stage at harvest, that in turn depends on the time the fruit was on the plant, while the firmness during storage depends on the initial firmness and on the storage temperature.

$$F = (F_{g,0} - F_{fix}) \cdot e^{-(k_g \cdot t_g + k \cdot t)} + F_{fix} \qquad \text{Eq. 3}$$

Both reaction rate constants (k and k_g) are defined by the same relation to temperature and are only different by the different temperatures applied: k at storage temperature after processing, k_g at the temperature in the greenhouse during growth. The reaction rate constants depend on temperature, presumably according to Arrhenius' law (Eq. 4) with the same activation energy (Ea) and the same reaction rate at reference temperature (k_{ref} at T_{ref}).

$$k = k_{ref} \cdot e^{\frac{Ea}{R} \cdot \left(\frac{1}{T_{ref}} - \frac{1}{T} \right)} \qquad \text{Eq. 4}$$

2.4. Data analysis

Based on equations 3 and 4 a non-linear regression analysis was performed (Genstat Rothamsted, UK), assuming a temperature of 20 °C during growth and at time zero. The data (averaged as well as individual) were analysed in their entirety without further transformation using stage of maturity, temperature and time simultaneously as explaining variables (multi-variate non-linear regression analysis). The kinetic parameters (k, k_g, Ea), the invariable firmness F_{fix} and the values of $F_{g,0}$ were estimated in common for all the slices. The values for t_g were estimated for the three stages of maturity at harvest separately. The t_g of the first sample (stage I) was used as a reference and was fixed to 0.

3. Results and Discussion

3.1. Statistical analysis

The results of the non-linear regression analysis are shown in Table 2. For the outer pericarp, based on average data, the explained part (R^2_{adj}) mounts up to 91.9%. For the inner pericarp the explained part is far less (76.7%), due to the higher variability in the data.

Table 2 - Result of statistical non-linear regresion based on Eqs. 3 and 4 using mean and individual values.

	Mean				Individual			
	Outer pericarp		Radial pericarp		Outer pericarp		Radial pericarp	
	Estimate	s.e.	Estimate	s.e.	Estimate	s.e.	Estimate	s.e.
$F_{g,0}$	7.762	0.146	4.563	0.151	7.798	0.122	4.614	0.107
F_{fix}	2.799	0.475	0.4	1.23	3.056	0.331	0.351	0.95
k_{ref}	0.0975	0.0183	0.0712	0.0328	0.1097	0.016	0.0691	0.0241
Ea	87.5	10.4	94.8	14.7	89.74	8.69	93.8	10.3
tg_1	0	fixed	0	fixed	0	fixed	0	fixed
tg_2	2.306	0.382	1.279	0.307	2.183	0.306	1.305	0.223
tg_3	6.21	1.74	2.742	0.582	6.85	2.35	2.807	0.41
R^2_{adj}	91.9		76.7		61.8		38.9	
T_{ref}	10		10		10		10	
N_{obs}	85		85		856		856	

The first analysis (mean column in Table 2) was conducted on the mean values. The firmness of the tomato slices decreased exponentially during storage, with a reference rate constant of 0.0975 $\pm$ 0.0183 day^{-1} for the outer pericarp and 0.0712 $\pm$ 0.0328 day^{-1} for the radial pericarp and an energy of activation of 87.5 kJ and 94.8 kJ for the outer and radial pericarp respectively (Table 2). Soliva-Fortuny *et al*., (2002) reported a linear decrease in the firmness of fresh-cut pears during storage. They discarded the consideration of a first-order kinetic mechanism although the textural values would consistently tend to stabilise after prolonged storage, which is a strong indication of an asymptotic end value. In the present study, an exponential decay with a fixed end value (Eq. 3) is considered to describe the firmness behaviour better, because it is very unlikely that the firmness will decrease to 0 (Tijskens *et al*., 1999b; Van Dijk and Tijskens, 2000). The asymptotic end value for firmness (F_{fix}) is a considerable 2.7 N equivalent to 1/3 of the initial firmness at stage I. This means that only 2/3 of the total firmness is available for change. For stages II and III, which presented lower initial firmness, these values are even smaller, representing respectively 1/2 and 1/3 of the initial firmness.

In essence, the factor t_g express the stage of maturity at harvest as the time necessary for the tomatoes in the greenhouse to mature to a particular stage. In Figure 1, the behaviour of firmness is shown for three stages of maturity, taking the shift in harvest time (t_g) into account. In this graph, all data of all stages of maturity and storage temperature are combined by using a conversion of the actual time at a certain stage and temperature to a "standardised" time at 20 ºC of $t_s = \frac{k \cdot t + k_g \cdot t_g}{k_g}$.

It is clear from this graph that the data at all stages of maturity at all temperatures of storage follow the same exponential behaviour towards a common asymptotic end values.

This graph (Fig. 1) also clearly shows that the firmness decay was more pronounced at stage I and decreased in the later stages of maturity. This is a direct consequence of the exponential behaviour: the higher the initial firmness, the more firmness is lost in the same time. The results reported by Holt (1970) cited by Sherman, (1973) indicate the first order mechanism used as plausible, at least for the postharvest period.

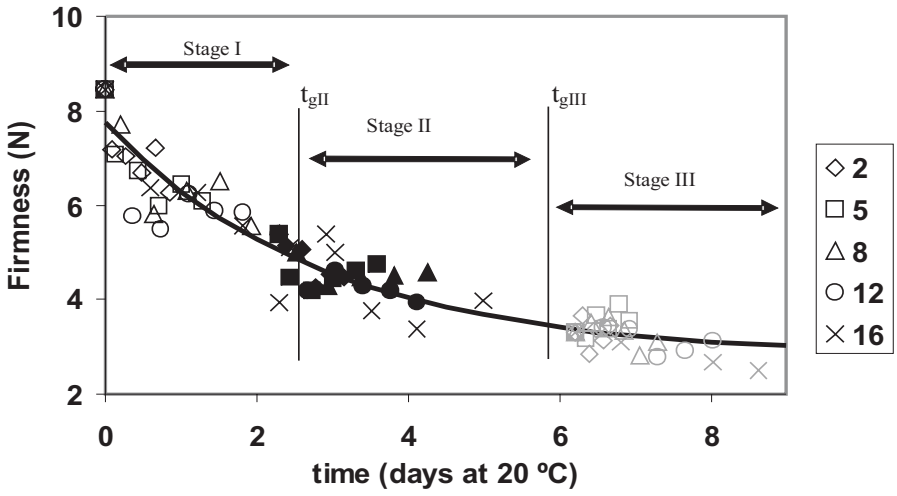

Figure 1 - Firmness of tomato slices (outer pericarp) as a function of stage of maturity at harvest (black opened symbols = stage I; black filled symbols = stage II; grey opened symbols = stage III), temperature of storage (see legend 2°C, 5°C, 8°C, 12°C, 16°C) and storage time. The symbols represent the mean of 5 replicates and the solid line represents the values simulated according to eq. 3 and the parameters values in Table 2. The time on the x-axis has been transformed to a standard temperature of 20 ºC using the conversion: $t_s = \frac{k \cdot t + k_g \cdot t_g}{k_g}$.

3.2. Classical versus integral analysis

When using a classical approach to analyse the data, where the effect of each temperature within each stage of maturity over time is taken separately (as depicted in Figure 2-4), it is difficult to perceive any trend in behaviour. The range in firmness change caused by the storage treatments is of the same magnitude as the variation between the measured firmness values. The major change in firmness was observed in the initial firmness at the different stages of maturity, as can be seen when the initial values in the three graphs are compared. Even pooling the data over the storage temperatures for each stage of maturity separately did not allow a reliable analysis (data not shown). However, when using the integral approach, all the available information can be incorporated in the model by pooling all data. In this case, successive maturity stages can be analysed in sequence as belonging to a same curve (Figure 1), by means of the incorporation of the maturity factor t_s, while taking the actual storage temperatures into account using the Arrhenius equation (Eq. 3). It also becomes clear that scattering of data points over the complete range appears to be far less than in the separate representation (Figure 2-4).

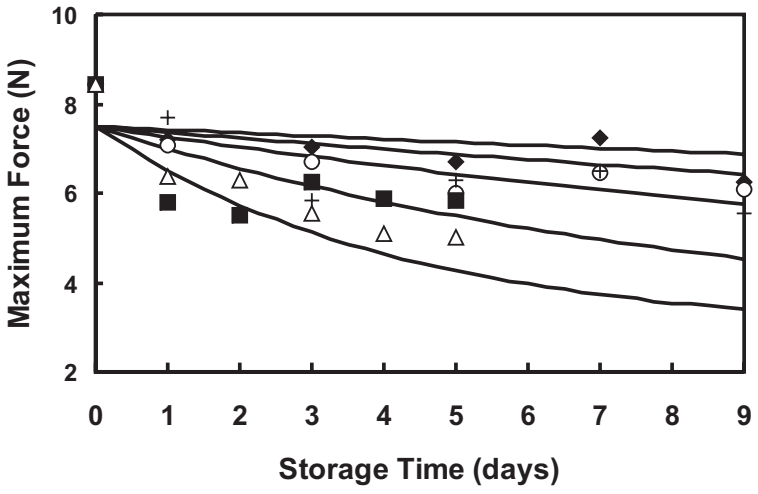

Figure 2 - Firmness of sliced tomato outer pericarp, processed at maturity stage I, expressed as maximum force to cause a deformation of 3-mm. Symbols represent measured values at ◆ 2°C; ○ 5 °C; + 8 °C; ■ 12 °C ; △ 16 °C. Solid lines represent values simulated according to eq. 3 and the parameters values in Table 2.

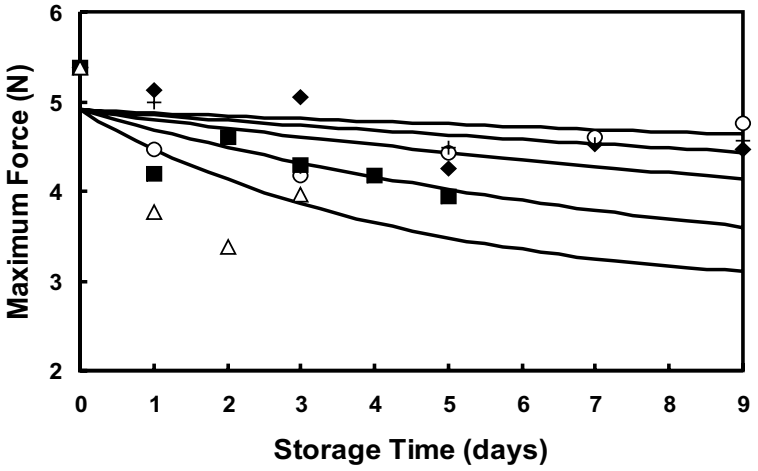

Figure 3 - Firmness of sliced tomato outer pericarp, processed at maturity stage II, expressed as maximum force to cause a deformation of 3-mm. Symbols represent measured values at ◆ 2°C; ○ 5 °C; + 8 °C; ■ 12 °C ; △ 16 °C. Solid lines represent values simulated according to eq. 3 and the parameters values in Table 2.

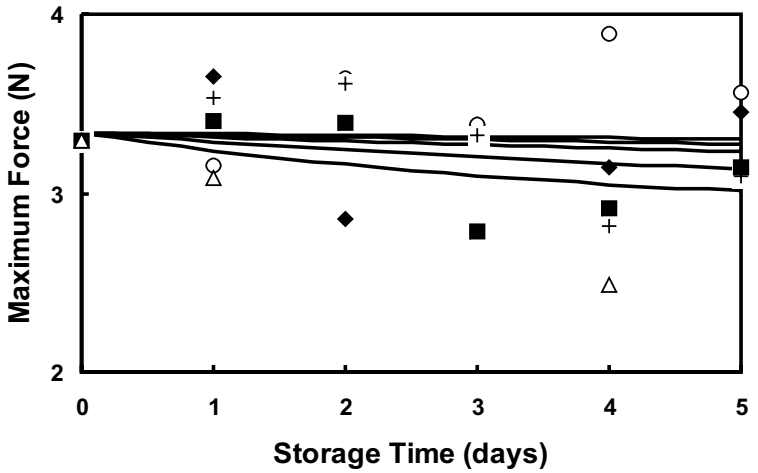

Figure 4 - Firmness of sliced tomato outer pericarp, processed at maturity stage III, expressed as maximum force to cause a deformation of 3-mm. Symbols represent measured values at ◆ 2°C; ○ 5 °C; + 8 °C; ■ 12 °C ; △ 16 °C. Solid lines represent values simulated according to eq. 3 and the parameters values in Table 2.

In Fig.5, the estimated versus measured firmness (top) and the residuals versus measured firmness (bottom) are shown for all measuring conditions. For the mean values the scatter is low enough to be acceptable, without indications of a structural discrepancy, except for the stage of maturity. Even for the individual data (not shown), no structural discrepancies can be observed. The scattering itself of the data however is huge (data not shown).

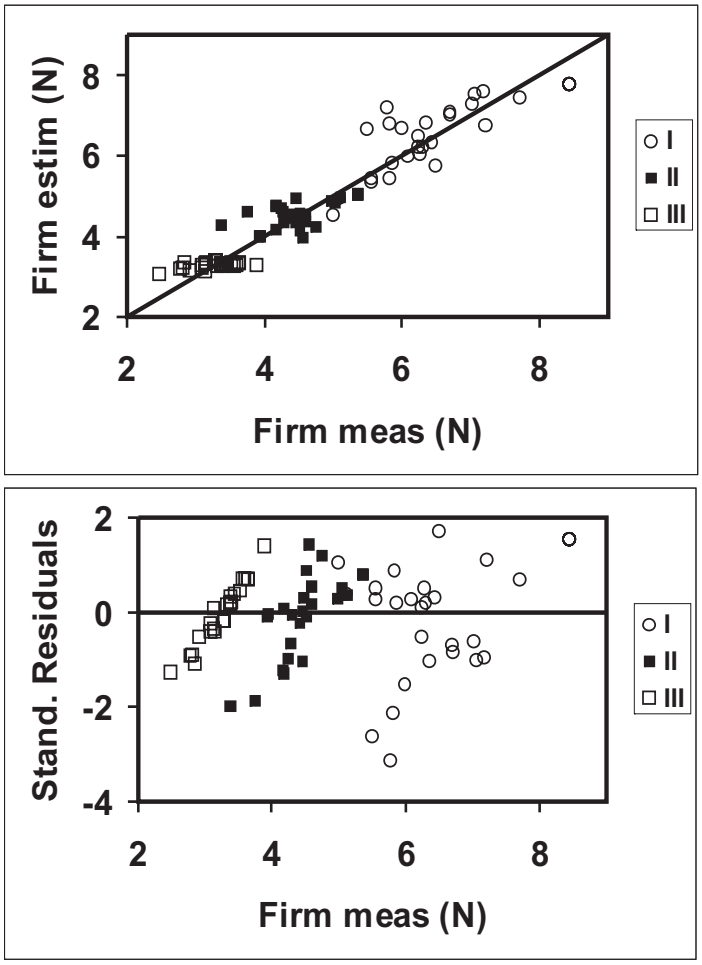

Figure 5 - Scatter plot (top) and residual plot (bottom) for mean data.

For statistical analysis, the pooling of data over the stages of maturity provides a large increase in the usefulness of the data by making the larger range of preharvest information available for the postharvest analysis, but constitutes at the same time a weakness. A mechanism has to be assumed for the firmness change during the preharvest growing period. By lack of suitable information regarding that (simplified) mechanism, the same exponential decay as used in postharvest storage was

assumed. It is however very unlikely that the same mechanism or process is active during growth as during storage. On the other hand, it is equally unlikely that a complete different mechanism or process is active. Including more extended mechanisms for the preharvest growth period will most affect only the values estimated for the individual t_g values. For the time being, until more detailed information becomes available, the same mechanism will be assumed for preharvest and postharvest period alike.

3.3. Changes in firmness during storage

Within a maturity stage, the firmness of the tomato slices decreased only slightly during storage. Especially at the lower temperatures (2 - 8 °C) the decrease was minimal. At higher temperatures, the decrease was more noticeable, but still very small. In Figure 2-4 the firmness of outer pericarp is presented. Similar graphs were obtained for the radial pericarp (not shown), although in this case the firmness was lower for all combinations stage of maturity and temperature. Far more difference could be noticed among the firmness of fruits harvested at different maturity stages (F_0), as can be taken from the overall representation (Figure 1) of the behaviour of firmness in tomato slices.

The same results regarding the slow decrease in firmness of sliced tomato at a certain maturity stage were obtained by Wu and Abbott (2002) when sliced tomatoes were stored at 5 °C. Although significant differences in firmness were observed among fruits from varying maturity stages, very small changes were observed over storage time for a given initial maturity stage. Other reports also showed that the changes in firmness of fresh-cut tomato were not as dramatic as expected (Artes *et al*., 1999; Hong *et al*., 2000; Gil *et al*., 2001).

3.4. Firmness and shelf-life

The observed decrease in the firmness of tomato slices in the present study was not enough to render the slices unacceptable due to excessive softening, except when the slices were spoiled (data not included in the model). In general, the end of the shelf life of cut tomato slices was caused by other reason than excessive softening. For all combinations of stage of maturity and temperature the translucency of the pericarp was the main change affecting the visual quality. It was expected that the translucency of the slices would be paralleled by a change in texture that could be measured by a decrease in firmness as reported for papaya (Karakurt and Huber, 2003) and pears (Soliva-Fortuny *et al*., 2002). However, in the case of tomato using the technique here described, this could not be observed. Tomato is susceptible to chilling injury at temperatures below 10-12 °C, what encompasses most of the temperatures used in the present work. Increased softening of whole tomato occurred as a consequence of chilling but only after transfer to non-chilling temperature (Jackman *et al*., 1990) a condition not encountered during this experiment.

3.5. Firmness measurement technique

A point that must be considered is the suitability of the method used to measure firmness. Using a method very similar to the one used in the present study, Wu and Abbott (2002) concluded that measuring only the maximum force to cause a pre-defined deformation was inadequate to detect the changes occurring in sliced tomatoes. They suggested that viscoelastic characteristics changed during storage and were better expressed as an empirical relaxation parameter. However the biological meaning of this parameter is not known, only that it affects the rate of change (radius or curvature in the stress/time curve) during the initial response to stress in the compression test. Besides that, Jackman *et al*. (1990) showed that different texture evaluation techniques present distinct sensibility to measure changes in firmness of chilled tomato tissue.

3.6 Variablity

In the present study, the first analysis (Table 2**.**, first columns) was conducted on the mean values over the different samples and replicates. When analysing the individual data (Table 2, last columns), roughly the same parameters values, and also about the same standard error of estimate (S.E..) were obtained. The major difference was a huge drop in the explained part: from 91.9% (outer pericarp) for the mean values to 61.8 % for the individual data. For the radial pericarp the variation was even larger and the explained part dropped from 76.7% to only 38.9%.

The high variability in the data prevented a reliable determination of the mechanism. Nevertheless, the high R^2_{adj} obtained when the mean values were used indicates a good and well-funded guess on the mechanism. When analysing individual data the residuals scattered all over, while for mean data they were quite acceptable Fig.5.

The large variation in the data reflects the natural variation within and between individual fruits, that results from differences in the composition of the fruit tissues (King Jr. and Bolin, 1989; Lesage and Destain, 1996; Artes *et al*., 1999). In the present study care was taken to use slices from a same position in the fruit and standardise the measurements in about the same position in the slice as described in the Material and Methods. However, a variation in composition and stage of maturity is also present in different sides of the same fruit (Lesage and Destain, 1996) and this variation could not be avoided. In the case of the radial pericarp another source of variation was present. In some slices the radial pericarp was narrower and the probe surface was not completely in contact with the slice surface what could lead to variations in the measurements. Additionally, Wu and Abbott (2002) observed that sliced tomatoes generally had higher coefficient of variation than those stored intact. It is not clear what the cause of this higher variation was.

For a real reliable determination of the kinetics of firmness decay, non-destructive data on the same individuals would be necessary (Tijskens *et al*., 1999a). In preliminary experiments (data not shown) non-destructive compression and puncture techniques were used to measure the firmness of

tomato slices. However, the use of a very small force (in the order of 0.3 N) rendered the test too insensitive. Besides that, other problems like warming up, dehydration and microbial contamination may occur when successive measurements are performed on the same individual slice and affect the rate of softening. Other non-destructive techniques such as those reported by Lesage and Destain (1996) and acoustic impulse-response techniques (Schotte *et al*., 1999) are, at the moment, not suitable for sliced tomato.

3.7 Correction for assay temperature

The firmness during the experiment was measured at the temperature the samples were stored at, and not equilibrated at the same standard temperature. Although this reflects more accurately the texture during storage, it can lead to both an under- or over-estimation of the firmness compared to the one that would be obtained if the firmness was always measured at a standard temperature (Johnston *et al*., 2001). To overcome this problem a correction for the effects of the storage temperature was introduced. The firmness at day 0 (as if measured at storage temperature) was extrapolated from the data collected during the subsequent days of storage, measured at storage temperature. The difference between the estimated and measured value was considered to be due to the effect of temperature on the measurement itself and was used to calculate the correction factor. This factor was dependent on temperature presumably according to a simple Arrhenius type dependence (Eq. 4) taken relative to the reference temperature of 10 ^{o}C.

$$F_c = F \cdot k_c \qquad \text{Eq. 5}$$

Where:

F_c = corrected value of firmness at reference temperature (in Newton)

F= measured firmness at measuring temperature (in Newton)

k_c = correction factor

The correction factor was subsequently used to express the firmness values at days other than 0 as if measured at room temperature. The activation energy for the temperature correction (measuring the slices at storage temperature) was very low, indicating that only a minor correction was necessary. However, when this correction factor was applied, higher firmness for slices stored at higher temperatures were obtained for the initial storage period. Johnston *et al*. (2001) reported a strong interaction between the physical effect of temperature and storage period. However in the present study it did not seem plausible that slices stored at 16 ^{o}C would be firmer than at 2 ^{o}C in the first two days of storage. Since the effect was found to be rather small and the actual function used could not be determined nor validated, the correction factor was not used.

4. Conclusions

In contrast to the expected behaviour, accelerated softening due to minimal processing was not found. The firmness measured as maximum force to cause a predefined deformation did not change significantly after slicing during short-term storage time. The major change in firmness was observed in the initial firmness at the different stages of maturity. The firmness decay of tomato slices during storage could be described by a first order reaction model that incorporates the stage of maturity of the fruit at harvest and both the temperature and time of storage. Pooling data in an integral analysis made it possible to analyse successive maturity stages in sequence as belonging to a same curve, and it had the advantage of using all available information, theoretical as well as experimental. Not only effects of time and temperature could be incorporated, but the different stages of maturity at harvest as well.

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CHAPTER 3

EFFECTS OF STORAGE TEMPERATURE AND STAGE OF MATURITY ON RGB COLOUR ASPECTS OF FRESH-CUT TOMATO USING VIDEO IMAGE ANALYSIS

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Abstract

Tomato fruit (*Lycopersicon esculentum* cv. Belissimo) were harvested at three different stages of maturity, sliced and stored at 5 different temperatures. RGB (Red-Green-Blue) images of the slices were taken regularly during storage, using an image processing system. For constructing a model, each of these colour aspects was considered to be built up by a variable part that changes according to a first order reaction mechanism and a fixed part that is invariable under the circumstances under study. All three colour aspects of the tomato slices (R, G and B) decreased exponentially during storage. The parameters of the model were estimated using multiple-response multiple-variate non-linear regression analysis using R, G and B simultaneously as response variables and time, temperature and maturity stage of the fruit at harvest simultaneously as explaining variables. To combine the information on the behaviour of the colour aspects during the preharvest and the postharvest period at different temperatures, it was assumed that the process of change during maturation was the same whether the fruit ripened on the plant or off-vine. So, the initial value for all three colour aspects ($Col_{0,R}$, $Col_{0,G}$ and $Col_{0,B}$) during the postharvest experiments depended on the time the tomatoes were allowed to ripen on the plant. By using this fundamental approach to build the model and using all available data and information it became possible to describe and simulate the behaviour of the colour aspects of tomato slices as a function of the maturity stage and the applied storage temperature.

Although the variance between replicates was high, the statistical analysis on the mean values of colour aspects over the replicates provided a percentage variance accounted for of 95%. The same model was validated with data of another experiment with another tomato cultivar (Durinta) over a larger range of maturity stages.

Keywords: *Lycopersicon esculentum*; minimally processed; image processing systems, modelling.

1. Introduction

Colour and firmness are considered good indicators of quality of fresh-cut fruits and vegetables. Understanding how these attributes change after processing is fundamental to establish good management practices in the industry and during distribution and marketing.

In a previous report (Lana *et al.* 2005) the change in firmness of tomato slices during refrigerated storage was described by a first order reaction model that incorporated the stage of maturity of the fruit at harvest and both the temperature and time of storage. Taking into account the concept of biological shift factor discussed next, it was possible to analyse successive maturity stages in sequence as belonging to a same curve. All data from different maturity stages and storage temperatures were combined for graphical representation by using a conversion of the actual time (days after processing) at a certain stage and temperature to a standardized time at 20 ^{0}C (days to mature at a particular stage). By applying the biological shift factor over the mean of harvest batches it is possible to express the stage of maturity at harvest as the time (in days) necessary for the tomatoes in the plant to attain a particular stage of maturity.

This concept had been previously used by Tijskens and Evelo (1994) to study the change in colour of intact tomato fruit after harvest. The tomato fruit colour expressed as a* value, was described by a logistic function where the effects of temperature during storage and stage of maturity of the fruit at harvest were both incorporated, and the colour development in every maturity stage could be

described by a same function. This approach was further developed by (Schouten *et al*., 1997) to describe changes in the colour of cucumber after harvest. A more comprehensive discussion about the use of this concept for modelling and understanding product behaviour after harvest can be found in (Tijskens *et al*., in press).

In the present report, the same approach was used to address changes in colour aspects of the same tomato slices as used in the previous report on firmness (Lana *et al*., 2005).

The rate of colour change of tomato fruit during maturation is known to depend on temperature and maturity stage (Tijskens and Evelo, 1994). Changes in colour induced by processing operations are also expected to depend on both factors (Beaulieu and Gorny, 2001). To pool all data over the stages of maturity as described in Lana *et al*. (2005) it was assumed that a same mechanism of change was active during both growth and storage. Studies with excised tissues indicates that this assumption is also plausible for colour changes. When intact tomato fruit and excised discs were first paired by initial skin colour, they both passed through the same colour stages as they ripened both in time and in location, apparently indicating that the regulatory mechanisms that determine the rates of pigment degradation and synthesis during maturation, are local and retained in pericarp discs (Campbell *et al*., 1990).

In the present report, the colour aspects of the tomato slices were expressed as Red (R), Green (G) and Blue (B) values obtained from digital RGB images of those slices. Although each individual RGB value alone does not represent any given perceived colour, the colour of each image is built up on these three variables. That means that any particular colour in an image can be represented by the relative amount of RGB present in that image and that two particular colours differ from each other in the value of at least one of these attributes. In this attempt to model changes in colour aspects of fresh-cut tomato applying the biological shift factor it was chosen first to work with the RGB values, here called the colour primaries or colour aspects, avoiding additional transformations to other colour spaces. From the function used to describe the changes in RGB values further inferences can be made about changes in the transformed variables like the CIELab system variables, obtained from the RGB values.

2. Materials and Methods

Two separate experiments were set up. The first one over a larger number of replicates to determine the basic structure of a model to describe the behaviour of the colour aspects R, G and B of cut tomato harvested at three stages of maturity; the second one to validate the findings over a larger range of maturity stages at harvest.

Legend of Symbols

Name	Dimension	Meaning
Ea	kJ/mol	activation energy
Col	-	colour aspect (R, G, B)
k	day^{-1}	rate constant of change of colour aspect
R	J/mol/K	universal gas constant (8.314 J/mol/K)
t	day	time
T	K (°C)	temperature
Subscripts		
0		initial
I		of stage I
II		of stage II
III		of stage III
IV		of stage IV
V		of stage V
s		during storage
fix		invariable part
g		during growth
ref		at reference temperature (=20 °C)

2.1. Harvesting and processing

2.1.1. Experiment 1

Tomatoes (*Lycopersicon esculentum* cv. Belissimo) grown in a greenhouse in Made (The Netherlands) were harvested in a single day on May 2003 in three colour stages, named here as I, II and III corresponding to stages 3, 5-6 and 9 of the tomato colour scale (kleur stadia-tomaten) from The Greenery (http://www.thegreenery.com)

On the same day of harvest, the fruit were washed in tap water, immersed for 60 s in sodium hypochlorite solution (1 mg / l, pH 6.8) and then rinsed in tap water. The next day, the fruit were sliced in 7 mm thick transversal slices. The first slice from the stem end was discarded while the following three were stacked in a plastic petri dish (diameter 90 mm and height 25 mm) in the same relative position they had in the fruit. The rest of the fruit (blossom end) was also discarded. For each combination of maturity stage x day of evaluation x temperature of storage, 5 replicates, corresponding to one petri dish with 3 slices from the same fruit, were used. The sliced tomatoes were stored at 2 ± 0.5 °C, 5 ± 0.6 °C, 8 ± 1.5 °C, 12 ± 0.3 °C and 16 ± 0.5 °C. Temperature data were recorded by an 8-channel thermocouple with a personal computer interface.

Only the central slice in the stack was used in the in order to get rid of the variation in colour

and appearance due to differences in maturity stage within the fruit, whitening of the cut surface of the top slice due to dehydration, or excessive water soaking of the pericarp tissue in the bottom slice. The R, G, B measurement was performed immediately after the evaluation of firmness (Lana *et al*., 2005).

Table 1 – Time between sampling in days for colour measurements of fresh-cut tomato slices – Experiment 1 and 2.

Experiment	Grade	2 °C	5 °C	8 °C	12 °C	16 °C
1	I	2	2	2	1	1
	II	2	2	2	1	1
	III	1	1	1	1	1
2	I	2	2	2	1	1
	II	2	2	2	1	1
	III	2	2	2	1	1
	IV	1	1	1	1	1
	V	1	1	1	1	1

The sampling scheme at each storage temperature is provided in Table 1.

2.1.2.Experiment 2

Tomatoes (cultivar Durinta) grown in a greenhouse in Wageningen (The Netherlands) were harvested in November 2002 in five maturity stages corresponding to the following grades of the tomato colour scale (kleur stadia-tomaten) from The Greenery (http://www.thegreenery.com): 3-4, 5, 7, 8 and 11. On the day of harvest, the fruit were washed in tap water, immersed for 60 s in sodium hypochlorite solution (1 mg / l, pH 6.9) and rinsed in tap water. The next day, the fruit were sliced in 7-mm thick transversal slices. The first and second slice from the stem end were discarded while the following two were stacked in a plastic petri dish (diameter 90 mm and height 15 mm) in the same relative position they had in the fruit. The rest of the fruit (blossom end) was also discarded. For each combination of colour stage x day of evaluation x temperature of storage 3 replicates, each corresponding to one petri dish with 2 slices from a same fruit, were used. The sliced tomatoes were stored at the same conditions of experiment 1. Only the bottom slice in the stack was used in the analyses and the R, G, B measurement was performed immediately after removal from refrigeration. The sampling scheme at each storage temperature is provided inTable 1. In both experiments, the first evaluation, denoted as day 0, was done at room temperature, before the slices were cooled.

2.2.Image Evaluation

In both experiments, the RGB images of the tomato slices were taken using an image processing system consisting of a 3 CCD Hitachi HV-C20 video camera with a Tamron SP 35-80mm objective, a computer and a lightning chamber as described by Schouten *et al*. (1997). Images were acquired under diffuse illumination provided by four fluorescent tube lamps (TL-D 18W/84) positioned in the higher part of the chamber.

The pictures were analysed using the KAS Software, developed by Agrotechnology and Food Innovations, NL. A colour learn set (Schouten at al, 1997) was created to enable the distinction between the background and the tomato slice and between different parts of the slice like the pericarp, the locular gel with the seeds and the columela. In the present report, only data from the pericarp were analysed. In this way the confounding effect of different changes happening in parts of the slice with distinct colour aspects (columela and locular gel) was avoided.

Colour aspects were expressed as the separate intensities of red, green and blue values (RGB). Although a non-destructive measuring technique was applied, different individuals were used in successive days of assessment, since destructive measurements of firmness (Lana *et al*., 2005) and lycopene (Lana *et al*., in press) were performed in the same slices, respectively in Experiment 1 and Experiment 2.

2.3.Model development

Each colour aspect (R, G and B) was considered to be built up by a variable part that changes according to a first order mechanism (simple exponential decay) and a fixed part that is invariable for the circumstances under study.

This results in the basic first order model as describe in Eq. 1:

$$Col = (Col_0 - Col_{fix}) \cdot e^{-k_s \cdot t} + Col_{fix}$$

Eq. 1

Where:

Col= colour aspect (R, G or B value) at time t after harvest

Col_0= initial colour aspect at harvest

Col_{fix}= invariable part of colour aspect

k_s= reaction rate constant (at storage temperatures)

t= time (in days), counting from the moment of harvest.

In each experiment, the tomato fruit were harvested from the same greenhouse at the same growing conditions but at different stages of maturity. Assuming that the same mechanism is active during both the growth and the postharvest period the initial colour aspect measured immediately after slicing the fruit (Col_0), depends on the time the tomatoes were allowed to ripen in the plant (t_g in Eq. 2).

$$Col_0 = (Col_{g,0} - Col_{fix}) \cdot e^{-k_g \cdot t_g} + Col_{fix}$$

Eq. 2

Where:

$Col_{g,0}$= the colour aspect of tomato at stage I when both t_g and t = 0

k_g = reaction rate constant at growth temperature

t_g= time of growth (days) arbitrarily counting from reaching state I.

Combining both equations results in a model description were the initial colour aspect depends on the maturity at harvest, which in turn depends on the time the fruit was on the plant (Eq. 3).

$$Col = \left(Col_{g,0} - Col_{fix}\right) e^{-\left(k_g \cdot t_g + k_s \cdot t\right)} \qquad \text{Eq. 3}$$

Both reaction rate constants (k_s and k_g) are defined by the same relation to temperature and are only different by the different temperatures applied: k_s at storage temperature after processing, k_g at the temperature in the greenhouse during growth (assumed to be 20 °C). The reaction rate constants depend on temperature, presumably according to Arrhenius' law (Eq. 4) with the same activation energy (Ea) and the same reaction rate at reference temperature (k_{ref} at T_{ref}).

$$k = k_{ref} \cdot e^{\frac{Ea}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)} \qquad \text{Eq. 4}$$

2.4.Data analysis

Based on equations 3 and 4 a non-linear regression analysis was performed (Genstat Rothamsted, UK), assuming a temperature during growth of 20 °C. The values for t_g were estimated for the three stages of maturity at harvest separately. The t_g of the first sample (stage I) was used as a reference and was fixed to zero.

The data averaged over the 5 replicates were analysed without further transformation using maturity stage, temperature and time simultaneously as explaining variables (multi-variate non-linear regression analysis). The kinetic parameters (k_{ref} and Ea) were estimated in common for all the slices, the invariable part Col_{fix} and the values of $Col_{g,0}$ were estimated separately for the three different colour aspects (R, G and B).

2.5. Validation

The model was validated using the data from experiment 2. The same procedure was followed on the mean values over the three replicates. Again, the kinetic parameters (k_{ref} and Ea) were estimated in common for all the slices, the invariable part Col_{fix} and the values of $Col_{g,0}$ were estimated separately for the three different colour aspects (R, G and B).

3. Results and Discussion

3.1.Raw data

The overall appearance of the tomato slices changed during refrigerated storage and this was paralleled by changes in the intensities of the individual aspects Red (R), Green (G) and Blue (B). In both experiments, the intensities of the individual aspects R, G and B decreased over time for all temperatures and all stages of maturity.

The value of the three aspects was significantly different for the three stages of maturity indicating that their initial differences in appearance were expressed by differences in RGB values. R, G and B decreased consistently from stage I to III and the differences among stages were roughly maintained during storage (Fig. 1). The effect of temperature although present was far less than the effect of stage of maturity. All aspects seemed to decrease faster at higher temperatures (8 and 12 ^{o}C) but the variation in the data for successive measuring days was very high and consequently, the effect of temperature was rather unclear. Despite the high variability, the changes tended to be less pronounced in riper fruit and at lower temperature (Fig. 1).

3.2.Analysis

The colour development after harvest of fruit and vegetables, expressed in the CIELab colour space, was previously described by a logistic function, which could be traced back to a possible autocatalytic mechanism (Tijskens and Evelo, 1994; Schouten *et al*., 1997). In the present study, the obtained R, G and B intensities could be very well described and analysed with a simple exponential model (first order kinetics) developing towards an asymptotic end value (see section 2.3. Model development).

When inspecting each combination of temperature and maturity stage separately over time it is difficult to perceive any trend in behaviour considering simultaneously the temperature, the time of storage and the maturity stage of the fruit. The information in the separate series (stages within temperatures or temperatures within stages) is fragmented. There is simply not enough information in the separate series at different temperatures and stages of maturity. Even pooling the data over the storage temperatures for each maturity stage separately did not allow an acceptable analysis (data not shown).

The first analysis was conducted on the mean values over the three attributes (Red, Green and Blue) separately (Table 2). The percentage variance accounted for (R^2_{adj}) was high for all three attributes (84 %, 94 % and 89 % respectively), and the standard error of estimates was acceptably small (around 10% or less).

When the 3 attributes were analysed separately, the estimated range parameters (Col_0 and Col_{fix}) highly depended on the colour aspect (Table 2). The kinetic parameters (k_{ref} and Ea), and the

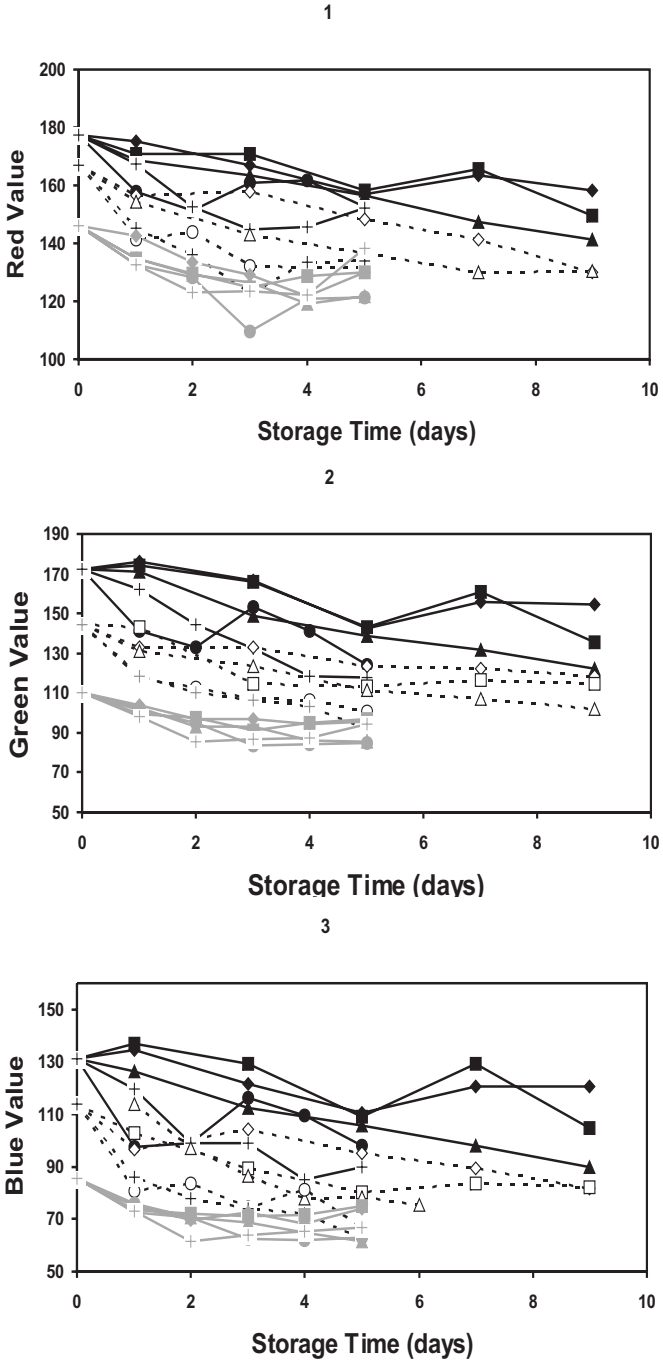

Figure 1 – Red Value (1) , Green value (2) and Blue Value (3) of cut tomato slices harvested at stages of maturity I (solid black line and solid black symbols), II (dotted black line and open symbols) and III (solid grey line and solid grey symbols) and stored at temperatures as indicated by the symbols 2 ^{o}C (◆), 5 ^{o}C (■), 8 ^{o}C (▲), 12 ^{o}C (●), 16 ^{o}C (+). N=5.

Table 2 - Results of the non-linear regression analysis for the colour aspects R, G and B separately, according to Eq. 3 and 4. Experiment 1.

	R		G		B	
	estimate	s.e.	estimate	s.e.	estimate	s.e.
Col_0	177.68	2.22	174.15	2.17	132.8	2.31
Col_{fix}	85.1	23.6	57.4	11.7	35.7	13.4
k_{ref}	0.0681	0.0261	0.0815	0.0142	0.0791	0.0188
Ea	53.48	7.69	76.38	6.35	83.17	8.56
t_{gI}	0	fixed	0	fixed	0	Fixed
t_{gII}	1.666	0.293	1.573	0.184	1.439	0.219
t_{gIII}	3.745	0.523	3.736	0.373	3.081	0.394
T_{ref}	10		10		10	
R^2_{adj}	84.1		93.6		88.9	
N_{obs}	90		90		90	

growth information (t_{gI}, t_{gII}, t_{gIII}) for the three colour aspects are, however, very similar in value certainly when the standard errors of estimates are taken into consideration. That would indicate that the dynamics are the same for the three colour aspects and that they occur quite parallel to each other, each colour aspect changing within its own specific boundaries of Col_0 and Col_{fix}. The growth information (t_g) should then be the same for each aspect, since it is related to the maturity stage at harvest of the individual fruit as a whole, irrespective of the attribute measured.

It should therefore be possible, again using Eq.3 and Eq.4, to pool all data in one integral analysis, estimating the kinetic parameters (k_{ref}, Ea) and the time of maturation (t_{gII} and t_{gIII}) in common for all the series of temperature, maturity stage and the three colour aspects, while allowing for separate ranges of changes for the three attributes defined by Col_0 and Col_{fix}. The results of this integral multi-response non-linear regression analysis are shown in Table 3. The estimated values for the range parameters are more or less identical to the previous ones (Table.2), except for $Col_{fix,R}$. The kinetic parameters are also quite the same. However, both types of parameters have now a much higher reliability (s.e.). Pooling all data makes a reliable estimation of the growth parameters possible: the explained part is higher (R^2_{adj}=95%), the standard errors of estimates are considerably smaller, and the t_g values are quite comparable to the ones estimated on firmness (0, 2.306, 6.210) of the same individuals (Lana *et al*., 2005).

Through the estimation of t_g, it became possible to analyse successive maturity stages in sequence as belonging to a same curve (Fig. 2). In this figure, all the data from the five different storage

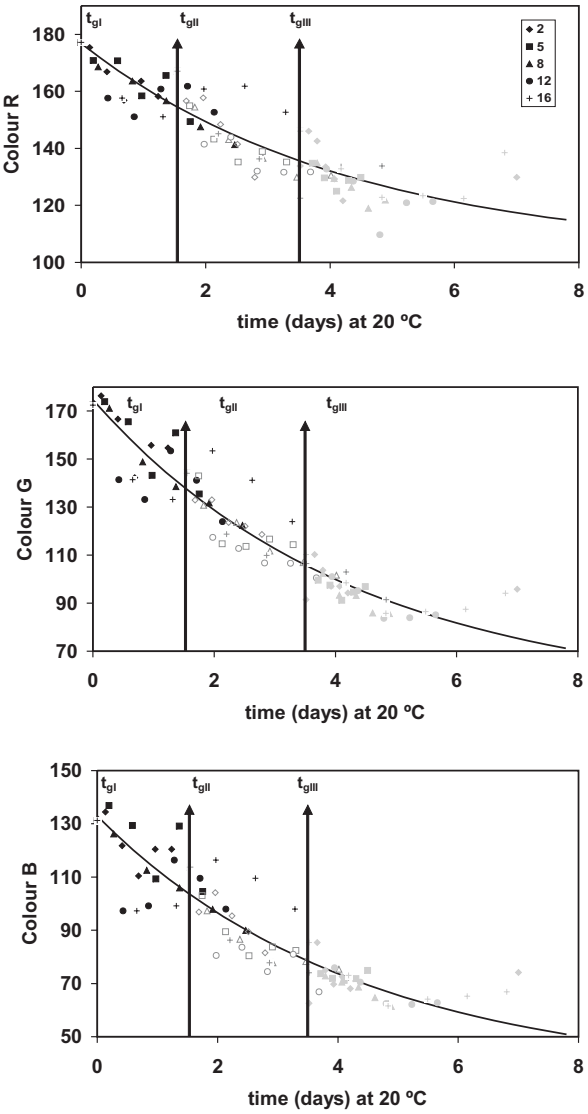

Figure 2 – Effect of maturity stage (black closed symbols= stage I; open symbols=stage II; grey closed symbols=stage III), temperature of storage (2 ^{o}C (◆), 5 ^{o}C (■), 8 ^{o}C (▲), 12 ^{o}C (●), 16 ^{o}C (+)) and storage time on the colour aspects Red (R), Green (G) and Blue (B) of sliced tomato pericarp tissue. The symbols represent the mean of 5 replicates and the solid line represents the values simulated according to Eq.3 and 4 and the parameters values in Table 3. The time on the x-axis is converted from the time at measuring temperature to a standard time at 20 ºC using the equation: $\frac{k_s \cdot t + k_g \cdot t_g}{k_g}$ (Experiment 1).

temperatures are combined using a conversion of time to the time at 20 ºC $\frac{k_s \cdot t + k_g \cdot t_g}{k_g}$, taking the

different temperatures into account in the value for k_s at the different temperatures. This pooling of data in statistical analyses provides a massive increase in the usefulness of the data by making the large preharvest information available for the postharvest analysis, but constitutes at the same time its weakness. A mechanism has to be assumed for the change in these properties during the preharvest growing period. By lack of suitable information regarding that (simplified) mechanism, the same exponential decay as used in postharvest storage was assumed, since it is to be expected that the behaviour in one stage is part of a continuum with previous and later stages, which are not perceived in the separated time series. This assumption is corroborated by the findings of Campbell *et al*. (1990). The temporal pattern of change in skin colour, as indicated by movement along the a* axis, was similar in intact fruit and excised discs. In the endocarp the sequence of colour change was also maintained,

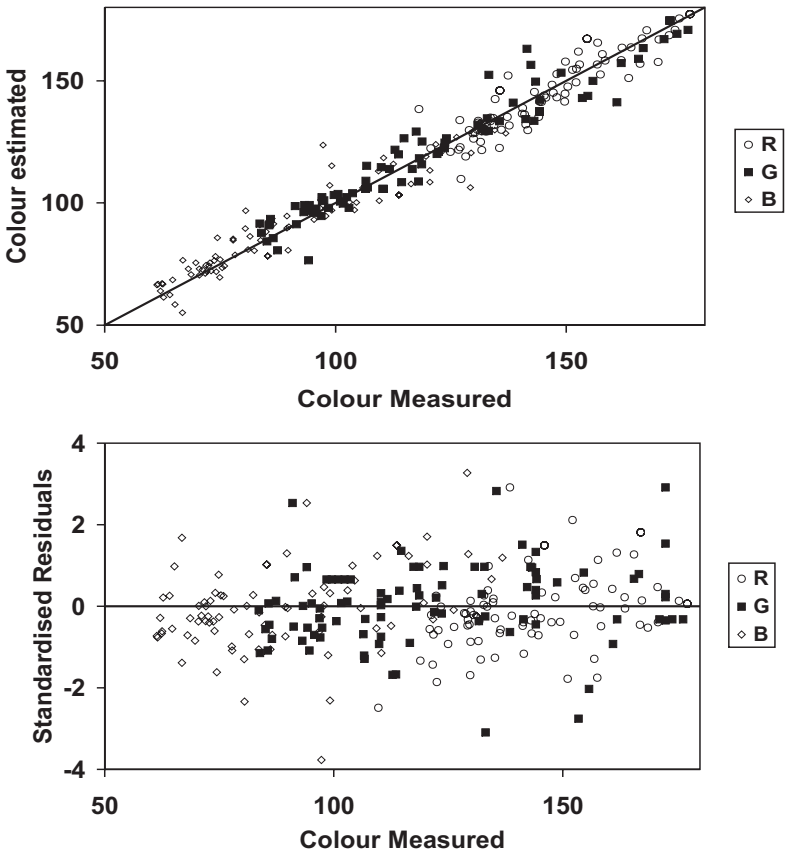

Figure 3 – Scatter plot (top) and residual plot (bottom) based on the combined analysis (Table 4) for Experiment 1. R, G and B refer respectively to Red, Green and Blue values.

although the changes in the endocarp appeared to decrease earlier in discs than in intact fruit. For the time being, until more detailed information becomes available, the same mechanism is assumed for preharvest and postharvest period alike.

In Fig. 3 (top), the estimated versus measured RGB values are shown (scatter plot). The residual plot (Fig. 3 - bottom) shows no regular pattern. Both graphs clearly show that despite the high variation present in the data, no trend in the residuals is present. This signifies that all major processes that could induce changes in these properties are taken into account. The large variation, observed when looking at times series at one stage of maturity and one temperature (e.g. Fig. 1) is in these plots somewhat masked by the larger range over which the colour aspects vary.

RGB values should be converted to into colour spaces as HIS (Antonelli *et al*, 2004) or CIELab (Mendoza and Aguilera, 2004) when a particular colour, as perceived by humans, needs to be identified. However, both HIS and CIELab are in this case derived from RGB values. Consequently, changes in perceived colour, expressed for example as changes in Hue angle or in a* value, necessarily reflect changes in the individual RGB values.

Table 3 - Results of the non-linear regression analysis for the colour aspects R, G and B combined, according to Eq. 3 and 4, based on mean data and individual data. Experiment 1.

	Mean R, G and B combined		Individ. R, G and B combined	
	estimate	s.e.	estimate	s.e.
$Col_{g,0,R}$	176.81	1.79	176.85	1.36
$Col_{g,0,G}$	174.66	1.98	174.67	1.51
$Col_{g,0,B}$	132.88	1.87	132.84	1.43
$Col_{fix,R}$	102.76	6.16	102.9	4.66
$Col_{fix,G}$	50.87	9.88	51.17	7.46
$Col_{fix,B}$	34.79	7.94	35.06	5.99
k_{ref}	0.0793	0.0106	0.08005	0.00809
Ea	73.84	4.36	73.17	3.31
t_{gI}	0	fixed	0	fixed
t_{gII}	1.55	0.128	1.5493	0.0974
t_{gIII}	3.519	0.244	3.528	0.186
T_{ref}	10		10	
R^2_{adj}	95		86.9	
N_{obs}	270		1347	

Assuming an exponential change in all three colour aspects, each with the same rate constant but changing over different ranges (Table 3), and taking ratios of R, G and B as shown in Eq. 3 will induce a logistic behaviour of colour, as humans perceive it (Schouten *et al.*,1997; Tijskens and Evelo, 1994). When the rate constants for the three colour aspects would be different, the behaviour of colour, as humans perceive it, could take any form. Since the behaviour of colour development has so often been described with the symmetrical logistic function, that would indicate that the rate constants for the three colour aspects have to be the same. So, this whole deduction indicates that the untransformed colour aspects R, G and B can be considered as primary properties, upon which colour is build up.

When converting RGB values into CIE-Lab values, again by taking ratios of the colour primaries R, G and B, the same effect will be obtained.

3.3.Harvest maturity expressed as time (tg)

In essence, the factor t_g expresses the time necessary for the tomatoes on the plant to ripen to a particular stage. It represents the biological shift in time, one has to consider when comparing produce from different stages of maturity. This preharvest time (counting from an arbitrary point, here chosen t_{gI}) represent the shift in postharvest time necessary to place data from successive maturity stages on a same curve of development as is shown in Fig. 2. As such t_g represents a direct link between the preharvest and the postharvest time (Tijskens *et al.*, 2003) (Tijskens *et al.*, in press).

In Fig. 2, the behaviour of the three colour primaries (R, G and B) is shown for the three stages of maturity, taking the shift in harvest time (t_g) into account. As can be seen, the decay was more pronounced at stage I and decreased in the later stages of maturity. This is a direct consequence of the exponential behaviour: the higher the initial value of the colour aspect, the more of it is lost in the same time. It also makes it clear that the effect of stage is much more pronounced than the effect of temperature (probably due to the higher temperature during growth) and that differences between stages plotted separately as in Fig. 1, are in reality distinct portions of a same curve.

From these results, it can be concluded that for sliced tomatoes in cold storage, the colour aspects R, G, and B do not change remarkably. The colour of the tomatoes at harvest, however, is a far more important quality attribute, and greatly determines the colour changes that happen during storage.

3.4.Validation

The same analysis procedure was applied to the data of the second experiment. Using the mean values over the three replicates, and estimating the kinetic parameters (k_{ref} and Ea) and the growth information (now over the five stages of maturity at harvest: t_{gI}, t_{gII}, t_{gIII}, t_{gIV}, t_{gV}) in common, while allowing for separate values at initial time and infinite time, a similar high explained part (R^2_{adj} 95.1%) was obtained (see Table 4). The most pronounced difference between the two experiments (on

Table 4 – Results of the non-linear regression analysis for the colour aspects R, G and B combined, according to Eq. 3 and 4, based on mean data and individual data. Experiment 2.

	Mean R, G and B combined	
	estimate	s.e.
$Col_{g,0,R}$	176.3	1.45
$Col_{g,0,G}$	166.15	1.77
$Col_{g,0,B}$	105.79	1.36
$Col_{fix,R}$	82.35	1.75
$Col_{fix,G}$	28.22	1.75
$Col_{fix,B}$	24.83	1.75
k_{ref}	0.03336	0.00268
Ea	67.75	7.45
t_{gI}	0	fixed
t_{gII}	5.541	0.769
t_{gIII}	11.8	1.44
t_{gIV}	26.11	3.07
t_{gV}	41.04	4.83
T_{ref}	10	
R^2_{adj}	95.1	
N_{obs}	456	

different cultivars) is found in the value of the rate constant at reference temperature (k_{ref}). Also for the late harvested samples, the estimated values for the maturity stages (t_{gIV} and t_{gV}) are very large. This is partly due to the fact that more ripe tomatoes (stage V) were used in the experiment. In that case, the initial value ($Col_{g,0}$) is already quite close to the asymptotic end value, and the development stage, expressed as time, is within the range of measuring error rather ill defined.

Another type of validation of the approach used can be found in comparing the values of t_g estimated for colour and firmness. Both firmness (Lana *et al*., 2005) and colour were measured for slices of the same tomatoes (Experiment 1), sharing consequently the same maturity stage. The values of the respective t_g should therefore be comparable for both quality attributes. In Table 5 the values for t_g from separate analyses shown a difference of about 1 day for stage II and about 3 days for stage III. This difference is certainly too large, even considering the standard error of estimates to be plausible. However, since both types of data, the colour aspects R, G and B and the firmness were determined on the same tomatoes they should share the same value for t_g, as this parameter represents the time the

Table 5 – Comparison for the t_g values, estimated on firmness and on colour. Experiment 1.

Firmness	Colour
0	0
2.31	1.55
6.21	3.52

Table 6 - Results of the non-linear regression analysis for the colour aspects R, G and B combined with firmness only joined by estimated days at the vine (t_g), according to Eq. 3 and 4, based on mean data. Experiment 1.

	Mean R, G, B and Firmness combined	
	estimate	s.e.
$Col_{g,0,R}$	177.49	2.10
$Col_{g,0,G}$	175.94	2.42
$Col_{g,0,B}$	133.95	1.89
$Col_{fix,R}$	107.98	4.88
$Col_{fix,G}$	59.45	7.65
$Col_{fix,B}$	41.52	6.05
F_0	7.55	0.10
F_{fix}	0.78	0.47
k_{ref}	0.089	0.010
Ea	76.864	5.073
$k_{f,ref}$	0.054	0.007
Ea_f	92.232	8.166
t_{gI}	0	fixed
t_{gII}	1.58	0.14
t_{gIII}	3.39	0.27
T_{ref}	10	
R^2_{adj}	90.60	
N_{obs}	360	

tomatoes were allowed to ripen at the vine. All data were therefore pooled and analysed together (standardised over their respective range of change), with only the biological time shift in common. The results are shown in Table 6. As can be seen, all parameters did obtain somewhat different values. The explained part (R^2adj) did hardly change and the standard errors of estimate are all acceptably low, some a little bit higher, some a little bit lower (see Table 6). The increase in information by pooling all data of the same samples did indicate that the t_g values reported for the firmness (Table 5 and Lana *et al*., 2005) were estimated too large by lack of sufficient information. The values for tg shown in Tables

4 and 6 are much more plausible values.

3.5.Changes in other colour aspects

During the experiments, it was noted that the slices of tomatoes became translucent after 2 – 4 days of storage depending on the combination maturity stage-storage temperature. This change from opacity to translucency is important in describing the quality and acceptability of sliced tomatoes. The problems associated with the colour measurement of translucent samples have been reported by Hutchings (1994) and MacDougall (2002). Besides measurement of colour, some measure of light scattering, e.g. Kubelka-Munk analysis, is required for adequate determination of colour appearance.

The occurrence of translucency or water-soaked areas in the pericarp of fresh-cut tomato was usually indicated as a symptom of chilling injury (Hong and Gross, 1998; Gil *et al*., 2002), rather than a result of wound injury. This was not confirmed by the present results since translucency was also observed at 12 and 16 ^{o}C, a range of temperatures not considered to cause chilling injury.

Whether this translucency affected the colour measurements in the present experiment is not known. Studies are now being conducted to study and quantify this phenomenon, and will soon be reported.

4. Conclusions

A novel approach to model the changes in colour and appearance of minimally processed tomato has been proposed. The result obtained with the untransformed RGB variables is promising and further investigation of it is application for other colour spaces are object of current investigation.

All three colour aspects R, G and B changed according to a simple first order mechanism, incorporating the stage of fruit maturity at harvest and both the temperature and time of storage. Pooling data in an integral analysis, revealing that all three aspects shared a common rate constant and a common temperature dependence, proved to have the advantage of using all available information, theoretical as well as experimental. The system of applying a biological shift factor proved to be very powerful. It even enable to combined data on different physical aspects (colour aspects and firmness) and different stages of plant and fruit development in one large analysis.

Converting the basic RGB values into colour systems (HIS, CIELab) more related to human perception induces a sigmoidal behaviour. The RGB system apparently represents the colour primaries and changes in a much more simple fashion.

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CHAPTER 4

ASSESSMENT OF CHANGES IN OPTICAL PROPERTIES OF FRESH-CUT TOMATO USING VIDEO IMAGE ANALYSIS. EFFECT OF FRUIT MATURITY STAGE.

Submitted as: M. M. Lana; L.M.M. Tijskens; A. De Theije; O. van Kooten. Assesment of changes in optical properties of fresh-cut tomato image analysis. Effect of fruit ripening stage. April 2005.

Abstract

Tomato fruit *Lycopersicon esculentum* (cv Belissimo) were harvest at three stages of maturity, sliced in 7-mm thick transversal slices and stored at 5 ± 0.5 ºC. Intact control fruits were stored at the same conditions. Digital images were taken immediately after processing, before cooling, and after 1, 2, 3, 5, 7 and 9 days under storage, after placing the slice in a double (half white, half black) background. The data were expressed as the separate average intensities per pixel of Red (R), Green (G) and Blue (B) for the white background and for the black background separately. Additional index were obtained through simple algebraic calculations from the original RGB values and through the conversion of RGB into L*a*b* values. The main change in the appearance of fresh-cut tomato during storage was the development of translucent areas in the radial and outer pericarp. This process was highly influenced by the maturity stage of the fruit when processed and the more ripened the fruit the faster and the more intense was the development of translucency. Using video image analysis, an increase in translucency could be accessed by a decrease in the amount of Red pixels when the sample was measured against a black background. Changes in colour due to maturation could be measured both by a decrease in the amount of Green pixels or by an increase in the proportion of Red pixels (R/(R+G+B)), when the sample was placed on a white background. Using the L*a*b* colour space, an increase in translucency corresponded to a decrease in lightness and changes in colour due to maturation resulted in an increase in a* value.

Keywords: Lycopersicum esculentum; Minimally processed; colour; translucency; appearance.

1. Introduction

The change from opacity to translucency is an important aspect of appearance and quality in fresh-cut fruits and vegetables, which has to be considered when the keeping quality and acceptability of these products are evaluated. It happens in fresh-cut tomato (Artes *et al*., 1999; Hong and Gross, 2000; Gil *et al*., 2001; Aguayo *et al*., 2004; Jeong *et al*., 2004); melon (Bai *et al*., 2001; Saftner *et al*., 2003) pears (Abbott and Buta, 2002), papaya (O'Connor-Shaw *et al*., 1994) and watermelon (Perkins-Veazie and Collins, 2004).

Beside the importance of translucency as an intrinsic quality attribute, it can cause particular problems for the measurement of colour as reported by (Hutchings, 1994) and (MacDougall, 2002). The adequate determination of colour appearance of translucent samples must include some measure of light scattering (MacDougall, 2002).

Studies with translucent samples were first concerned with the measurement of the hiding power of a colorant layer, which has an inverse relation with the translucency of this layer. This led to the development of many methods based on the measurement of the reflectance of a sample over a white background (Wb) and over a black background (Bb) (Judd and Wyszecki, 1975). The ratio Bb/Wb is known as the contrast ratio and the reciprocal of this ratio is taken as the hiding power of the material. Further development of this idea resulted in the Kubelka-Munk analysis which proved to be a reliable technique to estimate translucency (Hetherington and MacDougall, 1992; MacDougall, 2002; Talens *et al*., 2002).

Despite of its usefulness and applicability in many different fields (Cortat, 2004), this technique has some limitations when used with non-homogenous materials such as fruit and vegetables

(Hetherington and MacDougall, 1992).

In recent years, the application of image processing techniques using a CCD camera to evaluate the quality of food and plant material has increased consistently (Du and Sun, 2004). It has been used to estimate aspects as different as the degree of browning in fresh-cut lettuce (Zhou *et al*., 2004), the effect of drying on shrinkage, colour and image texture of apple discs (Fernandez *et al*., 2005), the development of brown core and red core on chicory (Zhang *et al*., 2003), the maturity stage of tomato fruits (Choi *et al*., 1995) among others.

Images captured by a CCD camera are commonly saved in the RGB (Red, Green, Blue) colour space (Du and Sun, 2004). In the RGB colour space any particular colour can be specified by the amount of each of the primary components present. The RGB colour space is considered a good one to reproduce images in computer, television and video systems but it is non-linear with visual perception and the RGB values themselves cannot readily be interpreted in terms of visual perception of colour by humans. Some authors therefore propose the transformation of RGB values in other tristimulus values such as HSI (Choi *et al*., 1995) or CIELab (Mendoza and Aguilera, 2004) to obtain a colour specification that better resembles the way human beings perceive colour. However, other authors found high correlations between the perceived appearance or colour and untransformed RGB values. The colour of cucumber fruits was expressed as the ratio of the blue to the red intensity (B/R) by (Schouten *et al*., 1997) while changes in colour of strawberry during ageing were well expressed as 100/R (Schouten *et al*., 2002). Colour of spores and pollen during maturation defined a consistent and reproducible trend on the red versus green intensity graph (Yule *et al*., 1998).

The changes in the RGB colour aspects of fresh-cut tomato during refrigerated storage were previously reported by (Lana *et al*., in press-b). The overall appearance of the tomato slices changed during refrigerated storage and that was parallel to changes in the intensity of the individual aspects RGB. The main change in appearance was the development of translucency (water soaked areas) but it was not clear then, whether and how the translucency interfered in the colour measurements and the need of further investigation was indicated.

The main objective of the present work is to describe the changes that occur in the optical properties of fresh-cut tomato during refrigerated storage and how these changes are related with the stage of maturity of the fruit at harvest. Additionally the potential to use video image analysis to measure translucency is evaluated and the need to transform RGB values in Lab in order to properly express changes in optical properties in fresh-cut tomato is examined. For that, the basic principle underlying the Kubelka Munk analysis was considered and the samples to be measured were placed over a double background (one half black, one half white). The differences in colour on both backgrounds are expected to reflect the intensity of translucency of the sample. The measured RGB values and derived values obtained by simple algebraic calculations from RGB values were considered together with Lab values obtained after transformation of the RGB values. This study was restricted to changes in the pericarp tissue, to avoid the confounding effect of other tissues (columela, locular gel with seeds) with different optical properties.

2. Material and Methods

2.1. Harvesting and processing

Tomato fruit *Lycopersicon esculentum* (cv Belissimo) were harvest in a commercial greenhouse (Made, The Netherlands) in September 2004. The fruits were harvested on a single day, when at three stages of maturity 3, 6 and 9 (named here as I, II and III) according to the "kleur stadia tomaten" from The Greenery (www.thegreenery.com) and transported immediately after harvesting and selection to Wageningen (The Netherlands). The same day, the fruits were washed in cold tap water in a sanitised room and stored overnight at room temperature.

The next day, fruits similar in colour, shape and size were paired and numbered. One fruit was stored intact while the other was sliced in 7-mm thick transversal slices. The first and last slices were discarded and the central four were stacked in the same relative position they had in the fruit. Intact and sliced fruits were placed in a white polystyrene tray (138 mm x 138 mm x 25 mm) covered with a plastic film (Magnetron) and stored at $5 \pm 0.5\,^{\circ}C$. For each maturity stage × cutting (or intact) × storage time combination 6 replicates were analysed. Only the second slice from the bottom of the stack was used. This set-up ensured that slices were taken from the same position in the fruit in successive measurements. It also avoided the confounding effect of possible whitening of the cut surface of the slice in the top of the stack and the influence of the amount of leakage in the bottom of the tray in the intensity of watersoaking. Changes in optical properties could therefore be ascribed solely to the effect of treatment.

Digital images were taken immediately after processing, before cooling, and after 1, 2, 3, 5, 7 and 9 days under storage. Intact fruits were sliced immediately before evaluation, in the same way the other fruits have been sliced previously. Temperature data were recorded by an 8-channel thermocouple with a personal computer interface.

2.2. Video image analysis

The digital images were obtained using an image processing system consisting of a 3 CCD Hitachi HV-C20 video camera with a Tamron SP 35-80mm objective, a computer and a lightning chamber. The samples were placed under diffuse illumination provided by four fluorescent tube lamps (TL-D 18W/84) positioned in the higher part of the chamber.

The images were later analysed using the KAS Software, developed by Agrotechnology and Food Innovations, NL. A colour learn set was created to enable the distinction between the background and the tomato slice. The slices were placed in such a way that half of the slice was over a white background and half of the slice over a black background. Because the colour learn set used was not able to differentiate between translucent pericarp in black background and locular gel, the pericarp

area (including outer and radial pericarp) was separated from the rest of the image manually. The data were expressed as the separate average intensities per pixel of Red (R), Green (G) and Blue (B) for the white background (wb) and for the black background (bb) separately.

2.3. Variables derived from RGB values

Additional variables were calculated from the RGB values. Difference between the measurements on both backgrounds was obtained for each colour aspect (R-dif, G_dif and B_dif) subtracting the value obtained on black background from that obtained on white background. The proportion of each of the three primaries in relation to the sum of R, G, and B (relative R, G, B), on black (bb) and white (wb) background, were obtained through the formulas:

$$x\_bb = Xbb / (Rbb + Gbb + Bbb)$$

$$x\_wb = Xwb / (Rwb + Gwb + Bwb)$$

where X refers to R, G or B value and x to r, g or b.

The sum of RGB on each background was calculated as:

$$sRGB\_bb = Rbb + Gbb + Bbb$$

$$sRGB\_wb = Rwb + Gwb + Bwb$$

*2.4. Calculations of L*a*b* Coordinates*

The RGB values were converted into L*a*b* coordinates using the procedure described in Hunter and Harold (1987) and in Mendoza and Aguilera (2004).

2.5. Calibration with colorimeter

To verify the accuracy of the transformation of RGB into L*a*b* values a separated calibration experiment was performed. For that, 225 colour sheets with hues varying from white to black from Pantone Formula Guide Coated/Uncoated (2nd Edition, 2004) were photographed and analysed in the same way the tomato images were analysed. The same colour sheets were measured later using a portable Data Colour (Mercury 2000). CIE L*a*b* values were obtained directly from the colorimeter. Data from both measurements (calculated and measure L*a*b*) were further correlated.

2.6. Translucency visual evaluation

Before being photographed, each slice was analysed visually and graded according to the scale shown in Table 1.

To have conditions of illumination as uniform as possible in successive measurements analyses were performed at about the same time of the day and at the same place in the laboratory.

Table 1 –Translucency scale for visual assessment

Grade	*Description*
0	Not translucent.
1	Incipient translucency in the whole pericarp or translucent spots in the inner pericarp and/or outer pericarp.
2	Light translucency in the whole pericarp.
3	Moderate translucency in the whole pericarp.
4	Intense translucency in the whole pericarp.

2.7. Statistical analysis

Analysis of variance (ANOVA) for the effects of cutting, stage of maturity and storage time and their interactions for each variable was conducted using PROC GLM in SAS 8.0 as described by Hatcher and Stepanski (1994), followed by LSD test for the significant interactions. For the translucency grade only data from sliced fruits were analysed, since for intact fruits the grade was 0 for all samples. Because the grades did not show a normal distribution the effect of time and stage of maturity was analysed by Kruskal-Wallis One-Way Analysis of Variance (Siegel, 1956) in GENSTAT 7.2.

Correlations between the translucency grades and all measured and derived RGB and Lab values were performed by SPEARMAN Test while correlations between RGB and Lab values were done by PEARSON Test, both in SAS 8.0.

To identify variables that best discriminate for stage of maturity and for differences between sliced and intact fruits, a Stepwise Discriminant Analysis was performed, using a procedure available in GENSTAT 7.2.

3. Results

3.1. Changes in appearance of fresh-cut tomato during storage

The main change in the appearance of fresh-cut tomato during storage at $5^{O}C$ was the development of translucent areas in the radial and outer pericarp accompanied by shrinkage of the locular gel. In some slices the columela also became water-soaked, but because of the much more

compact structure of this tissue, it rarely became really translucent. Translucent slices looked redder and darker than non-translucent slices, especially when the columela was water soaked.

The development of translucency during storage was highly dependent on the interaction between stage of maturity at harvest and storage time (P <.0001). Both, the intensity and the temporal pattern of change depended on the stage of maturity of the fruit when processed (Fig. 1). Fruits processed at stage I showed a small increase in translucency until day 2 when it levelled off. Fruits at stage II showed a gradual increase up to 7 days storage. Fruits at stage III showed a sharp increase in the first day of storage followed by a small increase up to day 5 when it levelled off. The intensity of translucency at the end of the storage period was on average light, moderate and high respectively for stages I, II and III. So, it means that the riper the fruit the faster and the more intense was the development of translucency.

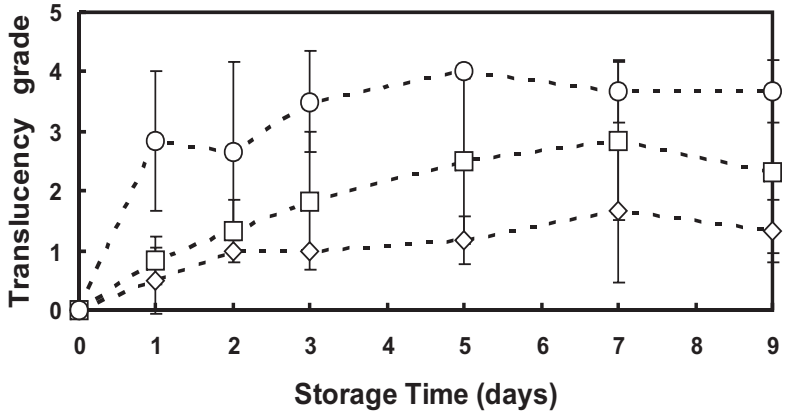

Figure 1 – Translucency grade of sliced tomato pericarp harvested at successive maturity stages I (◇), II (□) and III (○) and stored at 5°C. Points are the average of 6 samples ± SD.

3.2. Changes in measured RGB values during storage

The change in the appearance of the tomato slices during storage was accompanied by changes in the values of RGB (Fig. 2). Changes on time were mainly due to cutting (Table 2, for interaction cutting * time) and all RGB values decreased along storage for cut fruits while for intact fruits they did not change significantly or decreased at a lower rate. Changes on time also depended on the stage of maturity for some of the variables (Table 1, for interaction stage * time). The decrease in R_bb in cut fruit was more pronounced the more mature the fruit. The opposite interaction with maturity stage for observed for G_wb and G_bb. In this case, both decreased more, the less ripe the fruit.

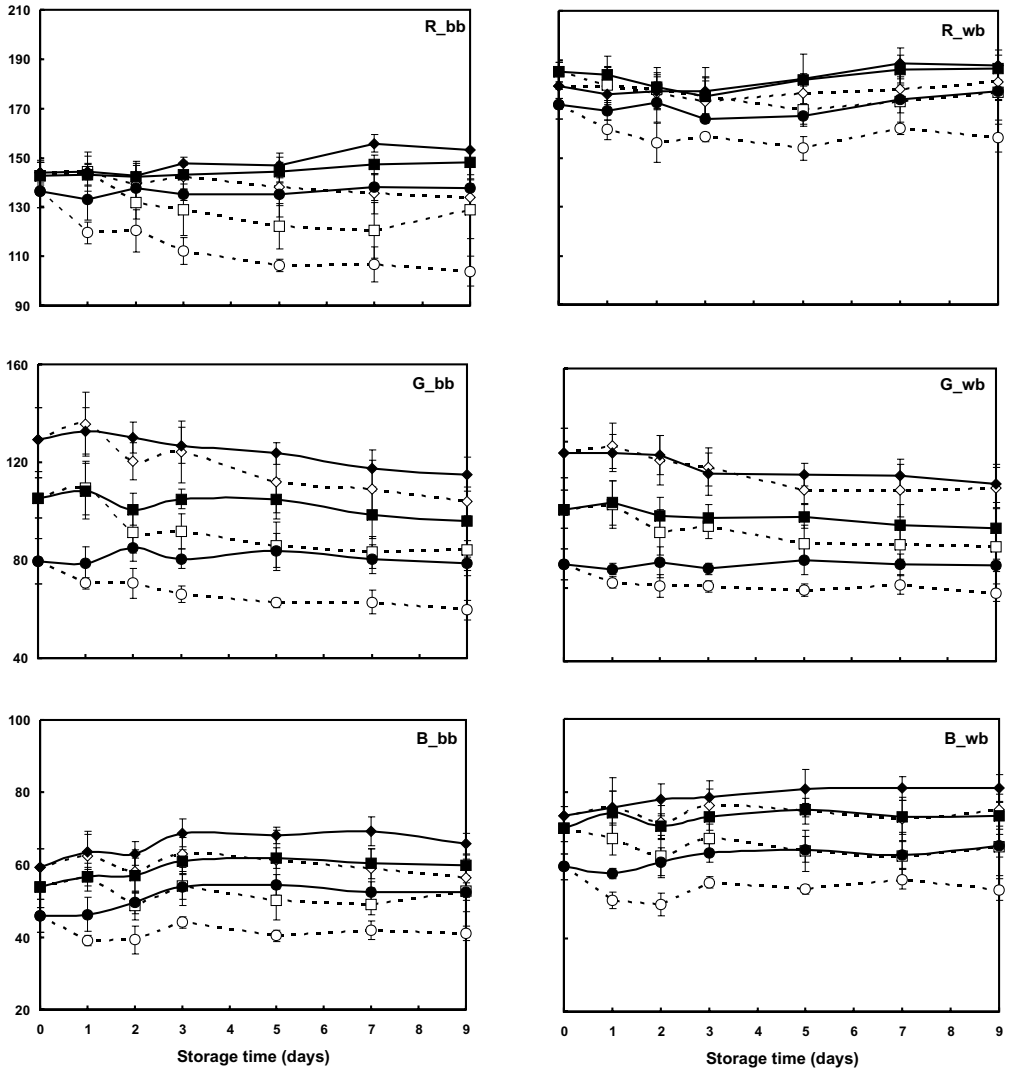

Figure 2 – Red (R), Green (G) and Blue (B) values on black (bb) and white (wb) background of intact (solid line and black symbols) and cut (dotted line and white symbols) tomato fruit harvest at successive maturity stages I (◆ ◇), II (■ □) and III (● ○) and stored at 5°C.

Although the three main effects and most of the two-way interaction were statistically significant (Table 2), the stage of maturity accounted for most of the variation in the RGB values (higher R^2). Exception is given to R_bb for which the effect of treatment and of maturity stage accounted for 31% and 26% of the observed variation.

Differences between both backgrounds were expressed in the absolute values of RGB (always higher at the white than at the black background). For G and B values about the same temporal pattern of change was observed on both backgrounds, while for R value changes on black background were more pronounced than on white background.

The three-way interaction was not statistically significant for any variable.

Table 2 – Analysis of variance for the effects of maturity stage, cutting (sliced x intact) and storage time on R (Red) G (Green) and B (Blue) measured values on white (wb) and black (bb) background. F values and R^2 (the percent of variance in the dependent variable that is accounted for by variability in the predictor variable) are shown for the sources of variation.

Variable	Statistics	Stage	Cutting	Time	Stage* Cutting	Stage* Time	Cutting* Time
R_bb	F value	170.23***	293.96***	7.16***	20.88***	3.46**	20.41***
	R^2	0.31	0.26	0.04	0.04	0.04	0.11
G_bb	F value	715.54***	87.31***	19.70***	4.67*	2.49**	5.65***
	R^2	0.74	0.05	0.06	0.00	0.02	0.02
B_bb	F value	315.51***	170.08***	5.66***	4.02*	1.45	8.18**
	R^2	0.56	0.15	0.03	0.01		0.04
R_wb	F value	121.7***	63.87***	7.64**	6.90**	1.39	4.67**
	R^2	0.39	0. 10	0.07	0.02		0.04
G_wb	F value	794.40***	29.49***	12.24***	4.33*	2.27**	2.45*
	R^2	0.81	0.02	0.04	0.00	0.01	0.01
B_wb	F value	402.89***	148.32***	3.27*	5.30	1.92*	5.96***
	R^2	0.64	0.12	0.02		0.02	0.03

***, **, *** indicates significance at $p < 0.05$, $p < 0.001$ and $p < 0.0001$ respectively**

3.3.Changes in derived RGB values during storage

The indexes obtained on the difference between both backgrounds (R_dif, G_dif and B_dif) were expected to express changes in translucency. However, there was no statistically significant effect of time for G_dif and no statistically significant effect of cutting and time for B_dif (Table 2), as it would be expected if they were related with translucency (results not shown). R_dif increased on time for cut fruits and remained practically constant for intact fruits (data not shown) without any significant interaction with maturity stage.

The relative R (r) and relative G (g) values were not influenced by cutting when measured on a black background (data not shown) and since they were not closely related with changes in colour or translucency (see items 5 and 7) were not further investigated.

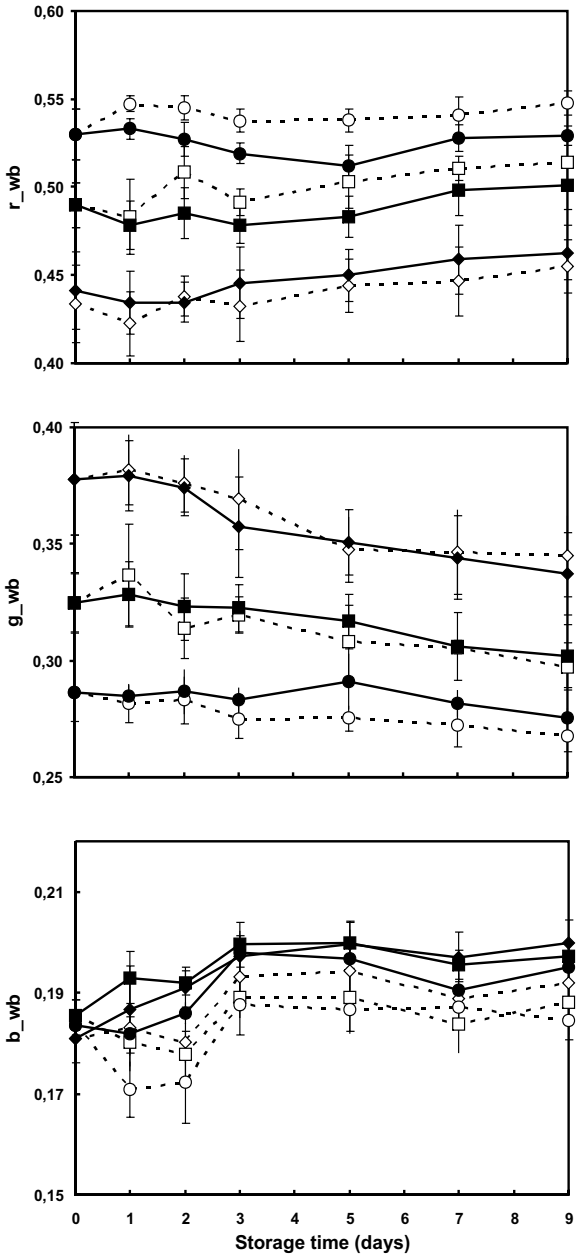

Figure 3 – Proportion of Red (r), Green (g) and Blue (b) values on white background (wb) of intact (solid line and black symbols) and cut (dotted line and white symbols) of tomato fruit harvest at successive maturity stages I (◆ ◇), II (■ □) and III (● ○) and stored at 5°C.

The values of relative Red (r_wb) increased while the values of relative Green (g_wb) decreased during storage (Fig. 3). Means of r_wb for the two-way interaction time x stage showed that the range of change in r_wb during storage was larger the less ripe the fruit. The same was observed for g_wb. The r_wb was also dependent on the interaction between cutting and stage of maturity. Cutting induced a significant increase in r_wb in fruits processed at stages II and III, while no statistical significant different was observed between cut and intact fruits at stage I.

Cutting induced a significant decrease in relative Blue (b) on both backgrounds. Although statistically significant for b_bb and b_wb, the interaction between storage time and treatment was not considered relevant in view of the trends showed in Fig. 3, and were not further investigated. The same applies for the interaction between maturity stage * time for b_wb.

In spite of the significant interactions described above most of the variation in the relative R and G values was accounted for by the stage of maturity, while storage time accounted for most of the variation in relative B values (see Table 2 for F and R^2 values).

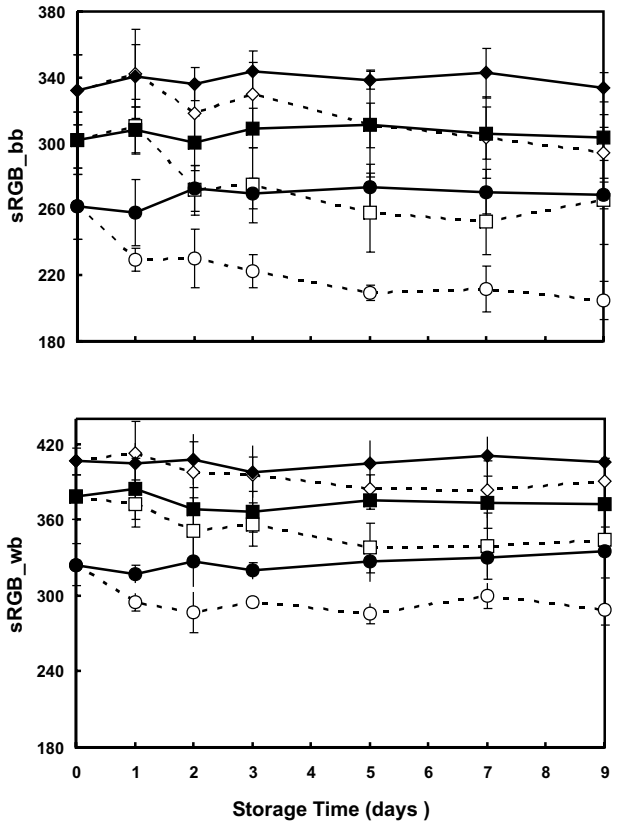

Figure 4 – Sum of Red (R), Green (G) and Blue (B) values on black (bb) and white (wb) background of intact (solid line and black symbols) and cut (dotted line and white symbols) of tomato fruit harvest at successive maturity stages I (◆ ◇), II (■ □) and III (● ○) and stored at 5°C.

Cutting induced a decrease in the sum of sRGB on both backgrounds (Fig. 4). There was a significant interaction between stage x cutting and time x cutting for both sRGB_bb and sRGB_wb (Table 3). Means for the interaction stage x cutting indicate that both variables decreased from stages I to III and were smaller for cut compared to intact fruits. Means for the interaction cutting x time indicated a significant effect of cutting from day 2 on for sRGB_bb and from day 5 on for sRGB_wb.

The three-way interaction was not statistically significant for any variable.

Differences between white and black background were less pronounced for the calculated relative RGB values than for the measured RGB values in what concerns both trends on time and absolute values.

Table 3 – Analysis of variance for the effects of maturity stage, treatment cutting and storage time on derived RGB values on white (wb) and black (bb) background. F values and R^2 (the percent of variance in the dependent variable that is accounted for by variability in the predictor variable) are shown for the sources of variation.

Variable	Statistics	Stage	Cutting	Time	Stage* Cutting	Stage* Time	Cutting* Time
R-dif	F value	13.08***	55.39***	5.47***	2.55	1.02	4.23**
	R^2	0.07	0.15	0.09			0.07
G_dif	F value	39.01***	12.49**	1.37	0.20	0.95	1.07
	R^2	0.24	0.04				
B_dif	F value	5.13*	0.73	1.82	1.44	0.67	1.00
	R^2	0.04					
r_bb	F value	731.47***	3.60	8.98***	0.25	5.74***	3.08*
	R^2	0.80		0.03		0.04	0.01
g_bb	F value	741.19***	0.17	33.79***	1.08	4.52**	1.59
	R^2	0.75		0.10		0.03	
b_bb	F value	5.38***	25.35***	95.19***	1.46	3.17**	2.96*
	R^2	0.01	0.03	0.064		0.04	0.02
r_wb	F value	852.64***	34.67***	10.40***	5.41	3.47**	1.81
	R^2	0.82	0.02	0.03		0.02	
g_wb	F value	743.48***	1.33	21.39***	2.96	3.05**	0.69
	R^2	0.79		0.07		0.02	
b_wb	F value	20.82***	182.38***	47.96***	4.31*	2.98**	6.06***
	R^2	0.05	0.22	0.35	0.01	0.04	0.04
sRGB_bb	F value	480.32***	197.52***	7.50***	10.40***	1.55	11.85***
	R^2	0.63	0.13	0.03	0.01		0.05
sRGB_wb	F value	568.84***	80.04***	4.21**	6.83**	1.18	4.72***
	R^2	0.75	0.05	0.02	0.01		0.02

***, **, *** indicates significance at $p < 0.05$, $p < 0.001$ and $p < 0.0001$ respectively**
R-dif, G_dif and B_dif refers to the differences in R(Red) , Green (G) and Blue (B) values on white and black background.
r, g, b refers to the values of R, G and B averaged divided by the sum of RGB.
sRGB refers to the sum of R + G+ B.

3.4. Changes in L a* b* values during storage*

Changes in L*_bb during storage were dependent on the interaction between stage x cutting ($p < .0001$) and time x cutting ($p < .0001$). The L*_bb of cut fruits decreased and the rate of decrease was significantly higher the riper the fruit, while it did not change for intact fruits. L*_wb was systematically higher than the correspondent L*_bb for each maturity stage x cutting x storage time combination (Fig. 5). Cutting also induced a decrease in L*_wb but the differences were much smaller than those observed for the black background and statistically significant for stages II and III but not for stage I. The initial value of both L_wb and L_bb was lower the less ripe the fruit.

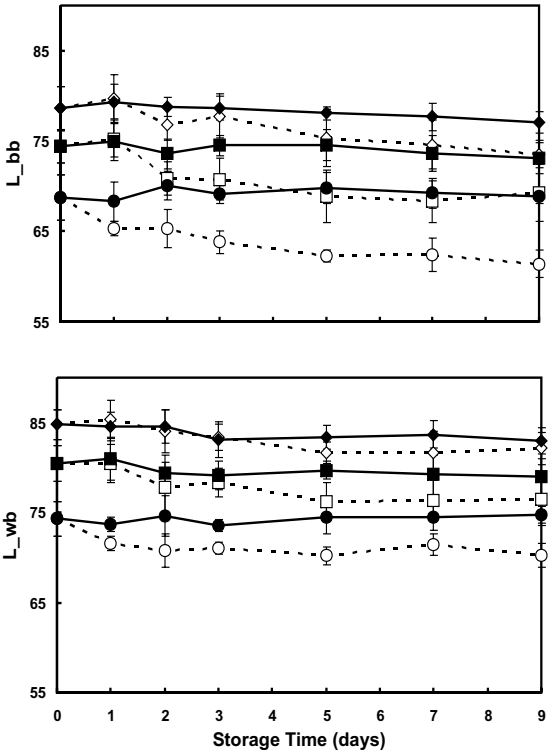

Figure 5 – L* value on black (bb) and white background (wb) of intact (solid line and black symbols) and cut (dotted line and white symbols) of tomato fruit harvest at successive maturity stages I (◆ ◇), II (■ □) and III (● ○) and stored at 5ºC.

The a* value increased along storage for stages I and II and hardly changed for stage III, indicating that even at temperature as low as 5 ^{0}C the fruits at stages I and II became redder during storage (Fig. 6). No significant effect of cutting was observed and the same temporal pattern of changes was observed for both cut and intact fruits (Fig. 6).

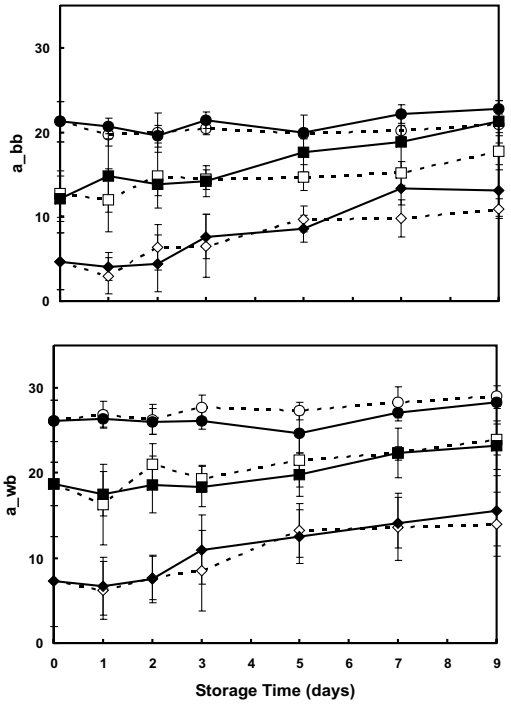

Figure 6 – a* value on black (bb) and white background (wb) of intact (solid line and black symbols) and cut (dotted line and white symbols) of tomato fruit harvest at successive maturity stages I (◆ ◇), II (■ □) and III (● ○) and stored at 5°C.

The interactions stage of maturity x treatment and treatment x storage time were statistically significant for b*_bb and b*_wb. However, it is clear from Fig. 7 that about the same temporal pattern of change is observed for each combination maturity stage x treatment, and only the effect of cutting in reducing b_bb was considered relevant. In view of that, these interactions were not further investigated.

3.5. Correlations between L a* b* values and RGB values*

The L* value was highly correlated ($R^2 = 0.99$) with the sum of R, G and B in each respective background (Fig. 8). L* value was also highly correlated with the individual values of Green ($R^2 = 0.99$ and $R^2 = 0.98$ respectively for white and black background) and moderately correlated with the individual values of Red ($R^2 = 0.73$ and $R^2 = 0.86$ respectively for white and black background). However, in this case, not only the coefficient of correlation was lower but also the spread of the data was higher, compared with the sum of RGB.

The a* value was highly positively correlated with the relative Red value (r), on both backgrounds ($R^2 = 0.98$ and $R^2 = 0.96$ respectively for white and black background)and highly negatively correlated with the relative Green value (g) on both backgrounds ($R^2 = 0.99$ and $R^2 = 0.99$ respectively

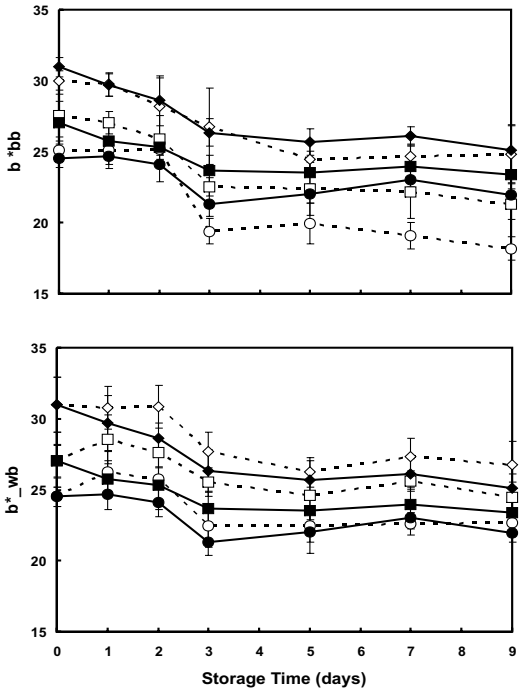

Figure 7 – b* value on black (bb) and white background (wb) of intact (solid line and black symbols) and cut (dotted line and white symbols) of tomato fruit harvest at successive maturity stages I (◆ ◇), II (■ □) and III (● ○) and stored at 5°C.

for white and black background) (Figs. 9-10). This indicates that both the changes in the proportion of R and G reflects the changes that happens in the a* axis.

3.6. Correlations between L a* b* values and translucency*

The translucency grade presented a moderate correlation with the L* _bb (R^2= 0.50). When the three stages of maturity were analysed separately the coefficient of correlation increased with maturity stages from 0.52 for stage 1 to 0.84 for stage III.

3.7. Correlations between RGB values and translucency

Among all the measured and calculated RGB variables, the one with highest correlation with translucency was the R_ bb (R^2 = -0.69). The correlation between translucency and the sRGB_bb was slightly smaller (-0.55) than that presented by R_bb. As observed for L*_bb the coefficient of - 0.74 and -0.87 respectively for stages I, II and III. The coefficient of correlation between translucency and the sRGB_bb was -0.57, -0.69, -0.87 respectively for stages I, II and III.

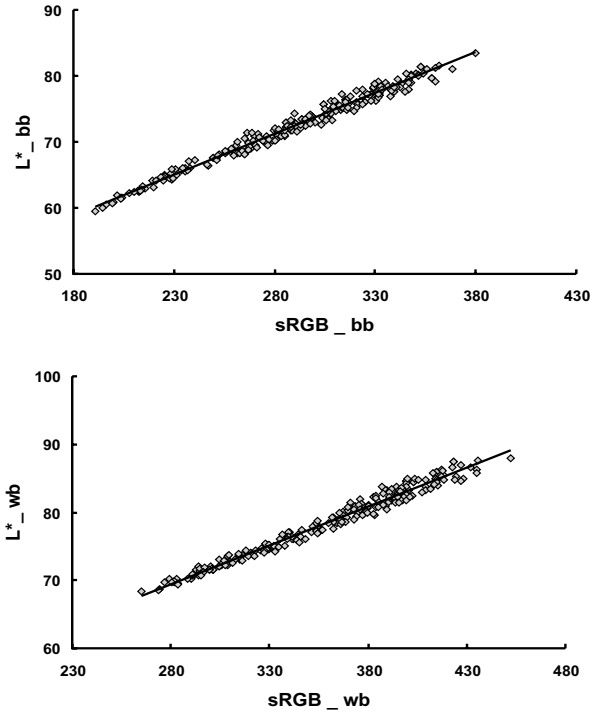

Figure 8 – Correlation between L* value and the sum of RGB values of cut and intact tomato pericarp harvested at 3 different maturity stages and stored at 5°C, measured over a black (bb) or white (wb) background.

3.8. Discriminant variables for stage of maturity and cutting (sliced vs intact fruits)

The results obtained with the stepwise discriminant analysis indicated R_bb as the most discriminatory variable to differentiate sliced from intact tomatoes. R_bb was also the variable that best discriminated between the scores for translucency when the complete data set was considered. When only cut samples were analysed, since the score for translucence is zero for practically all the intact samples, again R_bb was the most discriminatory variable for treatment.

The discrimination between stages by other hand was attained using a white background. Two variables, r_wb and G_wb, showed to have about the same discriminant power as indicated by the Wilk's Lambda index, respectively 0.1800 and 0.1868. The following most discriminant variables were L_wb and a*_wb, respectively with indexes equal to 0.2020 and 0.2125. When only intact fruits were analysed, so no possible confounding effect of translucency was present, maturity stages could better be discriminate by L_wb (0.1522) and G_wb (0.1574).

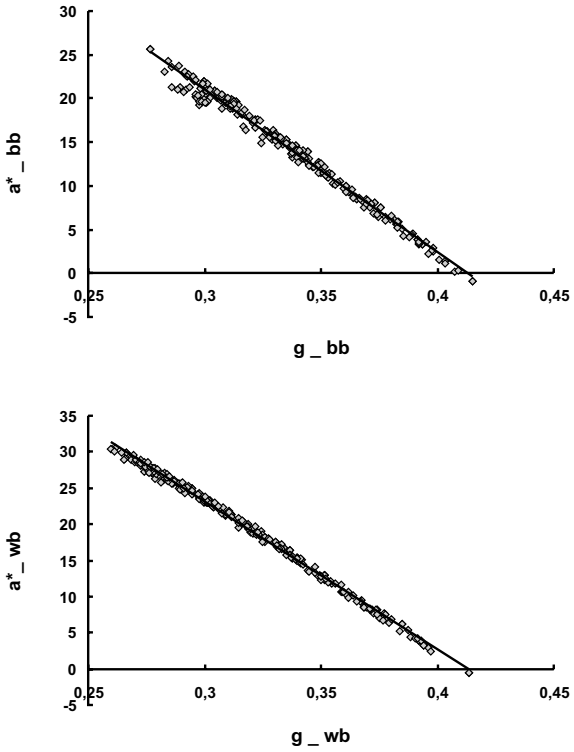

Figure 9 – Correlation between a* value and the relative G values (g) of cut and intact tomato pericarp harvested at 3 different maturity stages and stored at 5°C, measured over a black (bb) or white (wb) background.

*3.9. Correlation between calculated and measured L*a*b* values*

A high correlation was observed between the L*a*b* values of the Pantone charts measured using a colorimeter with the L*a*b* values converted from the RGB values of the digital images of the same Pantone charts (Fig. 11). The calibration using Pantone charts showed that L*a*b* values obtained from the calculation of the RGB values are very close in magnitude and highly correlated to those obtained using a colorimeter

4. Discussion

4.1. Relation between translucency development and maturity stage of the fruit at harvest

The processing of tomato fruits into transversal slices followed by refrigerated storage induced modifications in the optical properties of the tomato tissue. The main change in appearance

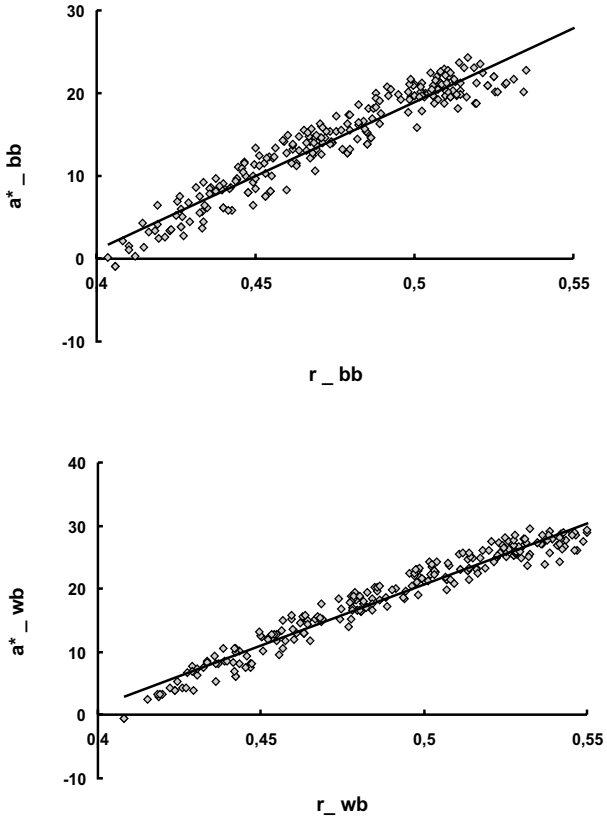

Figure 10 – Correlation between a* value and the relative R values (r) of cut and intact tomato pericarp harvested at 3 different maturity stages and stored at 5^{o}C, measured over a black (bb) or white (wb) background.

was due to change from opacity to translucency in the pericarp. The extent of development of translucency was higher in slices obtained from more ripened fruits. The same relation between maturity and translucency was reported by (Jeong *et al*., 2004). They also observed that watersoaking was consistently more rapid and severe in slices derived from the physiologically older blossom portion compared with the stem end of the fruit.

Translucency in the pericarp of fresh-cut tomato is most certainly due to the replacement of gas in the intercellular space by liquid. The pericarp tissue of mature green fruit has a compact structure with very small intercellular spaces. With maturity, the apoplastic volume in the pericarp increases (Damon *et al*., 1988; Hetherington and MacDougall, 1992) which results in an increase in translucency with maturity even in intact fruits (Hetherington and MacDougall, 1992).

Watersoaking of the flesh tissue in fresh-cut papaya (Karakurt and Huber, 2003) and fresh-cut pears (Soliva-Fortuny *et al*., 2002) as a consequence of wound injury, was ascribed to an enhancement in the activities of membrane and cell wall hydrolases with consequent leakage of cellular contents to the intercellular space. This model is consistent with a higher susceptibility of

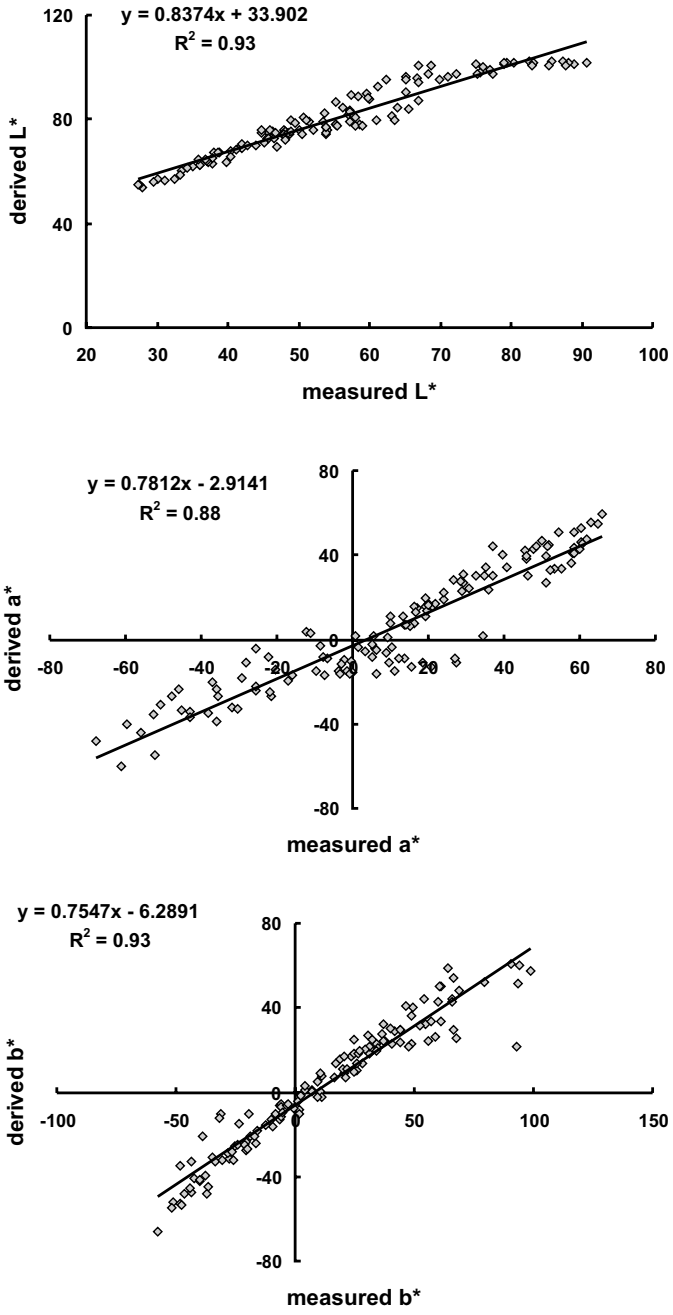

Figure 11 – Correlation between measured (using a colorimeter) and calculated (from RGB –Red-Green-Blue values obtained form Video Image Analysis) L* (top) a* (middle) and b* (bottom) values of Pantone Chart Colour.

more ripened fruits to translucency, since the enzymes activated by wounding are the same that are enhanced by maturation.

Results obtained by Lana *et al*.(in press-a) indicate that the liquid that floods the intercellular space in tomato pericarp is coming, not only from damaged cells, but also from the locular gel. In this case, the effect of stage of maturity would be simply a physical one. Because the intercellular space is higher in ripened fruits, the extent of the water flooding is larger. The tissue becomes more homogenous causing less distortion in the light pathway through the tissue and consequently reducing light scattering and making the sample looks translucent. Whether both processes occurs simultaneously or not remains to be elucidated.

4.2. Changes in colour

Fruits processed when partially ripe underwent changes in colour, with an increase in redness. The same magnitude and the same temporal pattern of changes was observed for fruits stored intact and sliced when colour was expressed as a* value. When expressed as r_wb, there was a small but significant increase in redness for cut fruits at stages II and III compared to intact fruits. This effect of cutting was not observed for g_wb. If this increase in redness was due to accelerated maturation it would be expected to happen for stage I (partially ripe) and not to stage III (fully ripe) and to be parallel to changes in the values of relative Green value. It is suggested that this change is due to translucency and not to maturation. Changes in the refractive index and homogeneity of the tissue (as is the case with translucent pericarp) have a great impact in the perceived colour even when there is no alteration in the tissue pigment composition or concentration.

So, it seems that the wound injury resulting from processing did not impair nor did accelerate the maturation process of sliced tomato tissue during refrigerated storage at 5^{o}C. Evidence that wound injury does not alter the rates of pigment degradation and synthesis during maturation were previously reported by Campbell *et al*. (1990). In tomato pericarp discs the colour developed in a pattern similar to intact fruit, indicating that the regulatory mechanisms involved in this process, are local and are retained in the pericarp discs. On the other hand reported an increase in ethylene production by tomato pericarp discs as a response to wound injury, what is expected to accelerate maturation compared to intact fruits. However, in that case the discs were kept at 20^{o}C, while in the present study the slices were kept at 5^{o}C. It is well known that the increase in respiration rate and ethylene production rate associated with wounding are minimized when the product is kept at low temperature (Brecht, 1995).

4.3. Assessment of translucency and colour using video image analysis

Using video image analysis, an increase in translucency could be accessed by a decrease in the amount of Red pixels when the sample was measured against a dark (black) background. The decrease in the values of R_bb happens because the more translucent the sample the higher the

proportion of light that is transmitted through the sample and absorbed by the black background, and is consequently, not reflected back.

Contrary to what was hypothesized, the difference in the colour aspects in white and black background was not a good estimator of translucency intensity. The lack of significant effect of storage time on the values of G-dif and B_dif clearly indicates that they are not good estimators, since the translucency increased significantly along storage. Besides that, B_dif was not influenced by cutting. The temporal pattern of changes in R_dif resembled that of translucency in the way that it increased for cut but not for intact fruits. However, the lack of interaction between stage*treatment and between stage*time indicates that R-dif is also not an adequate variable to estimate translucency, known to be dependent on both interactions.

Using the L*a*b* colour space an increase in translucency corresponded to a decrease in lightness. The appearance of translucent areas in cut cantaloupe melon was also related to a decrease in lightness (L*) (Bai *et al*., 2001; Portela and Cantwell, 2001; Saftner *et al*., 2003). Decrease in L* value indicates that the sample is becoming less bright and/or darker. The lower L value in black background can particularly be related with translucency because the translucent slices will look darker red on a black background what should be reflected in a lower L*.

The relation between translucency and R_bb was consistent through all the presented data analyses. The ANOVA indicated that R_bb was the variable most affected by cutting. About 26% of the variance in R_bb was accounted for by cutting, while for the other variables it was much smaller and never higher than 15% (Table 1). R_bb was the variable with a higher correlation with translucency intensity. The results of the discriminant analysis validated those obtained from the ANOVA and from the correlation tests. So, although in the ANOVA other variables were equally affected by the single effect of cutting, and in the correlation tests other variables were also highly correlated with translucency, intact (non-translucent) and cut (translucent) samples mainly differed from each other in relation to the values of R_bb.

In spite of these results, it was not possible to predict the translucency intensity using this variable, due to the large overlapping observed for different translucency classes. Possible reasons for that are:

- The intensity of translucency was graded with a numerical scale of 5 points that accounted only for large visual differences while the measurements of RGB were on a continuous scale.
- The scale used is probably not linear, and in this case an increase from 1 to 2 in translucency will not correspond to a same change in R_bb value as when the translucency increases from 3 to 4.
- Part of the variation in R_bb* is due to the colour of the sample and not to translucency and it is not clear how one process is interfering in the other.
- The backgrounds used were not perfectly white or perfectly black; what implies that part of the light transmitted through the samples was reflected back by the black

background, and absorbed by the white background, resulting in underestimation of the difference between translucent and opaque samples.

Although a quantification of translucency could not be achieved, the temporal pattern of changes in R_bb as in function of the treatments applied, reflected those observed for the translucency development. In view of that, the potential for the use of video image analysis for the assessment of translucency and colour in fleshy translucent tissue is indicated.

On other hand, to obtain information about colour the use of a white background is required. Again, the indication that r_w and g_wb were the two variables most related with change in colour from green to red was consistent through all the data analyses procedures.

*4.4. Conversion of RGB into L*a*b* values*

The conversion of RGB values into L*a*b* values was shown to be reliable in view of the calibration with the Pantone charts. However, to study temporal pattern of changes in colour and translucency of fresh-cut tomatoes, the RGB values were more discriminate for treatment and stage. By analysing changes in RGB values it was possible to identify clearly the factors that affected the appearance (maturity stage of the fruit, storage time and cutting). Therefore no conversion to CIELAB colour space was necessary, since the same conclusions could be drawn when assessing RGB and L*a*b* values.

Problems in measuring the colour of Pantone charts were observed with a few charts with high L value and very light and bright colours. These charts had RGB values of 0 when measured using video image analysis. Because these colours did not represent the colours of the tomato slices, this was not considered a problem in the present study, and these values were not considered in the correlation. However, it indicates that the system used here is not a universal one and is unable to measure the same range of colours measured by a colorimeter.

5. Conclusions

The changes in the appearance of sliced tomato during storage at 5 °C were due to changes in colour and development of translucency. Changes in colour due to maturation were more pronounced the more immature the fruit. These changes, represented by changes in the a* axis from green to red, could be assessed by a decrease in the amount of Green pixels or an increase in the proportion of Red pixels when the sample was measured against a white background. These changes were of low range in view of the low temperature at which the fruits were stored, and were not affected by cutting.

Changes in translucency on the other hand were caused by cutting and were more pronounced the riper the fruits. Although not related to production or degradation of pigments developing translucency also gives the appearance of more redness. An increase in the development of translucency

could be assessed by a decrease in lightness or by a decrease in the amount of Red pixels, when the sample was measured against a black background.

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CHAPTER 5

EVALUATION OF CHANGES IN OPTICAL PROPERTIES OF FRESH-CUT TOMATO USING VIDEO IMAGE ANALYSIS. EFFECT OF STORAGE TEMPERATURE.

Submitted as: M. M. Lana; L.M.M. Tijskens; M. Hogenkamp; O. Van Kooten. Assesment of changes in optical properties of fresh-cut tomato image analysis. Effect of storage temperature. April 2005

Abstract

Tomato fruit (*Lycopersicon esculentum* cv Belissimo) were harvested at the light-red stage, sliced in 7-mm thick transversal slices and stored between 5º and 13 ºC. Intact fruits, used as controls, were stored at the same conditions. Digital images from the slices were taken immediately after cutting, before cooling, and then every day up to 4 days after storage. To be able to evaluate both colour and translucency, the slices were placed in such a way that half of the slice was over a white background and half the slice over a black background. The values were expressed as the averaged amount of Red (R), Green (G) and Blue (B) pixels on white and on black background. Additional variables were calculated from the RGB values. Also the RGB values were converted in L*a*b* values. Before being photographed, each slice was analysed visually and graded according to the intensity of translucency. Translucency was the main change in appearance of cut tomato and it appeared within 24 hours after processing, independent of storage temperature. The increase in translucency in the pericarp tissue of cut fruits was highly correlated with a decrease in the value of R_bb i.e. the Red value with a black background (R^2 = -0.86) and a decrease in the sum of RGB also on black background (R^2 = -0.85). In the L*a*b* colour space, the translucency development was related to a decrease in lightness (L* value). During storage, the sliced tomatoes became slightly but significantly redder than the intact fruits, what could be expressed as an increase in a* value or both an increase in the amount of relative red (R/(R+G+B+) and a decrease in the amount of relative green (G/(R+G+B+). The increase in redness due to processing was considered mainly a result of translucency and not a result of enhanced maturation.

Keywords: Lycopersicum esculentum; Minimally processed; colour; translucency; appearance, wound injury, water-soaking, quality.

1. Introduction

The most studied changes in the colour and appearance of fresh-cut fruits and vegetables are those due to the production or degradation of pigments (Bolin and Huxsoll, 1989; Heaton and Marangoni, 1996; Gonzalez-Aguilar *et al*., 2000; Jamie and Saltveit, 2002; Loaiza-Velarde *et al*., 2003; Rocha and Morais, 2003). Additionally, minimal processing can induce changes in the refractive index and in the homogeneity of the tissue. These physical changes induce the development of translucency and have a great impact on colour and appearance although they do not involve changes in pigment composition or concentration. This seems to be particularly important for fleshy tissue such as tomato ; melon (O'Connor-Shaw *et al*., 1994; Bai *et al*., 2001; Portela and Cantwell, 2001; Aguayo *et al*., 2004a); papaya (O'Connor-Shaw *et al*., 1994) pears (Abbott and Buta, 2002; Soliva-Fortuny *et al*., 2002), and persimmon .

The occurrence of translucency or water-soaked areas in the pericarp of tomato fruit was first indicated as a symptom of chilling injury , rather than a result of wound injury, probably as a reference to what is known for intact fruits. The appearance of water-soaked areas in intact tomato fruits stored under low temperature was attributed to enhanced permeability of the cell membrane due to chilling injury. However, the translucency of tomato flesh as a result of chilling injury is visible only after removal of the fruit from chilling temperature while in the previously mentioned reports on fresh-cut tomato, the development of translucency occurred during refrigerated storage.

Some reports indicate that the development of water-soaking in cut tissue can be caused by other factors than low temperature and be attributed primarily to a response to wounding. The severity of wounding affected the development of translucency in cut melon fruit stored at 5 °C . Pieces of melon prepared with a sharp bore presented good or very good overall visual quality after 6 days storage, while those prepared with the blunt borer were on or below the limit of marketability due to translucency . observed translucency in excised pericarp tomato discs kept at 20-25°C, 2 days after preparation of the discs and reported the appearance of translucency in sliced tomato stored at 12°C and 16°C.

To measure the intensity of translucency in fresh-cut products the mostly used approach has been visual evaluation (O'Connor-Shaw *et al*., 1994; Bai *et al*., 2001; Portela and Cantwell, 2001; Gil *et al*., 2002; Bai *et al*., 2003; Jeong *et al*., 2004). The Kubelka-Munk analysis may be used to measure the absorption and scattering coefficients and hence the sample's optical properties by spectrophotometry (Hetherington and MacDougall, 1992; Hutchings, 1994). This technique found specific commercial use in the printing, painting and textile industries . Although it proved to be a reliable technique to estimate translucency in different plant and food products (Hetherington and MacDougall, 1992; MacDougall, 2002; Talens *et al*., 2002) it still presents limitations when used for non-homogenous samples such as fruits and vegetables (Hetherington and MacDougall, 1992).

In recent years, the use of image processing techniques using a CCD camera to evaluate the quality and appearance of food and plant material has increased considerably (Du and Sun, 2004). It has been used to estimate aspects of colour and appearance in products such as fresh-cut lettuce (Zhou *et al*., 2004), apple discs (Fernandez *et al*., 2005), chicory (Zhang *et al*., 2003), and tomato fruits (Choi *et al*., 1995) among others. The great interest in this technique lies among other reasons, in the possibility of application for non-homogenous samples and its non-destructive character.

The present study has two main objectives: to determine the influence of storage temperature on the development of translucency and to evaluate the potential use of video image analysis to measure translucency in sliced tomato fruit. For that, the basic principle underlying the Kubelka Munk analysis (Hutchings, 1994) was considered and the samples to be measured were placed over a double background (one half black, one half white).The differences in colour on both backgrounds are expected to reflect the intensity of translucency of the sample, while the measurements on white background are expected to reflect the colour without the interference of translucency. To avoid the confounding effect of other tissues (columela, locular gel with seeds) with different optical properties, the study was restricted to changes in the pericarp tissue.

2. Material and methods

2.1.Harvesting and processing

Tomato fruit (*Lycopersicon esculentum* cv Belissimo) were harvested in a commercial greenhouse in Berkel en Rodenrijs, The Netherlands in October 2004. The fruits were harvested when at stage of maturity 7 according to the "kleur stadia tomaten" from The Greenery (www.thegreenery.com) and transported immediately after harvesting and selection to Wageningen, The Netherlands. The same day, the fruits were washed in cold tap water in a sanitised room and stored overnight at room temperature.

The next day, fruits similar in colour, shape and size were sliced in 7-mm thick transversal slices. The first and last slices were thrown away and the central four were stacked in the same relative position they had in the fruit, in a white polystyrene tray (138 mm x 138 mm x 25 mm) covered with a plastic film (Magnetron) and stored at 5 ± 0.5, °C, 9 ± 0.7 °C or 13 ± 0.7 °C. Intact fruits, used as controls, were stored under the same conditions. For each temperature x treatment (intact or sliced) x storage time combination, 6 replicates corresponding to a tray with 1 fruit were analysed. Only the second slice from the bottom of the stack was used to avoid the possible confounding effect of whitening in the cut surface of the upper slice and excessive water-soaking in the bottom slice in contact with cell leakage and also to ensure that slices from the same position (stage of maturity) in the fruit were used. The evaluations were performed immediately after cutting, before cooling, and then every day up to 4 days of storage. Intact fruits were sliced immediately before evaluation, in the same way the other fruits have been sliced before. Temperature data were recorded by an 8 channel thermocouple with a personal computer interface.

2.2. Video image analysis

The digital images were obtained using an image processing system consisting of a 3 CCD Hitachi HV-C20 video camera with a Tamron SP 35-80mm objective, a computer and a lightning chamber. The samples were placed under diffuse illumination provided by four fluorescent tube lamps (TL-D 18W/84) positioned in the higher part of the chamber.

The images were later analysed using the KAS Software, developed by Agrotechnology and Food Innovations, NL. A colour learn set was created to enable the distinction between the background and the tomato slice. The slices were placed in such a way that half of the slice was over a white background and half the slice over a black background. Because the colour learn set used was not able to differentiate between translucent pericarp in black background and locular gel, the pericarp area (including outer and radial pericarp) was isolated from the rest of the image manually. The data were expressed as the separate average intensities per pixel of red (R), green (G) and blue (B) for the white background (wb) and for the black background (bb) separately.

2. 3. Calculations of RGB indexes

Additional variables were calculated from the RGB values.

Difference between the measurements on both backgrounds was obtained for each colour aspect (R-dif, G_dif and B_dif) subtracting the value obtained on white background from that obtained on black background.

The proportion of each of the three primaries in relation to the sum of R, G, and B (relative R, G, B), on black (bb) and white (wb) background, were obtained through the formulas:

$$x\_bb = Xbb / (Rbb + Gbb + Bbb)$$

$$x\_wb = Xwb / (Rwb + Gwb + Bwb)$$

where X refers to R, G or B value and x to r, g or b.

The sum of RGB on each background was calculated as:

$$sRGB\_bb = Rbb + Gbb + Bbb$$

$$sRGB\_wb = Rwb + Gwb + Bwb$$

*2.4 .Calculations of L*a*b* coordinates*

The RGB values were converted into L*a*b* coordinates using the procedure described in (Hunter and Harold, 1987) and used by (Mendoza and Aguilera, 2004).

In order to verify the accuracy of the transformation of RGB into L*a*b* values a separated calibration experiment was performed. For that, 225 colour sheets from Pantone Formula Guide Coated/ Uncoated (2nd Edition, 2004) with hues varying from white to black were photographed and analysed in the same way the tomato images were analysed. The same colour sheets were measured later using a portable Data Colour – Mercury 2000. CIE L*a*b* were obtained directly from the colorimeter. The results of this calibration were reported in (Lana *et al*., in press-b).

2.5. Translucency visual evaluation

Before being photographed, each slice was analysed visually and graded according to the scale shown in Table 1. Care was taken to perform the analysis at about the same time of the day and at same place in the laboratory, to have conditions of illumination as uniform as possible, in successive measurements.

Table 1 – Translucency scale for visual assessment

Grade	Description
0	Not translucent.
1	Incipient translucency in the whole pericarp or translucent spots in the inner pericarp and/or outer pericarp.
2	Light translucency in the whole pericarp.
3	Moderate translucency in the whole pericarp.
4	Intense translucency in the whole pericarp.

2.6. Statistical analysis

Analysis of variance for the effects of cutting, temperature, storage time and their interactions were done for each variable using PROC GLM in SAS 8.0 as described by (Hatcher and Stepanski, 1994), followed by LSD test for the significant interactions. Exception is given to the translucency grade. Because the grades did not show a normal distribution the effect of cutting, temperature and storage time was analysed by Kruskal-Wallis One-Way Analysis of Variance (Siegel, 1956) in SAS 8.0. Calculation of R^2 (the percent of variance in the dependent variable that is accounted for by variability in the predictor variable) was calculated according to (Hatcher and Stepanski, 1994).

Correlations between the translucency grades and all measured and derived RGB and Lab values were performed by SPEARMAN Test while correlations between all measured and derived RGB and Lab values were done by PEARSON Test, both in SAS 8.0.

3. Results

3.1. Translucency _ visual evaluation

The translucency of tomato slices increased during storage (P= <.0001) at all temperatures. The intensity of translucency was highly influenced by cutting (P <.0001) and did not depend on the temperature (P=0.2460). At all temperatures the same temporal pattern of change in translucency in cut fruits was observed, that is a sharp increase in the first day, followed by a small and almost linear increase afterwards (Fig. 1).

Few intact tomatoes showed small translucent spots in the pericarp (intensity always equal or lower than 1) but this was not related with storage time or storage temperature.

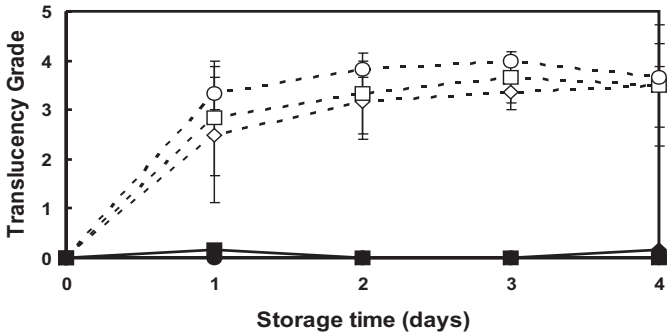

Figure 1 – Translucency intensity of intact (solid line and black symbols) and cut (dotted line and white symbols) light-red tomato fruit stored at 5 °C (◆ ◇) 9 °C (■ □) and 13 °C (● ○) for 4 days.

3.2. Changes in measured RGB values during storage

All RGB values, on both backgrounds, decreased during storage of cut fruits and remained practically constant for intact fruits (Fig. 2). All RGB values on white background were higher than the respective RGB values on black background. In general, differences between intact and cut fruits were more pronounced in black than in white background.

Although the effect of temperature was statistically significant for all variables, the variance accounted for by temperature was equal or lower than 2%, and consequently this effect was not considered relevant (Table 2). Equally from Fig. 2, it can be depicted that the effect of temperature was quite small, especially when compared with the effect of cutting. This holds true for both backgrounds.

The decrease in R, G and B on both backgrounds was mainly dependent on the interaction between cutting and storage time (P=0.0008 for G_wb and P <.0001 for all other variables). On black background, the R, G and B values decreased with time for cut fruits. A sharp decrease was observed 1 day after cutting, the same time necessary for the translucency in the slices pericarp tissue to become visible. R values did not change during storage for intact fruits, while G and B values showed a small but statistically significant decrease from day 3 on. On white background, the same temporal pattern of change was observed for all variables, but the changes were of lower magnitude than that observed on black background.

Interaction between cutting x temperature and the triple interaction cutting x temperature x storage time were not significant. Only for RGB values on white background the time x temperature interaction effect was statistically significant. Nevertheless, the time x temperature interaction means displayed no clear trend within each day or within each temperature and they are not presented.

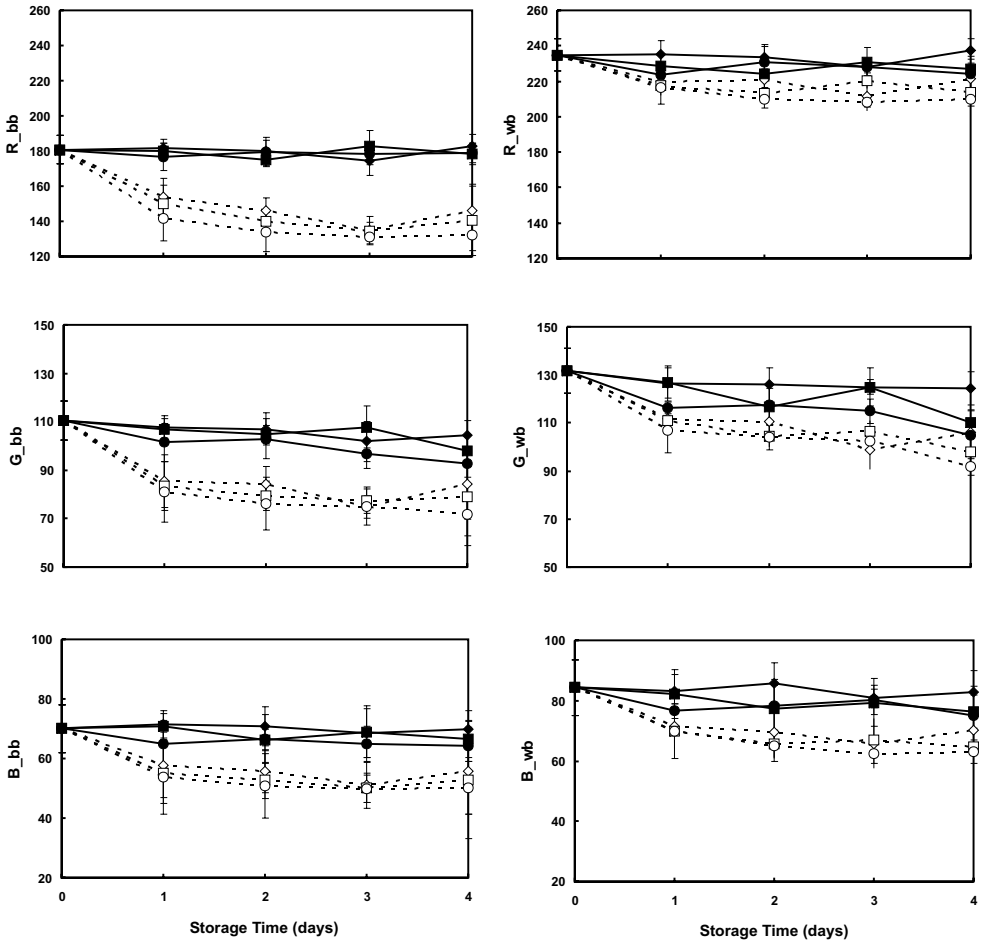

Figure 2 – Red (R), Green (G) and Blue (B) values on black (bb) and white (wb) background of intact (solid line and black symbols) and cut (dotted line and white symbols) of light-red tomato fruit stored at 5 ^{o}C (◆ ◇), 9 ^{o}C (■ □) and 13 ^{o}C (● ○) for 4 days.

3.3. Changes in derived RGB variables during storage

Differences between R, G and B values on both backgrounds were mainly affected by cutting and no significant effect of temperature was observed (Table 2). There was a two-way significant interaction in both R-dif and G_dif for storage time × temperature. The difference in Red value increased during storage for cut fruits and remained constant for intact fruits (Fig. 3). A significant increase in G_dif along storage was observed for cut fruits while for intact fruits a significant decrease occurred (data not shown). B_dif did not change on time and was not dependent on temperature (Table 2). A tendency for cut fruits to present higher B_dif values was observed but this effect was not consistent on time (data not shown).

Table 2 – Analysis of variance for the effects of temperature, cutting and storage time on R (Red) G (Green) and B (Blue) measured values on white (wb) and black (bb) background. F values and R^2 (the percent of variance in the dependent variable that is accounted for by variability in the predictor variable) are shown for the sources of variation.

Variable	Statistics	Time	Cutting	Temper	Time *Cutting
R_bb	F value	45.74***	565.61***	4.65*	38.38***
	R^2	0.17	0.52	0.01	0.14
G_bb	F value	41.50***	220.81***	6.22**	14.57***
	R^2	0.27	0.36	0.01	0.02
B_bb	F value	46.45***	407.76***	12.21***	26.56***
	R^2	0.21	0.46	0.01	0.03
R_wb	F value	23.44***	114.25***	9.41***	7.58***
	R^2	0.21	0.26	0.02	0.00
G_wb	F value	36.90***	71.95***	8.16**	5.01**
	R^2	0.34	0.17	0.02	0.01
B_wb	F value	63.04***	322.69***	16.25***	22.23***
	R^2	0.07	0.37	0.02	0.02

***, **, *** indicates significance at p < 0.05, p <0.001 and p < 0.0001 respectively**

The relative R, G and B values were not affected by cutting when measured on a black background (data not shown) and since they were not related with changes in translucency or colour (see item 5 and 6) they are not further discussed. When measured on a white background, cutting induced a statistically significant (P <.0001) increase in the relative R value and a decrease in the relative G (P=0.0001) and B value (P<.0001) comparably with intact fruits (Fig. 4). Although the level of significance was practically the same for all variables, cutting accounted for 21 % and 38% of the

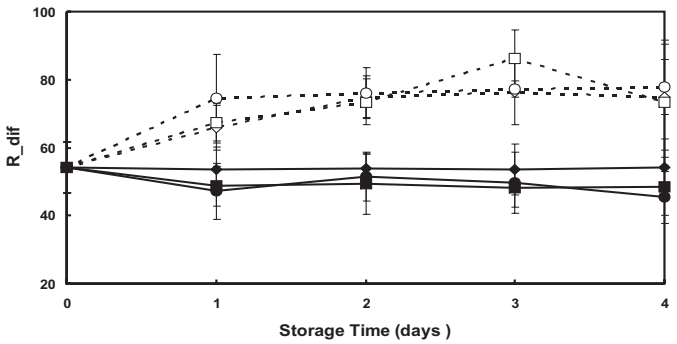

Figure 3 – Difference in Red values (R_dif) between white and black background of intact (solid line and black symbols) and cut (dotted line and white symbols) light-red tomato fruit stored at at 5 °C (◆ ◇), 9 °C (■ □) and 13 °C (● ○) for 4 days.

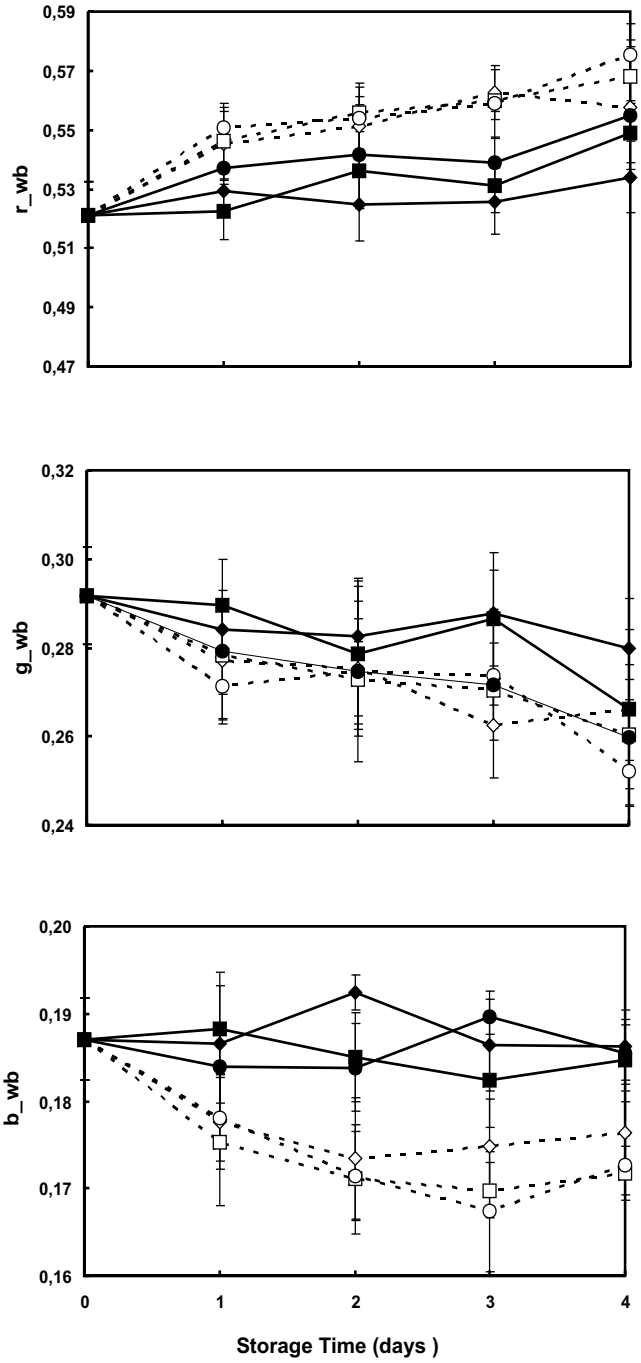

Figure 4 – Proportion of Red (r), Green (g) and Blue (b) values on white background (wb) of intact (solid line and black symbols) and cut (dotted line and white symbols) light-red tomato fruit stored at 5 °C (◆ ◇), 9 °C (■ □) and 13 °C (● ○) for 4 days.

variation in r_wb and b_wb respectively, but only 5% in g_wb. The single effect of temperature was significant for the three variables but since it accounted for less than 3% of the total variation it was not further investigated.

The sum of RGB decreased during storage on both backgrounds for cut and intact fruits (Fig. 5). There was a significant interaction between storage time and cutting (P<.0001) translated in a small but significant decrease in sRGB_wb and sRGB_bb for intact fruits and a much larger decrease for cut fruits. Differences between intact and cut fruits were of higher magnitude on black background.

LSD means for the main effect of temperature indicated no significant differences for sRGB_bb. The sRGB_wb was significantly higher at 5 °C compared to 13 °C.

*3.4. Changes in derived L*a*b* variables during storage*

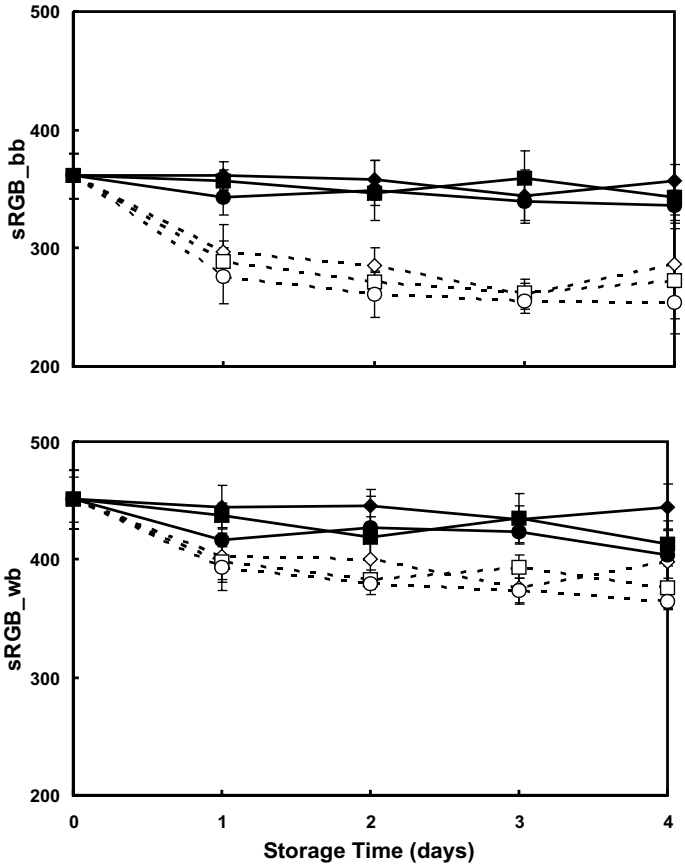

Figure 5 – Sum of Red (R), Green (G) and Blue (B) values on black (bb) and white (wb) background of intact (solid line and black symbols) and cut (dotted line and white symbols) light-red tomato fruit stored at 5 °C (◆ ◇), 9 °C (■ □) and 13 °C (● ○) for 4 days.

There was a significant two-way interaction (P <.0001) between storage time and cutting for L* values on both backgrounds. L_bb and L_wb decreased along storage time for both intact and cut fruits, but at a higher rate for cut fruits, which presented lower lightness than intact fruits throughout storage. Difference between cut and intact fruits was more pronounced on black background. A rapid decrease in L*_bb of cut fruits occurred in the first day after cutting, followed by a smaller decrease until day 3. Intact fruits presented a very small but significant decrease in lightness on day 3 (Fig. 6).

The means of L_bb for the main effect of temperature were not significantly different from each other, while L_wb at 13ºC was significantly lower than at 5ºC.

The changes in a*_wb were mainly due to the effect of storage time (P<.0001) and cutting (P=0.0001) and to a lesser extent to the effect of temperature (P=0.0259). No interaction between these factors was significant, meaning that a*_bb increased on time for all temperatures and for both cut and intact fruits in the same way (Fig. 7).

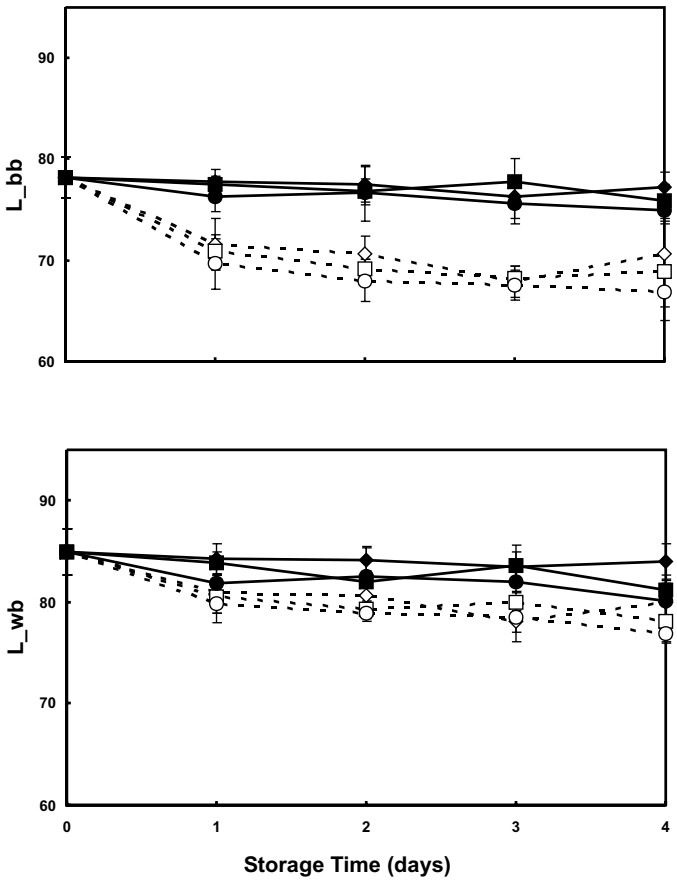

Figure 6 – L* value (lightness) on black (bb) and white (wb) background of intact (solid line and black symbols) and cut (dotted line and white symbols) light-red tomato fruit stored at 5 ºC (◆ ◇), 9 ºC (■ □) and 13 ºC (● ○) for 4 days.

Cutting induced an increase in a*_wb, meaning that the sliced fruits became redder compared with intact fruits. Means of a*_wb for the main effect of temperature (P <.0001) were slightly but significantly higher at 13°C than at 5°C. Means for the interaction storage time × treatment (P <.0001) showed that for both cut and intact fruits the value of a*_wb increased during storage, but this increase was faster and higher for cut fruits (Fig. 7).

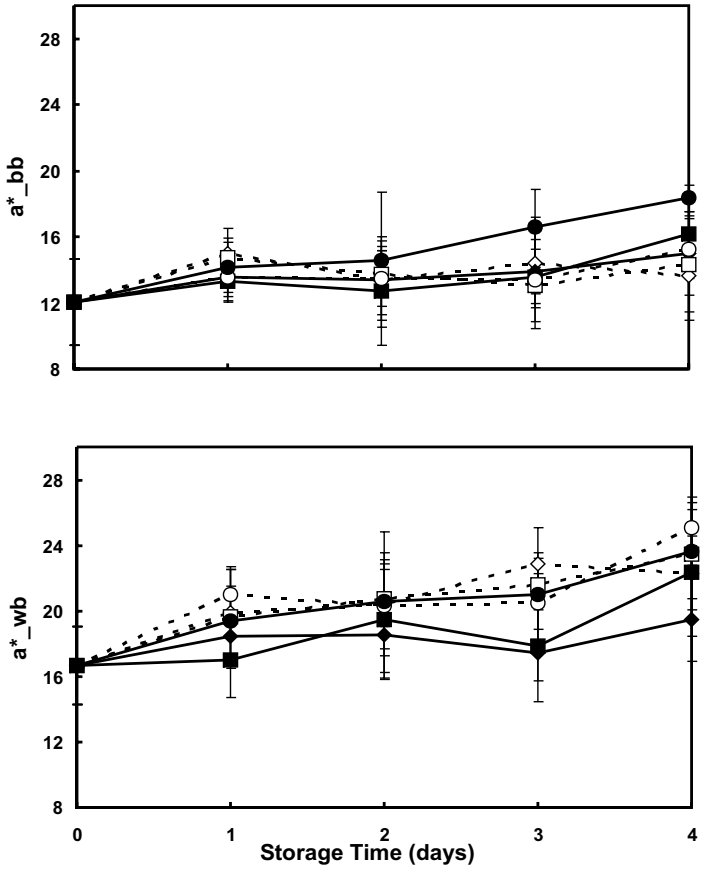

Figure 7 – a* value on black (bb) and white (wb) background of intact (solid line and black symbols) and cut (dotted line and white symbols) light-red tomato fruit stored at 5 °C (◆ ◇) 9 °C (■ □) and 13 °C (● ○) for 4 days.

3.5. Correlation between translucency development and RGB values

The increase in translucency of tomato pericarp of cut fruits was highly correlated with a decrease in the value of R_bb (R^2 = -0.86) and a decrease in the sum of RGB also on black background (R^2 = -0.85). In spite of the high correlation, the dispersion of the data around each translucency grade

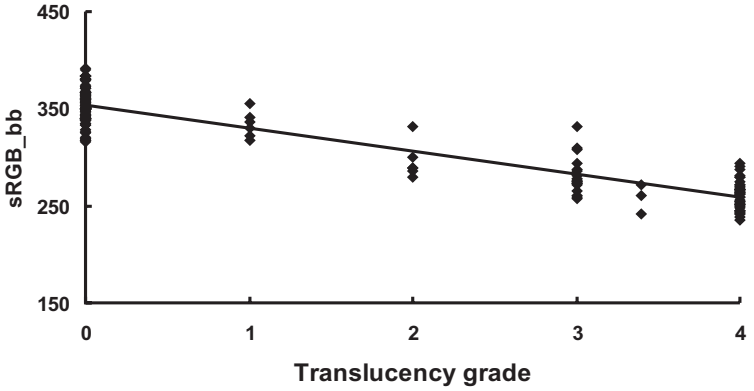

Figure 8 – Correlation between translucency intensity evaluated visually and the sum of R(Red) Green (G) and Blue (B) values on black background. Includes data from intact and cut tomato fruit stored at 5 °C, 9 °C and 13 °C for 4 days.

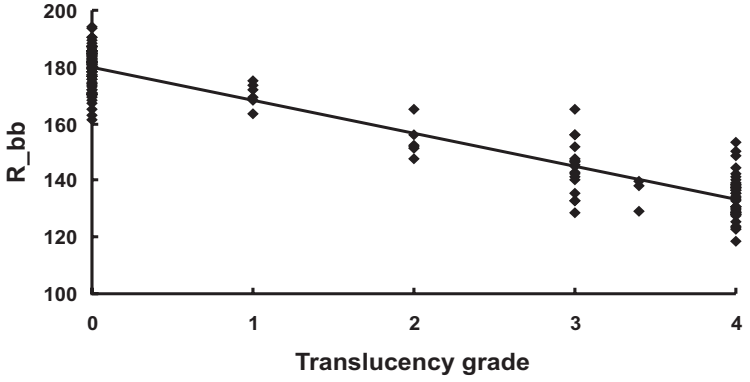

Figure 9 – Correlation between translucency intensity evaluated visually and R(Red) value on black background. Includes data from intact and cut tomato fruit stored at 5 °C, 9 °C and 13 °C for 4 days.

was very high and a large overlapping of sRGB_BB and R_bb values was observed for adjacent translucency grades (Figs. 8-9).

Similar high correlation was observed between translucency and the following variables: B_bb (R^2 = -0.83), G_bb (-0.82), R_diff (R^2 = 0.82) and B_wb (R^2 = -0.81).

*3.6. Correlation between L*a*b* and RGB values*

The lightness (L*) of fresh cut and intact tomato fruit was highly correlated (R^2 = 0.99) with

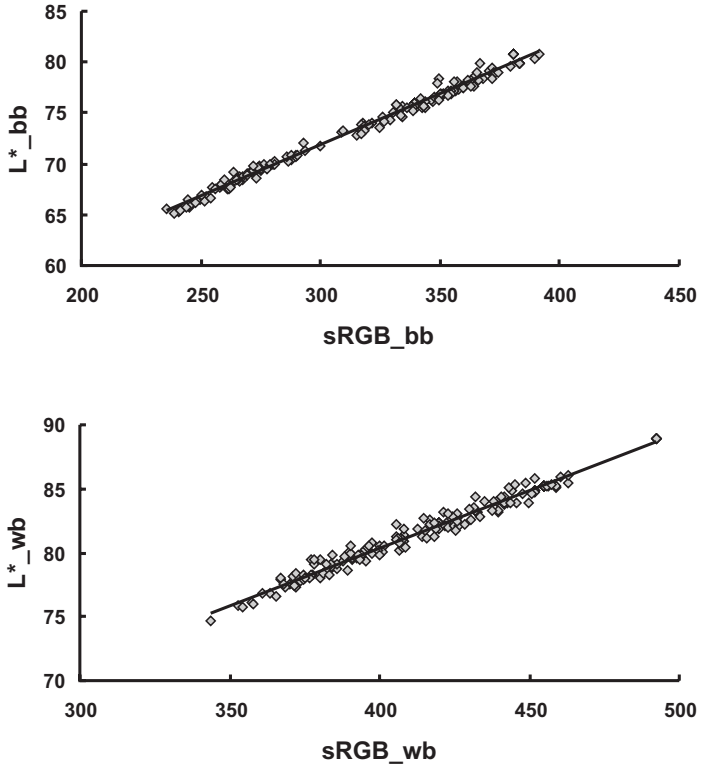

Figure 10 – Correlation between L* value and sum of R(Red) Green (G) and Blue (B) values respectively on black (bb) and white (wb) background. Includes data from intact and cut tomato fruit stored at 5 ^{o}C, 9 ^{o}C and 13 ^{o}C for 4 days.

the sum of RGB in each respective background (Fig. 10). The correlation with the individual values of RGB on each background was also always higher than 0.90.

The a* value was highly correlated with the relative Green value g (-0.96 and 0.99 on black and white background respectively) and to a lesser extent with the relative Red value (-0.91 and 0.91 on black and white background respectively).

4. Discussion

4.1. Relation between translucency development and storage temperature

A change from opacity to translucency was the most important alteration in the appearance of light-red sliced tomato during refrigerated storage. It occurred within 24 hours after processing, independent of the storage temperature.

The development of translucency in the pericarp of refrigerated fresh-cut tomato was first thought to be a symptom of chilling injury (Hong and Gross, 2000; Hong and Gross, 2002; Aguayo *et al*., 2004b). It seemed to be a reasonable assumption since tomatoes are sensitive to chilling injury when stored at temperatures lower than 12°C and water-soaking of the pericarp is one of the symptoms of chilling injury described in the literature (Sharom *et al*., 1994).

The data obtained in the present study do not support this conclusion since the translucency in the pericarp tomato was also observed at 13°C, a temperature not considered chilling injurious to ripe tomatoes and intact fruits stored at 5°C did not develop translucency in the pericarp. The effect of temperature on the visual intensity of translucency was statistically not significant and the tendency observed was of a slightly higher translucency at higher temperature, what is the opposite of what would be observed if this were the result of chilling injury. Translucency in tomato pericarp was previously also observed in slices stored at 12 °C and 16°C (Lana *et al*., in press c), (Lana *et al*., 2005).

Besides that, (Lana *et al*., in press-b) observed that the more ripe the fruit from which the slice is prepared, the higher its susceptibility to translucency development. According to Autio and Bramlage, 1986 cited by the tomato fruit is more susceptible to chilling injury at the mature-green stage than in the more ripened stages.

Evidence that the development of translucency is not a result of chilling injury but of wound injury was also presented by (Jeong *et al*., 2004) and (Bai *et al*., 2003).The presence of translucent spots in a few intact fruit is in accordance with what was previously reported by (Hetherington and MacDougall, 1992). They measured a change from opacity to increasing translucency as intact tomato fruit turned from green to red.

4.2. Assessment of translucency using video image analysis

The results obtained in the present study confirmed those obtained in a previous experiment (Lana *et al*., in press-b), that is, an increase in translucency is accompanied by a decrease in the sum of R, G and B value when the slice is placed on a black background. The most pronounced changes occurred in the R values. The value of RGB decreased because the more translucent the sample the higher the proportion of light that is transmitted through the sample and absorbed by the black background, and consequently not reflected back. When measured on a white background, the light transmitted through the sample is reflected back by the background.

The increase in translucency was also highly correlated with a decrease in lightness (L* value), especially in the black background. This same correlation was observed for other fresh-cut products (Bai *et al*., 2001; Portela and Cantwell, 2001; Saftner *et al*., 2003). The decrease in lightness indicates that the sample is becoming less bright and/or darker. The lower L* value in black background is particularly related with translucency because the translucent slices will look darker red on a black background what is measured as a lower L*.

Contrary to what was first hypothesized the indexes obtained by the difference between

white and black background (R_dif , G_dif and B_dif) were not the ones more correlated with translucency. That means that the development of translucency was better expressed as decrease in the colour aspects values themselves (RGB) when the measurement was done placing the sample on a black background than in differences between both backgrounds.

Although the development of translucency was highly correlated with a decrease in the sum of RGB_bb and a decrease in R_bb, in the present study it was not possible to estimate the grade of translucency through the RGB values due to the large overlapping of RGB values in successive classes of translucency. That means, that a particular translucency grade (for example 2 = light) does not correspond to a specific value of RGB, but to a range of RGB values that overlap with slices at stage 1 for the higher values and stage 3 for the lower values in the range. Nevertheless, it is clear that the increase in translucency is expressed as a decrease in the sum of RGB, and both follow the temporal pattern of change that is, a higher rate of change in the first day after processing, followed by a slower rate and a tendency to level off.

The numerical scale used for the visual evaluation in the present study (like all scales for visual inspection) only accounts for large differences in translucency and this is one of the possible reasons for the observed overlapping between RGB values and translucency grades.

The same reasoning can explain why the effect of temperature was not significant when the translucency intensity was considered, although it was significant for the R_bb and sRGB_bb, considered to express the development of translucency. Changes in colour (hue) and lightness in fresh-cut melon were visible immediately after processing in areas that later became translucent (Portela and Cantwell, 2001). That means that the colour measurement accounted for differences not perceived by the visual evaluation.

Despite these limitations, these results suggest that the analysis of video images presents a high potential to be used to evaluate translucency in fresh cut products. The sensibility of the system for small but significant changes in translucency can be further improved by the use of more perfect white (more reflective) and black (more absorbing) background and by the use of a more sensitive digital camera.

4.3. Changes in colour

When measuring colour in the CIELAB colour space, an increase in a* value of tomato fruits indicates they are becoming more red (Tijskens and Evelo, 1994). When using the RGB colour space an increase in redness could be expressed as an increase in the proportion of Red (r) or a decrease in the proportion of Green (g), since both were highly correlated with a*.

It should be emphasized that measurements on black background were highly correlated with the translucency development and should be not used to assess colour since in this case the measurements will reflect differences in translucency that will make the evaluation of colour inaccurate. Because of that, measurements on the white background should be considered when measuring

colour of translucent samples (HunterLab, 2000).

During storage, the sliced tomatoes became redder than the intact fruits. This increase in redness is most likely to be due to an increase in the absorption of light by the pigments in the tissue (mainly lycopene) than to the production of lycopene as a response to wound injury. Evidence that cutting or wound injury does not result in an enhancement in the lycopene production was presented by (Parkin, 1987; Campbell *et al*., 1990; Lana *et al*., in press-a).

On the other hand, alterations in the level of opacity or translucency of a given product result in changes in the light absorbed/reflected by that product and consequently in its colour and appearance even when there is no change in the composition or amount of pigments. An increase in the absorption of light occurs in the water-soaked tissue due to a more homogenous medium (reduction of light scattering) enhancing the contribution of the pigments components in the perceived colour, without a concomitant change in the amount of pigments. When a red tomato becomes more translucent there is a reduction in the light scattering and from the transmitted light the tomato absorbs more of the blue and green components, and eventually predominantly the red component is reflected back from the white background. A similar phenomenon was observed in cut melon by (Portela and Cantwell, 2001), where the development of translucency was associated with a decrease in hue, making the product looks more orange immediately after cutting. On other hand, on a black background the tomato may appear less colored (more dark) upon becoming more translucent. This is because the red component, which is the only component that is not already absorbed by the tomato itself, will eventually be absorbed by the background.

The fact that a very small but significant increase in redness was observed for intact fruits indicates that at least part of the change in colour was due to maturation. However, for the reasons discussed above, enhanced maturation is not expected to have contributed to the increase in redness due to cutting.

5. Conclusions

The development of translucency in the pericarp tissue was the main change in appearance occurring in sliced tomato during refrigerated storage. When the fruits were cut at light-red stage the changes from opacity to translucency occurred within 24 hours after cutting and increased little after that. The time for the development of translucency as much as its intensity was not affected by the storage temperature in the range of 5-13^{O}C. In view of that, the water-soaking of the pericarp seems to be a direct result of wound injury and not of chilling injury as previously thought.

The development of translucency could be assessed by video image analysis when the sample was placed on a black background and expressed as a decrease in the amount of RGB pixels. Measurements of colour on the other hand demand the use of a white background in order to avoid the interference of translucency.

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CHAPTER 6

APPLICATION OF KUBELKA-MUNK ANALYSIS TO THE STUDY OF TRANSLUCENCY IN FRESH-CUT TOMATO

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Abstract

In order to assess the development of translucency in fresh-cut tomato (*Lycopersicum esculentum* cv. Belissimo) during refrigerated storage, two experiments were conducted. In the first one, tomato slices obtained from fruits at breaker and at red stage were stored at 5 ± 0.5 °C and monitored at regular intervals for 9 days. In the second one, slices obtained from fruits at the light-red stage were stored at 5 ± 0.5 °C , 9 ± 0.7 °C and 13 ± 0.7 °C for 4 days. Intact (control) fruits were stored at the same conditions and sliced immediately before the evaluations. In both experiments, translucency was assessed using Kubelka-Munk analysis and through visual evaluation using a scale from 0-4. The translucency of cut tomato slices increased during storage in both experiments. Fruits at red stage got translucent faster than fruits at breaker stage and the intensity of translucency was also higher for more ripe fruit. The storage temperature did not influence significantly the development of translucency, indicating that the watersoaking of the pericarp tissue is not a result of chilling injury. The K/S ratio increased during storage for cut fruits and remained practically constant for intact fruits, reflecting the effects of treatment observed visually. Additional experiments indicated that the removal of the locular gel combined or not with washing and drying the slice cut surface inhibited the development of translucency.

Keywords: *Lycopersicum esculentum;* spectrophotometry; water-soaking; wound injury; minimally processed.

1. Introduction

The operations necessary to prepare fresh-cut fruits and vegetables (peeling, cutting, shredding, etc.) can induce undesirable changes in the colour and appearance of these products during storage and marketing. Degradation and oxidation of pigments like chlorophylls and carotenoids are likely to occur as a consequence of wounding (Heaton and Marangoni, 1996; Jamie and Saltveit, 2002). Browning of cut surfaces due to the production of wound-related compounds has been extensively studied and reported for many different products (Bolin and Huxsoll, 1989; Rocha and Morais, 2003), (Gonzalez-Aguilar *et al*., 2000; Loaiza-Velarde *et al*., 2003). Loss of water causes loss of sheen and gloss at the cut surface of cut pears (Gorny *et al*., 2000) and a whitening or dehydrated surface develops in carrots (Barry Ryan and O' Beirne, 1998), shredded green papaya (Techavuthiporn *et al*., 2003) and sliced tomatoes (Artes *et al*., 1999). Equally important but less studied, are changes in the refractive index and in the homogeneity of the tissue that result in the development of translucency or a water-soaked appearance after processing and have a great impact in colour and appearance of the product. This seems to be particularly important for fleshy tissue as tomatoes (Artes *et al*., 1999; Gil *et al*., 2002; Lana *et al*., 2005), melon (O'Connor-Shaw *et al*., 1994; Aguayo *et al*., 2004a), papaya (O'Connor-Shaw *et al*., 1994) and pears (Soliva-Fortuny *et al*., 2002).

Translucency is the property by which light can penetrate and disperse into and / or through a material (Hutchings, 1994). A translucent material is one that both transmits and scatters light. How much translucent an object is depends on the extent to which the light entering a sample is reflected, scattered or absorbed. As a phenomenon, translucency occurs between the extremes of transparency and opaqueness (Hutchings, 1994).

The reflection of light from opaque and translucent materials depends on the ratio of absorption to scatter as affected by pigmentation, refractive index and the light-scattering properties

of the material (MacDougall, 2002b). The interaction of light scatter and absorption is particularly important in influencing the perceived appearance. Besides that, translucency is one of the most important sources of structural error in the measurement of colour, since it can lead to severe confusion in both visual assessment and instrumental measurement. Its confounding effect in the colour appearance of such products as fruit juice, dairy products and meat was extensively studied by (MacDougall, 2002a). Because of that, the measurement of colour of translucent samples requires in addition to colour measurement the inclusion of some measure of light scatter, in order to obtain an adequate definition of colour appearance (Judd and Wyszecki, 1975; MacDougall, 2002a).

The Kubelka-Munk analysis was originally derived in 1931 by P.Kubelka and F. Munk in order to predict the optical properties of any given material. It found an extensive use in the industry of paints, inks and others (Cortat, 2004). In food science and horticulture the Kubelka-Munk analysis has been used in the study of optical properties of a range of different products (Law and Norris, 1973; Knee *et al*., 1988; Budiastra *et al*., 1998; MacDougall, 2002a; Pauletti *et al*., 2002; Talens *et al*., 2002; Rozycki, 2003).

The changes in optical properties of tomato fruit during maturation were assessed by (Hetherington and MacDougall, 1992) using the Kubelka-Munk analysis. They measured a decrease in scatter from green to red tomatoes and this was translated into a change from opacity to increasing translucency as the fruits turned red. Because the Kukelka-Munk analysis assumes homogeneity of the sample being analysed, the fruit was dissected into different parts (skin, outer pericarp, inner pericarp and locular contents), since they all scatter and absorb light in a different way. For all stages of maturity the outer pericarp was the most translucent tissue (less opaque). The columela and locular content are opaque and densely packed, albeit in a jelly surrounding in the case of locular contents.

The assessment of translucency in fresh-cut products has been made by visual evaluation by (O'Connor-Shaw *et al*., 1994; Bai *et al*., 2001; Portela and Cantwell, 2001; Gil *et al*., 2002; Bai *et al*., 2003; Jeong *et al*., 2004).

In the present study the changes in optical properties in the pericarp of fresh-cut tomato slices were assessed using the Kubelka-Munk analysis. The effects of the stage of maturity of the fruit at harvest and the storage temperature on the development of translucency are discussed. Additionally, the translucency development after cutting is proposed, based on additional experiments as described in the next sections.

2. Material and Methods

2.1. Experiment 1. Effect of Stage of Maturity

Tomato fruit (*Lycopersicon esculentum* cv Belissimo) were harvest in a commercial greenhouse in Made (The Netherlands) in September 2004. The fruits were harvested at two stages of maturity 3 (breaker) and 9 (red) according to the "kleur stadia tomaten" from The Greenery (www.thegreenery.com)

and transported immediately after harvesting and selection to Wageningen, The Netherlands. The same day, the fruits were washed in cold tap water in a sanitised room and stored overnight at room temperature.

The next day, fruits similar in colour, shape and size were paired and numbered. One fruit was stored intact while the other was sliced in 7-mm thick transversal slices. The first and last slices were thrown away and the central four were staked in the same relative position they had in the fruit. Intact and sliced fruits were placed in a white polystyrene tray (138 mm x 138 mm x 25 mm) and covered with a plastic film (Magnetron) and stored at 5 ± 0.5 °C. Because the translucency is related with the amount of cell leakage, the slices were kept in horizontal position in order to have uniform translucency in the entire pericarp. To avoid interference of possible whitening of the cut surface due to water loss, the slices on the top of the stack were not measured. This set-up also ensured that slices from the same position in the fruit were taken, so variations due to stage of maturity within the fruit were avoided. Therefore changes in translucency could be ascribed solely to the effect of treatment.

For each maturity x treatment (intact or sliced) x storage time combination, 6 replicates corresponding to a tray with 1 fruit were analysed. Only the second slice from the bottom of the stack was used. The evaluations (spectrophotometric measurements and visual evaluation) were performed immediately after processing, before cooling, and after 1, 2, 3, 5, 7 and 9 days under storage. Intact fruits were sliced immediately before evaluation, in the same way the other fruits have been sliced previously. Temperature data were recorded by an 8 channel thermocouple with a personal computer interface.

2.2. Experiment 2. Effect of Storage Temperature

Tomato fruit (cv Belissimo) were harvested in a commercial greenhouse in Berkel en Rodenrijs,The Netherlands in October 2004. The fruits were harvested when at maturity stage 7 (light-red) according to the "kleur stadia tomaten" from The Greenery (www.thegreenery.com) and transported immediately after harvesting to Wageningen, The Netherlands. The same day, the fruits were washed in cold tap water in a sanitised room and stored overnight at room temperature.

The next day, fruits similar in colour, shape and size were sliced and packaged as described in experiment 1 and stored at 5 ± 0.5 °C, 9 ± 0.7 °C or 13 ± 0.7 °C. Intact fruits, used as controls, were stored at the same conditions. For each temperature x treatment (intact or sliced) x storage time combination, 6 replicates corresponding to a tray with 1 fruit were analysed. Only the second slice from the bottom of the stack was used. Because most of the changes in the appearance of sliced tomato in Experiment 1 happened at the beginning of the storage time, the measurements (spectrophotometric measurements and visual evaluation) were performed immediately after processing, before cooling, and then every day for up to 4 days. Intact fruits were sliced immediately before evaluation, in the same way the other fruits have been sliced previously. Temperature data were recorded by an 8 channel thermocouple with a personal computer interface.

2.3. Experiment 3. Effect of the Presence of the Locular Gel and of Rinsing and Drying of the Cut Surface on the Ddevelopment of Translucency in Tomato Slices

To investigate whether the development of translucency is affected by the presence of both cell contents at the cut surface and the locular gel, the slices were submitted to different treatments after cutting, and evaluated visually after 2 days at 5 °C. Tomato fruit from the same batch as Experiment 2 were washed and sliced as described previously. Treatment was applied immediately after slicing to the 3 central slices from each fruit as follows:

1.1 – sliced

1.2 – sliced and rinsed superficially with tap water to remove the contents of cut cells present at the cut surface

1.3 – as before followed by drying superficially, by gently pressing the slice on a paper cloth to remove the water used to rinse the cut surface.

The same treatments were then applied to slices from which the locular gel had been removed gently with a spatula, as follows:

2.1 – sliced, followed by the removal of the locular gel

2.2 – sliced, followed by the removal of the locular gel and rinsing with tap water, as described before

2.3 – sliced, followed by the removal of the locular gel, rinsing with tap water and drying superficially with a paper cloth, as described before

After treatment, the slices were stacked in a covered plastic Petri dish (diameter 90 mm and height 25 mm) in the same relative position they had in the fruit and stored at 5 °C. After 2 days they were evaluated for the development of translucency as described below (item 5. Translucency: visual evaluation). After the evaluation, the locular gel of the slices of the first set of treatments (1.1, 1.2 and 1.3) was removed and transferred to the correspondent slices without locular gel (respectively treatments 2.1, 2.2 and 2.3). The slices were returned to cold storage and revaluated after 2 days storage at 5°C as before. For each treatment 6 replicates, correspondent to one Petri dish with 3 slices was used. Only the central slice in the stack was evaluated.

2.4. Translucency: Spectrophotometric Approach

The sample preparation and the spectrophometric measurements were conducted in the same way for experiments 1 and 2.

2.4.1. Sample Preparation

Tomato samples were taken from the outer pericarp and placed in a sample holder either with a white or a black background. The white background was made of Perspex and the black background

was made of Teflon. In the present study the measurements were focused on the outer pericarp. Although the translucency typically initiates in the radial pericarp, the outer pericarp comprises the major part of the flesh tissue in the slice, and contributes most to the appearance of translucency or water-soaking. Besides that, the other tissues present different optical properties and when using all of them the effect of treatment on translucency development would be confounded with the effect of kind of tissue.

2.4.2. Spectral Reflection Measurements

The reflection spectrum was measured with a Cary 5E spectrophotometer equipped with a reflecting sphere (type RSA-CA-50 byLabsphere) in the visible light range (400-700 nm) at 1 nm interval. Calibration of the instrument was done considering the reflectance of the reflecting sphere without sample holder to be 100%, and that with the black sample holder to be 0%, by using the common procedure for baseline correction.

2.4.3. Calculation of Kubelka–Munk index

The ratio between the absorption coefficient (K) and the scattering coefficient (S), was obtained applying the Kubelka-Munk theory (Eq. 1) as described by (Judd and Wyszecki, 1975). In this equation, R_8 is the reflectance of an infinitely thick layer of the material, determined through the equations (2) - (4) in terms of the reflectance R of the sample layer backed by a white background with a known reflectance R_g and the reflectance R_0 of the sample with an ideal black background. The K/S ratio was calculated for each wavelength of the measured spectra.

$$\frac{K}{S} = \frac{(1 - R_\infty)^2}{2R_\infty} \tag{1}$$

$$R_\infty = a - b \tag{2}$$

$$a = 1/2\left(R + \frac{R_0 - R + R_g}{R_0 R_g}\right) \tag{3}$$

$$b = \left(a^2 - 1\right)^{1/2} \tag{4}$$

2.5. Translucency: Visual Evaluation

Before sample preparation for the spectrophotometric evaluation, each slice was evaluated visually and graded according to the scale shown in Table 1.

To have conditions of illumination as uniform as possible in successive evaluations, they were performed at about the same time of the day and at same place in the laboratory.

Table 1 – Translucency scale for visual assessment.

0	Not translucent.
1	Incipient translucency in the whole pericarp or translucent spots in the inner pericarp and/or outer pericarp.
2	Light translucency in the whole pericarp.
3	Moderate translucency in the whole pericarp.
4	Intense translucency in the whole pericarp.

2.6. Statistical Analysis

The effect of time, temperature and stage of maturity on the development of translucency in cut tomatoes was analysed by Kruskal-Wallis One-Way Analysis of Variance (Siegel, 1956) in GENSTAT 7.2.

3. Results

3.1. Effect of stage of maturity

3.1.1. Translucency: Visual Evaluation

The translucency of tomato slices increased during storage (Fig. 1). The extent of increase was highly dependent on the interaction between storage time and maturity stage of the fruit (P= <.0001). Fruits at red stage (grade 9) got translucent faster than fruits at breaker stage (grade 3) and the intensity of translucency was also higher for riper fruits.

The development of translucency typically initiated as very translucent spots in the radial pericarp or in the junction between radial and outer pericarp and more rarely it started along the outer pericarp. In the next stage, it spread to the outer pericarp, initially close to the locular gel and then outwards, increasing in severity along time on both radial and outer pericarp. The shrinkage of the locular gel occurred in parallel with the development of translucency in the pericarp. The columela could become water soaked especially in more ripe fruits, but because of its more compact structure it would seldom become really translucent.

Fruits processed at breaker stage showed a small increase in translucency until day 2 and

then levelled off. At this stage, the translucency rarely spread in the outer pericarp being then restricted to the first stage described in the previous paragraph. Fruits at red stage showed a sharp increase in the first day of storage followed by a slower increase up to day 5 when it levelled off. This implies that there was an effect of maturity stage on both the time for appearance and the intensity of translucency.

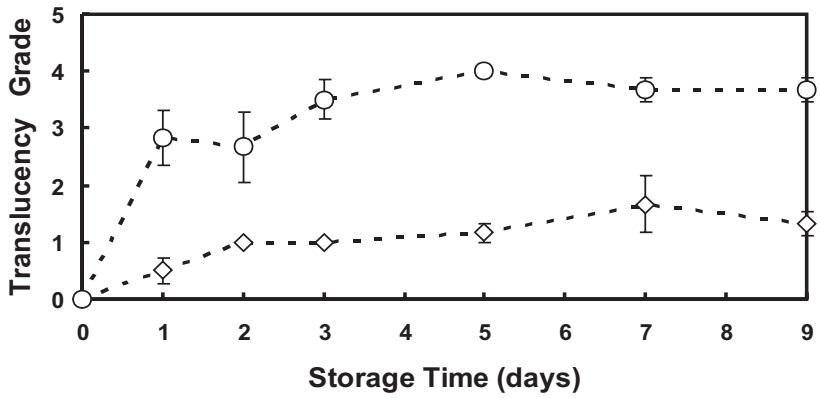

Figure 1 – Translucency grade of cut tomato slices obtained from fruits harvested at stage of maturity 3 (◇) (breaker) and 9 (○) (red) and stored at 5°C for 9 days. Points are the average of 12 measurements ± standard deviation.

3.1.2. Translucency: Kubelka-Munk Analysis

The ratio between the Kubelka-Munk absorption coefficient (K) and the Kubelka-Munk scattering coefficient (S) of tomato pericarp increased during storage for cut fruits and remained practically constant for fruits stored intact (Figs. 2-3). The increase in K/S values was concomitant to the increase in translucency observed visually (Fig. 1).

Differences between both maturity stages in relation to the time for translucency development and the magnitude of increase were expressed in the values of K/S. On average, fruits harvested and processed at breaker stage did show a progressive and slow increase in K/S during storage (Fig. 2).

Fruits harvested and processed at red stage showed a sharp increase in the first day and a second large increase on day 5 (Fig. 3). The initial values of K/S were higher for fruits harvested at red stage and the magnitude of the increase during storage was also higher for more ripe fruits.

The spectra of individual slices for three select days are shown in Figs. 4-5. The variation in K/S for intact fruits was very small, while the variation for cut fruits increased along storage especially for slices at red stage.

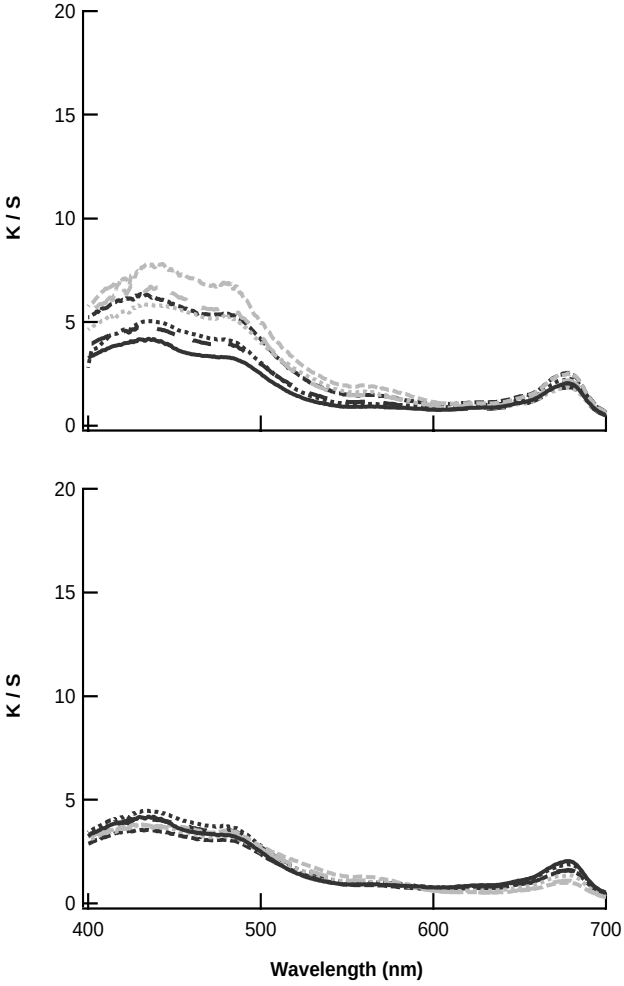

Figure 2 – Average K/S value of pericarp of cut (top) and of intact (bottom) tomato slices obtained from fruits harvested at stage of maturity 3 (breaker stage) and evaluated after 0 (—), 1 (···), 2 (---), 3 (– –), 5 (···), 7 (---), and 9 (– –) days under storage at 5 °C.

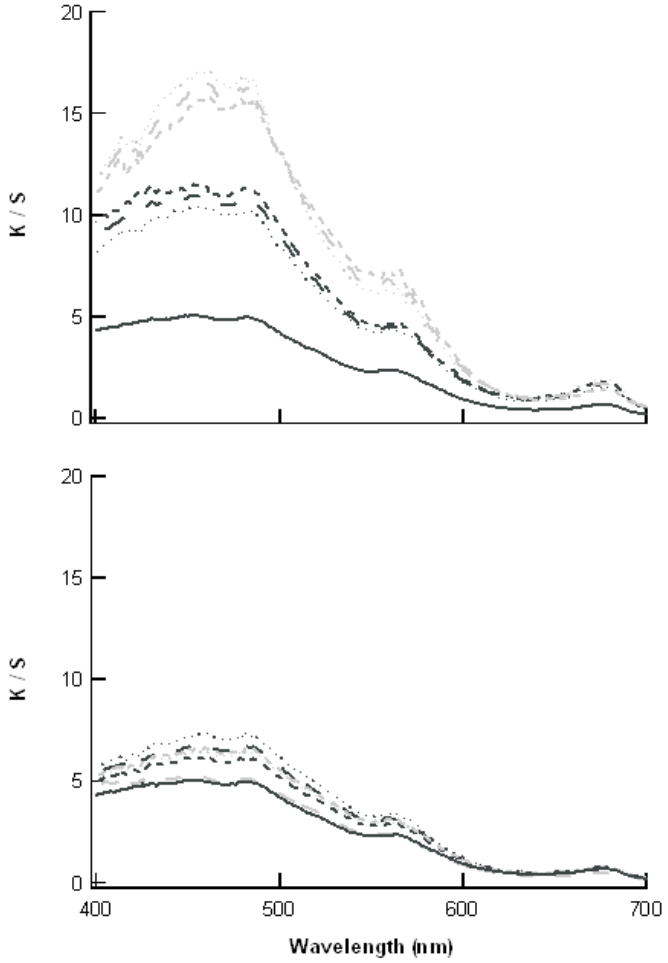

Figure 3 – Average K/S value of pericarp of cut (top) and of intact (bottom) tomato slices obtained from fruits harvested at stage of maturity 9 (red stage) and evaluated after 0 (—), 1 (···), 2 ( ),3 (– –), 5 (···), 7 ( ), and 9 ( ) days under storage at 5 °C.

3. 2. Effect of temperature

3.2.1.Translucency: Visual Evaluation

The translucency of tomato slices increased during storage (P= <.0001) at all temperatures. The intensity of translucency did not depend on the temperature (P=0.2460). At all temperatures the same temporal pattern of change in translucency in cut fruits was observed, that is a sharp increase in the first day, followed by a small and almost linear increase afterwards (Fig. 6).

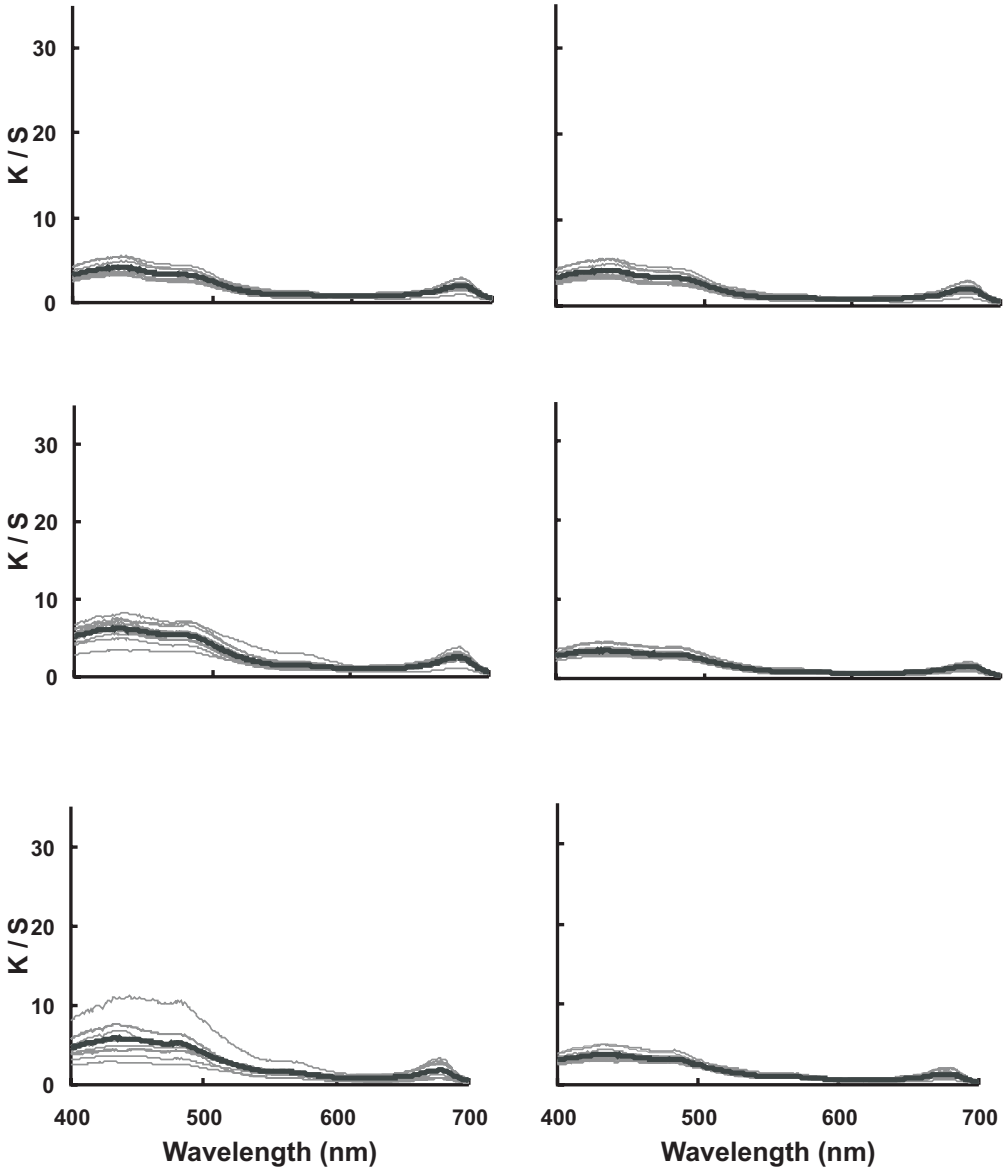

Figure 4 – K S value of pericarp of cut (left) and intact (right) tomato slices obtained from fruits harvested at stage of maturity 3 (breaker stage). Grey lines represent the individual slices, and the black line represents the average. Top graph day 0, middle graph day 2, bottom graph day 5.

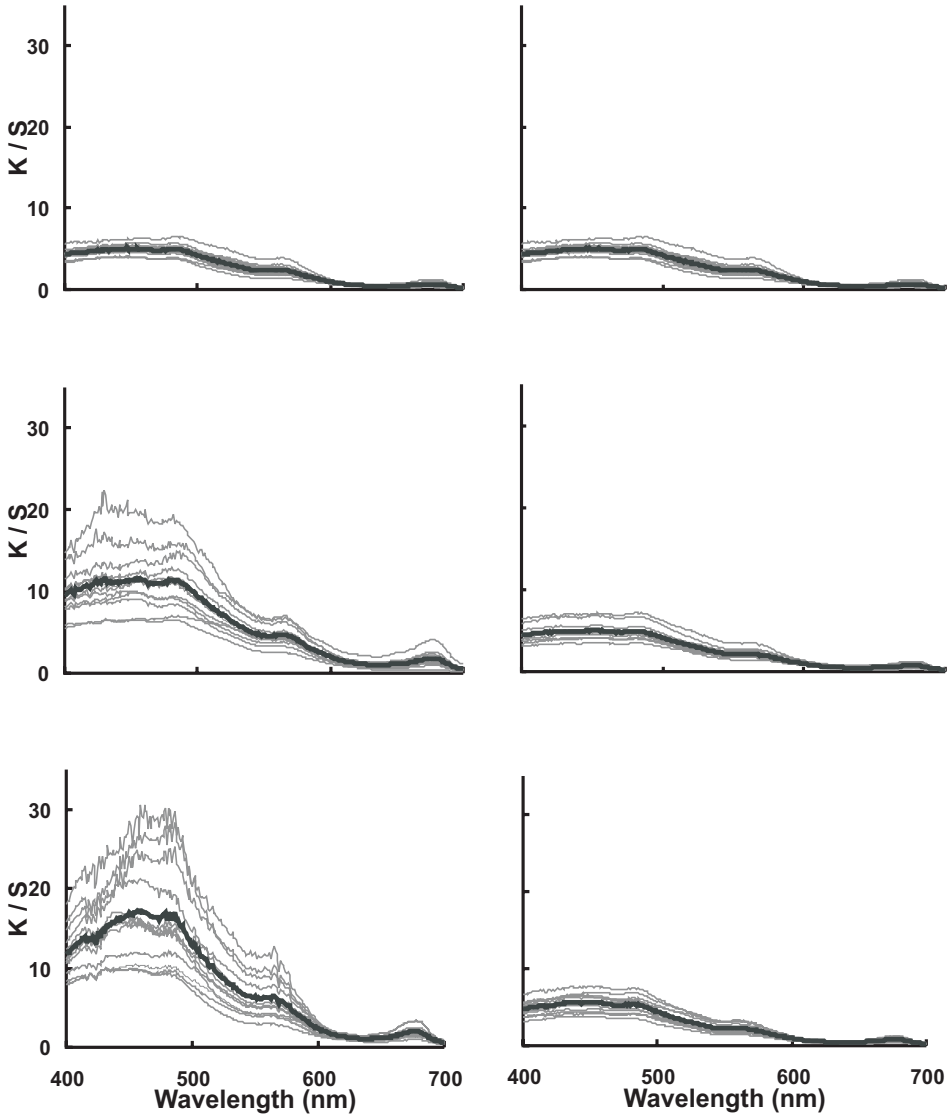

Figure 5 – K/S value of pericarp of cut (left) and intact (right) tomato slices obtained from fruits harvested at stage of maturity 9 (red stage). Grey lines represent the individual slices, and the black line represents the average. Top graph day 0, middle graph day 2, bottom graph day 5.

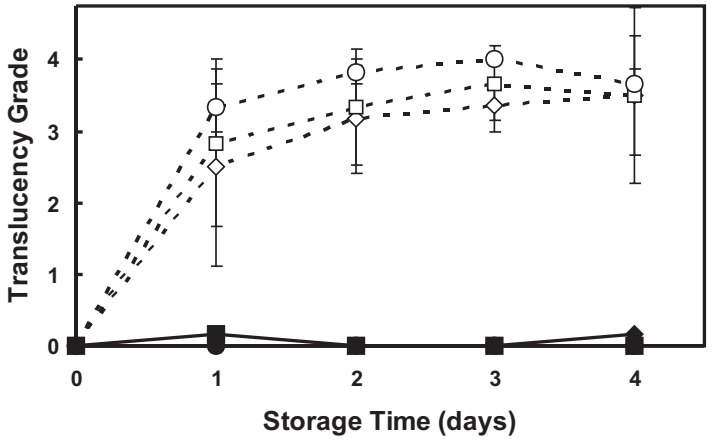

Figure 6 – Translucency intensity of intact (solid line and black symbols) and cut (dotted line and white symbols) light-red tomato fruit stored at 5 °C (◆ ◇), 9 °C (■ □) and 13 °C (● ○) for 4 days.

3.2.2. Translucency: Kubelka-Munk Analysis

The increase in translucency in cut fruits was accompanied by an increase in the K/S values (Fig. 7). As in the visual evaluation, there was no remarkable effect of temperature on the changes in K/S value, while a clear difference between intact and cut fruits was observed after storage for 1 day (Fig. 7). After that, the K/S value did not change remarkably as can be observed comparing the results obtained 2 and 4 days after cutting (Fig.7). The same temporal pattern of change was observed visually (Fig.6).

3.3. Effect of rinsing, drying and removing of the locular gel

Rinsing the cut surface of the tomato slices immediately after processing reduced the severity of translucency (Table 2). Rinsing and drying reduced it even further and almost prevented it from happening in the period of 2 days. The removal of the locular gel inhibited the appearance of translucency to a greater extent than only rising, but to a lower extent than rising and drying. A synergistic effect in reducing translucency was observed when two or more treatments were combined.

Adding the locular gel to the slices previously without it, induced translucency in all treatments and reversed the effect of the previous treatments in reducing translucency. On the other hand, the removal of the locular gel after the slices were translucent did not reverse its effect, and the slices retained their water-soaked appearance (Table 2).

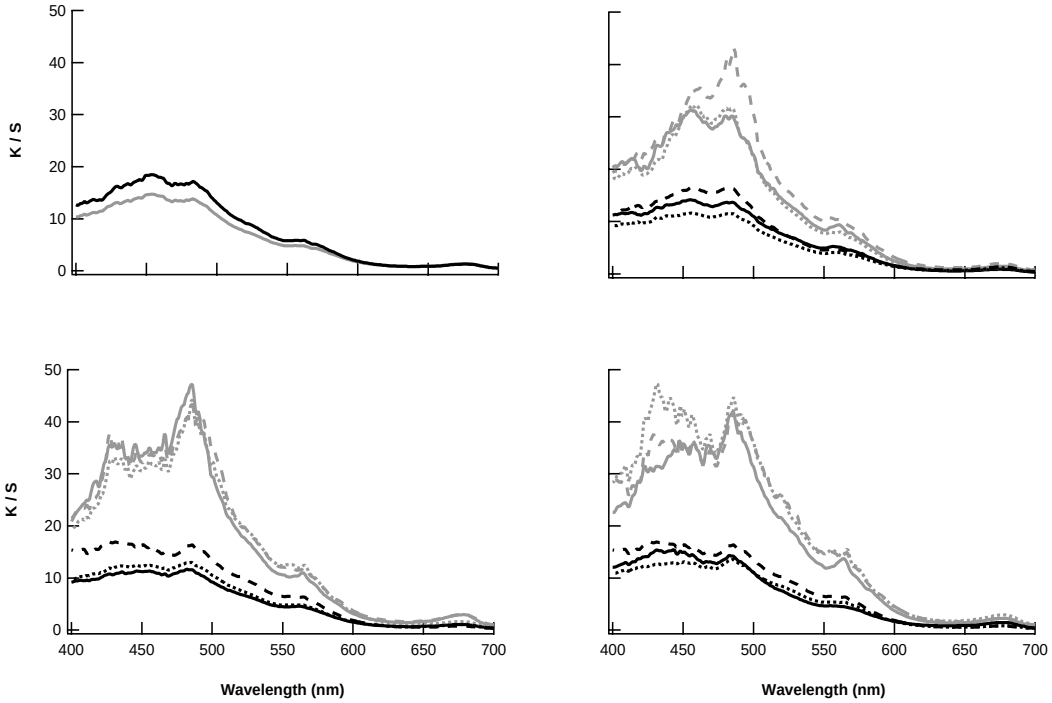

Figure 7 – Average K/S value of pericarp of cut (grey lines) and intact (black lines) of tomato slices obtained from fruits harvested at stage of maturity 7 (light-red stage) and stored at 5°C (— and —), 9°C (⋯ and ⋯) and 13°C (--- and – –) for 0 (top left), 1 (top right), 2 (bottom left) and 4 (bottom right) days after cutting.

Table 2 – Translucency grade of tomato slices submitted to different treatment combinations of rinsing, drying and removing the locular gel after slicing.

		Slice originally with locular gel	Slice originally without locular gel
Translucency after 2 days at 5°C	Sliced (control)	4.00 ± 0.0	1.40 ± 0.5
	Rinsed	2.60 ± 0.1	0.30 ± 0.4
	Rinsed and dried	0.40 ± 0.4	0.20 ± 0.45
Translucency 2 days after removal of locular gel (3rd column) or after addition of locular gel (4th column)	Sliced (control)	3.50 ± 0.6	3.50 ± 0.6
	Rinsed	3.25 ± 0.5	4.00 ± 0.0
	Rinsed and dried	2.00 ± 0.8	2.75 ± 1.3

4. Discussion

4.1. Development of Translucency

The development of translucency in the pericarp of sliced tomatoes can be explained, at least partially, by the replacement of gas by liquid in the intercellular space, resulting in a more homogeneous refractive index in the tissue and consequently the promotion of light absorption in the product surface and concomitant reduction in light scattering. A similar process was described by (Talens *et al*., 2002) to explain the development of translucency in processed kiwi fruit.

Micro structural observations of fresh-cut pear tissue (Soliva-Fortuny et al., 2002) indicated a total flooding of intercellular spaces what was supposed to be the consequence of a diminution of membrane integrity, which in turn would cause the decompartimentalization of texture-related enzymes and their substrates. A similar process was described in fresh-cut papaya by (Karakurt and Huber, 2003) which rapid deterioration was described to involve membrane and cell wall catabolism accelerated or otherwise altered in response to physical wounding. Both products develop translucency after cutting.

In the present study, the presence of the locular gel was important for the development of translucency in the pericarp of tomato slices. It remains to be elucidated whether this is a purely physical effect, in which at least part of the liquid that fills the intercellular space in the pericarp comes from the locular gel, or whether there are chemical compounds/enzymes in this tissue that promote the degradation of cell membranes and cell wall what results in modification of the apoplastic environment. Evidences in favour of a purely or predominantly physical effect are the lack of temperature effect in the development of translucency (expected if an enzymatic process was predominant) and the observed shrinkage of the locular gel parallel to development of translucency. Additionally, the wound-induced modification of apoplastic conditions in papaya (Karakurt and Huber, 2003) and melon (Soliva-Fortuny *et al*., 2002) was associated with accelerated softening after cutting, while for cut tomatoes the changes in firmness at low temperature were small (Lana *et al*., 2005).

The removal of the locular gel alone was sufficient to reduce translucency significantly, although in this case the contents of the cut cells were not removed from the cut surface. When combined with rinsing and drying the slice superficially after cutting, the removal of the locular gel practically prevented the development of translucency. However, in this case the effect of rinsing and drying seemed to be the main effect with only a marginal effect by removing the locular gel. However, since the whole slice was pressed against the paper cloth it is very likely that it also dried part of the locular gel.

The reduction in translucency because of rinsing alone indicates that the release of cell contents when the fruit is sliced may play a role in the development of translucency by promoting the degradation of membranes and enhancing leakage. Washing after cutting had a great impact on physicochemical measures of quality in sliced green pepper (Toivonen and Stan, 2004). It was suggested

that this effect was mediated by the removal of stress-related compounds produced as a response to the wound-injury.

Drying had an additional effect in reducing translucency because part of the superficial water at the surface that would otherwise be absorbed by the slice was removed and probably also, because part the locular gel was superficially dried in this operation.

4.2. Visual perception and instrumental measurement

When light reaches the surface of an object it is partially reflected back from the surface and partially penetrates the object. The light that penetrates the object is partially reflected at each surface it encounters. This process of multiple reflection and refraction thoroughly diffuses the light and returns much of it to the surface of the object where it leaves in all directions. The process of diffusing the light is called light scattering. When the tomato slice becomes water-soaked there is a reduction in light scattering due to a more homogenous medium (in a very simple way, about the same refractive index between the interior of the cell and the intercellular space) and at the same time there is an increase in absorption of light by the pigments present in the tissue.

The basic principle underlying the Kubelka-Munk analysis is depicted in Fig.8. The reflectance spectra of a highly opaque hypothetical red sample is about the same when the sample is placed on a white and on a black background. When the sample is translucent, more light is transmitted through the sample, due to the reduction in scattering. The light that reaches the white background is reflected back while the light reaching the black background is absorbed by it. This results in a reduction in reflection from the translucent sample, when measured on a black background, especially in the range from 630 to 700 nm. Together with the reduction in scattering, there is an increase in the absorption of light in the region of absorption of the pigments present in the sample. Consequently, the K/S ratio increases for translucent samples in the 400-630 nm region of the spectrum, while it is practically 0 for in the region over 630 nm.

The measured changes in K/S value in the pericarp of tomato fruit reflected the observed visual changes in optical properties, that is, an increase in translucency of stored cut slices. The effect of maturity stage and temperature also gave different results in the Kubelka-Munk analysis. The observed increase of translucency in red fruits compared to partially ripened fruits was reflected in higher values of K/S for red fruits, and the lack of significant effect of storage temperature was reflected in similar K/S values for slices stored under different conditions.

4.3. Effect of maturity stage and temperature on the development of translucency

The development of translucency as a consequence of cutting was highly dependent on the stage of maturity of the fruit when processed. Changes in optical properties of the different tomato fruit tissues in relation to maturity were first addressed by (Hetherington and MacDougall, 1992). The

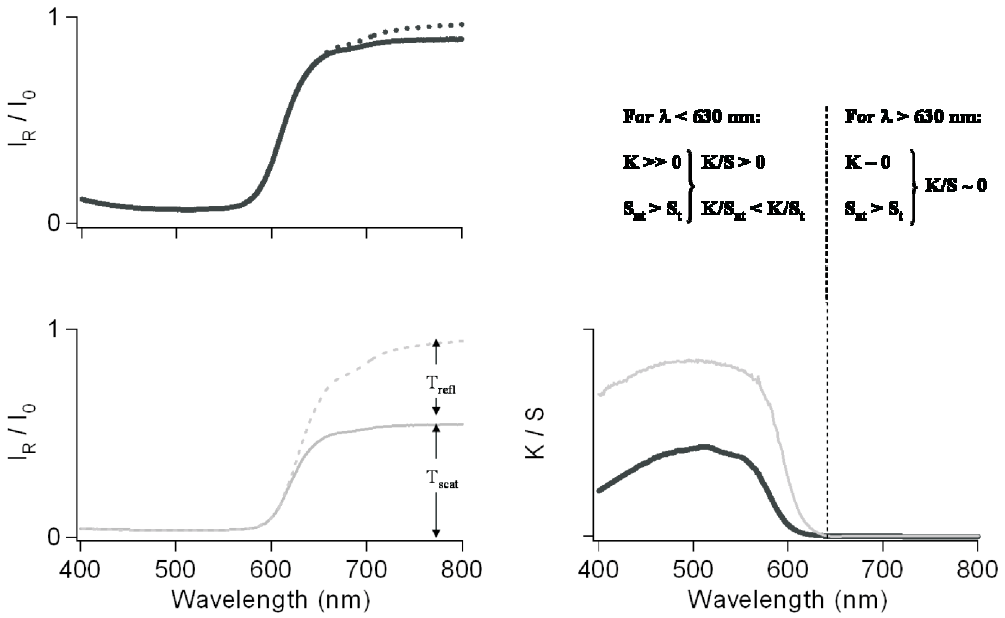

Figure 8 – K/S ratio of a hypothetical non-translucent (black line) and a hypothetical translucent (grey line) red sample obtained from their respective reflectance Spectra (I_R/I_0) (top left graph = non-translucent and left bottom graph = translucent). T_{refl} = transmission reflected from white background; T_{scat}= transmission reflected from white background; S_{nt} = scattering of non-translucent sample; S_t = scattering of translucent sample

cellular structure of mature green tomato is compact with very small intercellular spaces, and light is highly scattered and reflected. The apoplastic volume of tomato pericarp tissue increases sharply at the later stages of maturity (Damon *et al*., 1988). In red fruits the cells are engorged with fluid and there are more intercellular spaces, causing the light path to be less distorted. Thus more light is transmitted rather than scattered or reflected. This results in a decrease of K/S values from green to red fruits (Hetherington and MacDougall, 1992) and a higher susceptibility of ripe fruits to develop translucency after cutting. The higher susceptibility to translucency with maturity was also observed by (Jeong *et al*., 2004). Additionally, they observed that watersoaking was consistently more rapid and severe in slices derived from the physiologically older blossom portion compared with the stem end of the fruit.

The previously discussed watersoaking of the flesh tissue in fresh-cut papaya (Karakurt and Huber, 2003) and fresh-cut pears (Soliva-Fortuny *et al*., 2002) as a consequence of wound injury, was ascribed to an enhancement in the activities of membrane and cell wall hydrolases with consequent leakage of cellular contents to the intercellular space. This model is consistent with a higher susceptibility of more mature fruits to translucency, since the enzymes activated by wounding are the same that are enhanced by maturation. There is also the possibility that the effect of maturity stage would be simply

a physical one. Because the intercellular space is higher in ripened fruits, the extent of the water flooding is higher and the medium becomes more homogenous causing less distortion in the light pathway through the tissue and consequently reducing light scattering and making the sample looks translucent. The possibility of both processes happening in succession or simultaneously however can not be excluded at the present stage. One physical related with the flooding of the intercellular space with leakage from cut cells and from the locular gel that happens immediately after cutting; and a chemical one due to degradation of the cell wall and cell membrane with consequent cell leakage and further flooding of the intercellular space.

The appearance of translucency in stored fresh-cut tomato was first considered to be the result of chilling injury by (Hong and Gross, 2000) and (Aguayo *et al*., 2004b). The present results indicate that this is not the case since it was observed at temperatures that are not inducing chilling injuries, that is 13 ^{o}C in the present study and 12 ^{o}C and 16 ^{o}C in previous studies (Lana *et al*., 2004; Lana *et al*., 2005). Besides that, translucency was not observed in intact fruits stored under the same conditions. Evidence that the development of translucency in fleshy cut tissue is not a result of chilling injury were similarly obtained by (Jeong *et al*., 2004) and (Bai *et al*., 2003). Translucency in sliced tomato stored at 5 ^{o}C was progressively greater as the temperature during the initial 24 hours after slicing increased from 5 ^{o}C to 10 ^{o}C and 15 ^{o}C (Jeong *et al*., 2004). Besides that, the tomato slices stored at 10 ^{o}C developed severe translucency within 4 days while its development was delayed by three days in 5 ^{o}C storage. Similarly, the development of translucency in honeydew melon cubes was higher at 10 ^{o}C than at 5 ^{o}C (Bai *et al*., 2003).

In the present study there was no significant effect of temperature in the range 5 to 13 ^{o}C on the rate of development of translucency. This indicates that an enzymatic process involving cell wall and membrane degradation, although not excluded, is unlikely to be playing the major role in the process of development of translucency.

4.4. Limitations of the Spectroscopic Technique

In Experiment 2 very noise spectra, with extremely high and negative K/S ratios were obtained. This can be explained by the fact that when absorption by pigments reduces the intensity of the light beam to less than 0.001% (spectrophotometer specifications), the noise level of the detection is dramatically increased. When measuring highly translucent samples on a black background the same problem of very low signal intensities will appear. In the second experiment, there are some evidences that the second situation occurred. The extreme values of K/S ratios were obtained for cut fruit samples (translucent) but not for intact fruit samples (not translucent). The very low or negative reflectance was observed in the range 400-550 nm (strong absorption) but not in the range 600-700nm (no absorption). Very translucent slices can also present problems because in this case the vascular bundles become more visible having a different colour from the rest of the sample. Problems in using the Kubelka-Munk analysis with very translucent and heterogeneous tissues were discussed by

(Hetherington and MacDougall, 1992). In the first case, the problems were due to very low S values and relatively high K values. In the second case, problems were due to the inhomogeneity of the highly translucent parenchyma (low scattering) and very opaque (high scattering) vascular bundles.

Spectra with an extremely high noise level were not considered when calculating the average for each treatments x storage day combination. This seems to be justified since when considering only the low noise spectra, the results (K/S values) were in accordance with the visual evaluation of translucency as observed in Experiment 1.

5. Conclusions

The main change in the appearance of tomato slices during storage was the development of translucency in the pericarp. It was shown that the changes in K/S from the Kubelka-Munk analysis of the reflection spectra of the sliced tomatoes paralleled the changes in translucency observed visually. The observed effects of storage temperature and maturation stage were equally reflected in changes of K/S during storage of cut tomato. Control fruits, that did not develop translucency, showed no remarkable changes in K/S values, while for cut fruits they increased on time.

Tomato slices become translucent 1 to 2 days after processing. The intensity and the rate of development of translucency was higher the more mature the fruit and was not influenced by storage temperature in the range 5-13 ^{o}C. This indicates that the development of translucency is not a symptom of chilling injury as first suggested but rather a consequence of wound injury. Removal of the locular gel inhibited the development of translucency in the pericarp. At the moment, it is not clear whether this is simply a physical effect, where the liquid that floods the intercellular space comes from the locular gel, or whether it is related with the removal of stress-related compounds produced in response to cutting. Similarly, the inhibition of the development of translucency by rinsing and drying of the cut surface can also be due to the removal of stress-related compounds:

Acknowledgements

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CHAPTER 7

MODELLING RGB COLOUR ASPECTS AND TRANSLUCENCY OF FRESH-CUT TOMATOES

Submitted as: Lana, M.M.; Tijskens, L.M.M.; van Kooten, O. Modelling colour aspects and translucency of fresh-cut tomatoes. May 2005.

Abstract

Translucency is one of the major problems in fresh-cut fruits. This phenomenon seriously limits the use of fruits by the fresh-cut industries. Techniques for measuring translucency in this kind of product are not readily available. As a consequence, the processes that are important in the development of translucency are little understood, let alone described in detail.

Based on techniques used in the industry of paint, paper and textiles, a measuring technique using Video Image Analysis (VIA) involving light reflection roma sample placed in a double white and black background was used to assess the development of translucency and its interference with colour measurement in fresh-cut tomatoes. The effects of stage of maturity at the moment of processing as well as the effect of storage temperature were studied in two separate experiments. The data were expressed as the average intensities per pixel of red (R), green (G) and blue (B) for the white and for the black background (bb) separately. A model was developed and presented that describes the change in the appearance of tomatoes after cutting and during storage. In the model the observed effects were considered to be the result of two processes namely changes in colour due to the production or degradation of pigments and development of translucency (i.e. physical water-soaking). Both processes resulted in changes in each one of the colour aspects R, G and B. Each colour aspect was considered to be built up by a variable part that changes according to a first order reaction mechanism and a fixed part that is invariable under the circumstances under study. The data obtained on both experiments were used to estimate the model parameters by multiple non-linear regression analysis using R,G and B as response variables and time, temperature, stage of maturity and background simultaneously as explaining variables.

The change from opacity to translucency in the pericarp was the main change in appearance of tomato slices during refrigerated storage, for all stages of maturity (Experiment 1) and storage temperatures (Experiment 2). Changes in colour were much less pronounced and related to a small increase in redness for all stages. The susceptibility to develop translucency was very much dependent on the maturity stage of the fruit at harvest and rather independent on temperature.

Keywords: Lycopersicum esculentum; water-soaking, video image analysis, minimally processed, water soaking.

1.Introduction

The appearance of fresh-cut products changes on time due to the production and/or degradation of pigments (Artes *et al*., 1999; Rocha and Morais, 2003) and due to physical changes such as loss of water (Barry Ryan and O' Beirne, 1998) and water-soaking (Jeong *et al*., 2004). For fresh-cut tomato, two different processes, maturation (Artes *et al*., 1999) and the development of translucency (Aguayo *et al*., 2004; Jeong *et al*., 2004), result in changes in appearance after processing.

The colour and other optical properties that contribute to the appearance of food products can be assessed using video image analysis (VIA). The images are captured with a CCD camera and saved in the RGB (Red, Green, Blue) format (Du and Sun, 2004).

The changes in the overall appearance of tomato slices during refrigerated storage were assessed using VIA by Lana *et al*. (2004). Changes in appearance, attributed then mainly to changes in colour, were parallel to a decrease in the RGB values. All three colour aspects R, G and B changed

according to a simple first order mechanism, which incorporated the maturity stage of the fruit at harvest and both the temperature and storage time. Each colour aspect was considered to be build up by a variable part that changes according to a first order mechanism (simple exponential decay) and a fixed part invariable under the circumstances under study. Pooling data in an integral analysis revealed that all three aspects shared a common rate constant and a common temperature dependence. This approach showed to be a powerful one to use all the available information and to understand how the process of colour change in cut tomato depends simultaneously on all the factors considered (temperature, maturity stage and storage time).

In the above mentioned experiment, the tomato slices became translucent after 2 to 4 days of storage. Occurrence of translucency will interfere in colour measurements. The problems associated with the colour measurement of translucent samples were discussed in detail by Hutchings (1994) and by MacDougall (2002). Translucency is probably the most important source of structural error during colour measurement. It can lead to severe confusion in both visual assessment and instrumental measurement. The colour measurement of translucent samples are sensitive to ambient light, path length changes, background changes, and small differences in the optical configuration of the instrument.

To assess the contribution of maturation and translucency to the final appearance of the tomato slices experiments were performed where the samples to be photographed were placed over a half white and half black background. This approach was based on techniques previously used to measure the hiding power of a colorant layer, which has an inverse relation with the translucency of that layer. The hiding power can be calculated as the reciprocal of the ratio between the reflectance of a sample over a white background and over a black background (Judd and Wyszecki, 1975). In the present work, it was expected that the difference in colour aspects (RGB) between both backgrounds would be closely related with the intensity of translucency and eventually could be used to quantify the translucency development.

The main objective of the present study was to expand the model previously reported (Lana *et al*., 2004; Lana *et al*., 2005) in order to include both the changes in colour and translucency that happens in fresh cut tomato during storage. Doing so, it is expected that it will be possible to describe how both processes happen after cutting and how they depend on storage time, storage temperature and maturity stage of the fruit. The second objective is to identify how the changes in appearance due to translucency and maturation are expressed in changes in the RGB colour aspects

2. Material and Methods

2.1. Harvesting and Processing

2.1.1. Experiment 1: Effect of Stage of Maturity

Tomato fruit *Lycopersicon esculentum* (cv Belissimo) were harvest in a commercial greenhouse in Made, The Netherlands in September 2004. The fruits were harvested on a single day, when at three stages of maturity 3, 6 and 9 (named here as I, II and III) according to the "kleur stadia tomaten" from The Greenery (www.thegreenery.com) and transported immediately after harvesting and selection to Wageningen (The Netherlands). The same day, the fruits were washed in cold tap water in a sanitised room and stored overnight at room temperature.

The next day, fruits similar in colour, shape and size were paired and numbered. One fruit was stored intact while the other was sliced in 7-mm thick transversal slices. The first and last slices were discarded and the central four were stacked in the same relative position they had in the fruit. Intact and sliced fruits were placed in a white polystyrene tray (138 mm x 138 mm x 25 mm) and covered with a plastic film (Magnetron) and stored at 5 ± 0.5 °C. For each combination of maturity stage x processing (intact or sliced) x storage time, 6 replicates, corresponding to a tray with one fruit, were analysed. Only the second slice from the bottom of the stack was used. This set-up ensured that slices from the same position in the fruit were taken in successive measurements. It also avoided the confounding effect of possible whitening of the cut surface of the slice at the top of the stack and the influence of leakage in the bottom of the tray on the intensity of watersoaking. In this way, changes in optical properties could be ascribed solely to the effect of treatment.

Digital images were taken immediately after processing, before cooling, and after 1, 2, 3, 5, 7 and 9 days under storage. Intact fruits were sliced immediately before evaluation, in the same way the other fruits have been sliced previously. Temperature data were recorded by an 8 channel thermocouple with a personal computer interface.

2.1.2. Experiment 2: Effect of Storage Temperature

Tomato fruit (cv Belissimo) were harvest in a commercial greenhouse in Berkel en Rodenrijs,The Netherlands in October 2004. The fruits were harvested when at stage of maturity 7 according to the "kleur stadia tomaten" from The Greenery (www.thegreenery.com) and transported immediately after harvesting and selection to Wageningen, The Netherlands. The same day, the fruits were washed in cold tap water in a sanitised room and stored overnight at room temperature.

The next day, fruits similar in colour, shape and size were sliced and packaged as described in experiment 1 and stored at 5 ± 0.5 °C, 9 ± 0.7 °C or 13 ± 0.7 °C. As controls, intact fruits were stored at the same conditions. For each combination of temperature x processing (intact or sliced) x storage time, 6 replicates corresponding to a tray with 1 fruit were analysed. Only the second slice from the bottom of the stack was used. Because most of the changes in appearance of sliced tomato in previous

experiment occurred early during storage, the measurements were performed immediately after processing, before cooling, and then every day for 4 more days after storage. Intact fruits were sliced immediately before evaluation, in the same way the other fruits have been sliced previously. Temperature data were recorded by an 8 channel thermocouple with a personal computer interface.

2.2. Video Image Analysis

The digital images were obtained using an image processing system consisting of a 3 CCD Hitachi HV-C20 video camera with a Tamron SP 35-80mm objective, a computer and a lightning chamber. The samples were placed under diffuse illumination provided by four fluorescent tube lamps (TL-D 18W/84) positioned in the higher part of the chamber.

The images were later analysed using the KAS Software, developed by Agrotechnology and Food Innovations, NL. A colour learn set was created to enable the distinction between the background and the tomato slice. The slices were placed in such a way that half of the slice was over a white background and half the slice over a black background. Because the colour learn set used was not able to differentiate between translucent pericarp in black background and locular gel, the pericarp area (including outer and radial pericarp) was isolated from the rest of the image manually. The data were expressed as the average intensities per pixel of Red (R), Green (G) and Blue (B) for the white background (wb) and for the black background (bb) separately.

To avoid the confounding effect of tissues with different optical properties (columela, locular gel with seeds) only the pericarp was evaluated in the present study.

2.3. Translucency Visual Evaluation

Before being photographed the slices evaluated visually and graded in relation to the intensity of translucency, according to the scale shown in Table 1.

Table 1 – Translucency scale for visual assessment.

Grade	Description
0	Not translucent.
1	Incipient translucency in the whole pericarp or translucent spots in the inner pericarp and/or outer pericarp.
2	Light translucency in the whole pericarp.
3	Moderate translucency in the whole pericarp.
4	Intense translucency in the whole pericarp.

2.4. Model development

2.4.1 The mechanism

The model developed in the present study is an extension of the previously presented model on changes in RGB colour aspects of fresh cut tomatoes during refrigerated storage (Lana *et al*., 2004). According to that model, all colour aspects (R, G and B) change according to a first order mechanism, which results in an exponential decay towards an end value at infinite storage time. In the present extension of that model, two separate processes are considered; one of colour change due to the production or degradation of pigments (maturity), and one of physical change from opacity to translucency development due to flooding of intercellular spaces. Both processes are reflected in changes in the RGB values as will be later detailed.

Changes in the appearance of tomatoes after cutting and during storage will be the result of these two processes that clearly differ with respect to the time at which they start. The colour change due to maturation is a continuation of the same (or very similar) mechanism that happens in the plant (Lana *et al*., 2004) while the process of increasing translucency starts from the moment the tomatoes are cut into slices. So the complete but massively simplified mechanism can be represented as:

$$cc \xrightarrow{k_c} ripening$$
$$ST + st \xrightarrow{k_t} tr + st \qquad \text{Eq. 1}$$
$$\mathrm{S} + \mathrm{st} \xrightarrow{\mathrm{k_s}} 2 \cdot st$$

Where:

cc = concentration of colouring compounds (pigments).

kc = rate constant of pigment production and/or degradation.

ST = potential intensity of translucency.

st = susceptibility of the tissue to become translucent.

kt = rate constant of translucency development.

tr = actual translucency.

S = potential level of susceptibility of the tissue to become translucent.

ks = rate constant of translucency susceptibility development.

The first reaction represents the change in colour aspects during maturation due to the production or degradation of pigments (cc) that will result in changes from green to red colour. The second reaction represents the changes in colour aspects due to the development of translucency (tr) (i.e. physical water-soaking). ST represents the potential amount of translucency that can be converted into actual translucency (tr). Both processes will result in changes in the appearance of the product that will be reflected in changes in each one of the colour aspects R, G and B when using video image analysis to assess optical properties (Lana *et al*., 2004). The third reaction represents the sensitivity to

translucency increasing with increasing maturation. The term S refers to the potential level of susceptibility of the tomato slice that will be eventually converted in st which represents the actual susceptibility of the tissue to become translucent. It is assumed that translucency develops in parallel with the normal maturation process, and is therefore represented by the autocatalytic mechanism that eventually results in a logistic behaviour (Tijskens and Evelo, 1994; Schouten *et al*., 1997).

2.4.2. Development of translucency

Based on the mechanism described by Eq. 1, and assuming that translucency only occurs in cut fruits, the analytical solution of the appearance of translucency can be derived as:

$$tr = (tr_{max} - tr_0) \cdot (1\text{-}e^{-k_t \cdot st_0 \cdot t}) + tr_0 \qquad \text{Eq. 2}$$

Where

tr = translucency grade as measured by visual evaluation at time t.

tr_{max} = maximal value translucency can take on the visual score.

tr_0 = initial translucency at the moment of cutting.

k_t = rate constant of the development of translucency on the visual scale.

st_0 = the sensitivity as developed during maturation at the plant at the moment of cutting.

t = time.

According to this model, the translucency increases exponentially with time towards a maximal end value (tr_{max}). The factor st_0 expresses the sensitivity to translucency development increasing with increasing maturity at harvest, while the rate constant is the same for all stages. The initial level of translucency (tr_0) was fixed to zero, since no translucency is observed in intact fruits, before cutting. Eq. 2 expresses translucency on its own scale, that is, the visual scale, which express the visual intensity of translucency. To express the development of translucency in terms of changes in the colour aspects RGB (or the intensity of translucency in terms of RGB values) this equation has to be converted.

2.4.3 Development of colour due to maturation

The changes in composition and / or concentration of colouring pigments due to maturation can be represented as in Eq. 3.

$$cc = cc_0 \cdot e^{-k_c \cdot t} \qquad \text{Eq. 3}$$

where

cc = concentration of colouring pigments at time t.

cc_0 = initial concentration of colouring pigments at time zero.

k_c = rate constant for the change of concentration of colour pigments.

These changes will be reflected in changes in the RGB values, so that fruits harvested at different stages will differ with respect to the initial level cc_0 and eventually in respect to the level cc at any time t. However, the RGB values also depend on the development of translucency. For translucent samples it also depends on the background used during measurement, as will be described later. To accommodate for the difference in expressions (chemical concentration of pigments expressed in concentration units versus physical properties expressed in RGB values) Eqs. 2 and 3 have to be converted into a set of differential equations, which can be solved for constant external conditions as applied in the present experiments. This in fact represents the conversion from the chemical to the physical expression of this process. The simplest conversion is a linear transformation (see section 4.5). A constant final value (at infinite time) was added to account for the invariable part in the RGB. When both converted Eqs. 2 and 3 are combined, it will be possible to describe the changes in appearance resulting from both processes in terms of RGB values (see section 2.4.5).

2.4.4. Effects of temperature

To study the effect of temperature (Experiment 2) the same models (Eq. 2 and Eq.3) were used, but now all reaction rate constants were assumed to depend on temperature according to Arrhenius law.

$$k_i = k_{i,ref} \cdot e^{\frac{Ea_i}{R}\cdot\left(\frac{1}{T_{ref}}-\frac{1}{T}\right)} \qquad \text{Eq. 4}$$

All the references to stage of maturity were removed from the analysis, since only one stage was available.

2.4.5. Combining colour and translucency - Overall model on colour aspects

At the same time the translucency develops (on the visual scale) (Eq. 2), the values for the individual colour aspects R, G and B changes according to Eq. 3. Converting both equations to the physical dimension (RGB) by applying a linear transformation, results in the final expression:

$$ca = \left(ca_0 - ca_{fin}\right) e^{-\left(k_c + k_t \cdot st_0\right)t} + ca_{fin} \qquad \text{Eq. 5}$$

where

ca = condition for each colour aspect (R, G or B) at time t.

ca_0 = initial condition for each colour aspect at time zero. It depends on the maturity stage and on the background.

ca_{fin} = final value of each colour aspect at infinite time. It depends on the maturity stage and on the background.

k_c = rate constant for the change of colour aspect due to changes in the composition or concentration of pigments.

k_t = rate constant of the development of translucency on the physical scale. It depends on

the background (see Eq. 6).

st_0 = the sensitivity as developed during maturation at the plant at the moment of cutting. It depends on the maturity stage.

Additionally, during maturation at the plant, the tissue gets increasingly susceptible to water-soaking and translucency development, expressed as increasing levels of the susceptibility st. This eventually results in different ranges of ca_0 - ca_{fin} for different maturity stages. So, both ca_0 and ca_{fin} depend strongly on the maturity at harvest. Since intact fruit did not develop significant signs of translucency, the compound rate constant kt was fixed to zero for intact fruits (Eq. 6, 2nd line).

$$kt = kt \quad \text{if cut}$$
$$kt = 0 \quad \text{if intact} \qquad \text{Eq. 6}$$

Due to the expected interaction between the process of colour change and the development of translucency, both affecting the measured RGB values, the system of combining all data over the stage of maturity, as presented in previous papers (Lana *et al*., 2004; Lana *et al*., 2005) could not be applied here. All initial and final values had to be estimated separately for each stage of maturity. It was not possible either to pool all the data in one integral analysis as in Lana *et al*. (2004) since the separate colour aspects (R, G and B) did not show the same behaviour.

Based on the first analysis of all data combined, using Eq. 5, estimating the initial and final values separate for each background and stage of maturity, while estimating the rate constant and the sensitivity in common, it was noticed that the difference between both backgrounds was very similar in value for all three stages of maturity (see section 3.2.2). That would mean that the difference between black and white backgrounds is the same for all three stages of maturity. Combining this information into the model finally applied in the regression analysis produces Eq. 7. For the white background, the values for the differences can be taken as zero, for the black background the estimated values can be applied.

$$ca = \left(\left(ca_0 - ca_{0,dif}\right) - \left(ca_{fin} - ca_{fin,dif}\right)\right) e^{-(k_c + k_t \cdot st_0)t} + \left(ca_{fin} - ca_{fin,dif}\right) \qquad \text{Eq. 7}$$

where

ca = condition for the colour aspect (R, G or B) at time t.

ca_0 = initial condition for the colour aspect on a white background at time zero. It depends on the colour aspect (RGB) and on the stage of maturity.

$ca_{0,dif}$ = initial difference between white and black background at time zero.

ca_{fin} = final value of the colour aspect at infinite time on a white background. It depends on the colour aspect (RGB) and on the stage of maturity.

$ca_{fin,dif}$ = final difference between white and black background at time zero.

Notation

Name	Dimension	Meaning
ca	-	colour aspect (R, G, B)
cc	arbitrary	concentration of colouring pigments
Ea	kJ/mol	activation energy
kc	day^{-1}	rate constant of change of colour aspect due to pigments
kt	day^{-1}	rate constant of change of colour aspect due to translucency
R	J/mol/K	universal gas constant (8.314 J/mol/K)
T	day	Time
T	K (^{o}C)	Temperature
Subscripts		
0		initial
b		black background
fin		invariable part at infinite time
I		of stage I
II		of stage II
III		of stage III
ref		at reference temperature (= 10 ^{o}C)
w		white background

3. Results and Discussion

3.1. Raw data

The change from opacity to translucency in the pericarp was the main change in the appearance of tomato slices during refrigerated storage, for all stages of maturity (Experiment 1) and storage temperatures (Experiment 2). Changes in colour were much less pronounced and related to a small increase in redness for all stages. The development of translucency was not observed in intact fruits except for few replicates in Experiment 2 which showed small translucent spots in the pericarp (intensity always equal or lower than 1). No clear effect of temperature or storage time was apparent in this case.

Both the rate of development and the intensity of translucent were very dependent on the stage of maturity. The riper the fruit the faster it became translucent and the more intense was the translucency (Figure 1). In Experiment 2, a tendency to an increase in translucency with an increase in temperature was observed in the first days of storage, but after 4 days all treatments had attained about the same grade (Figure 2). The effect of time after cutting was by far more important than the effect of temperature. The same temporal pattern of change in translucency in cut fruits was observed

at all temperatures, that is, a sharp increase in the first day, followed by a small and gradual increase afterwards towards a common end value. The development of translucency was similar to what was observed on Experiment 1 for maturity stage III, that is, after 1 day storage most of the slices were already moderately translucent.

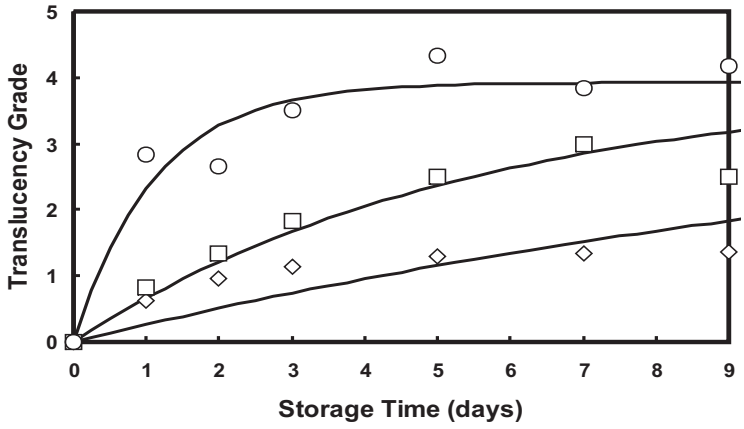

Figure 1 – Translucency grade of cut tomato slices obtained from fruits harvested at three maturity stages. Symbols represent measured values for ◇ (stage I), □ (stage II) and ○ (stage III). Solid lines represent values simulated according to Eq.2 and the parameters values in Table 3.

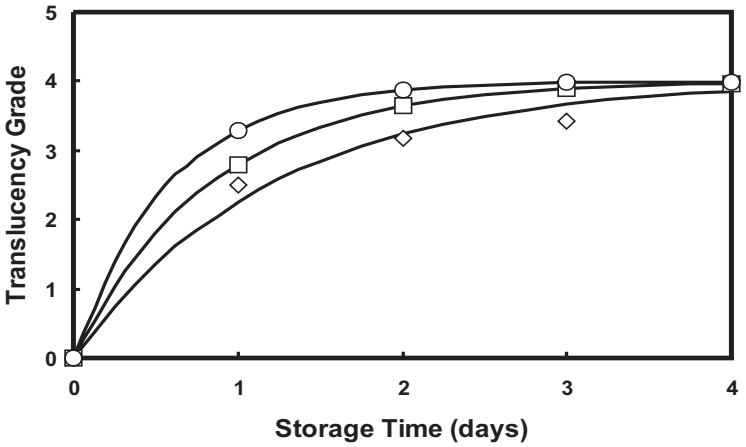

Figure 2 - Translucency grade of cut tomato slices stored at 5 °C (◇), 9 °C (□) or 13 °C (○) Symbols represent measured values and solid lines represent values simulated according to Eq. 2 and 4 and the parameters values in Table 4.

Differences in colour due to maturation (initial condition in Experiment 1) were expressed in differences in the RGB values, with lower values the riper the fruit, on both backgrounds. The changes from opacity to translucency were also accompanied by changes in the values of the RGB colour aspects. In general, the RGB values decreased along storage for cut fruits and remained practically constant for intact fruits on both experiments. Changes on black background were much more pronounced than on white background, although in both cases the temporal pattern of change was quite similar. Differences between both backgrounds were much larger for the Red colour aspect compared with Green and Blue aspects.

3.2. Modelling

3.2.1. Translucency versus time

Based on Eq.2, the data of translucency intensity, graded visually on a scale from 0 to 4 were analysed. The value of st for the ripe harvested samples (st_{III}) was fixed to 1 and the st for the other two stages together with k_t were estimated. The results are shown in Table 2 and Fig.1. The increasing values of st from stages I to III confirms the observation that the intensity of translucency is higher the more ripened the fruit.

Table 2 – Results on non-linear analysis of translucency development in cut tomato slices as a function of fruit maturity stage at harvest.

	Mean		Individual	
	estimate	s.e.	estimate	s.e.
tr_0	0	fixed	0	fixed
tr_{max}	3.924	0.2	3.924	0.208
st_I	0.0778	0.0195	0.0778	0.0203
st_{II}	0.2059	0.0453	0.2059	0.0472
st_{III}	1	fixed	1	fixed
k_t	0.897	0.201	0.897	0.21
R^2	93.3		67.2	
Nobs	21		125	

Assuming a temperature dependence of the rate constant k_t (Eq.4), the data of the Experiment 2 were also analysed using Eq. 2 and the results are shown on Table 3 and Fig.2. The dependence of temperature was low, but significant with an Ea of about 60 kJ/mol, indicating a moderate increase in rate constant with temperature. The estimated values for the rate constant k_t (both at 5 ^oC) in Experiments 1 and 2 are very similar, certainly considering that the maturity stage in the second experiment was slightly lower that the most ripe harvested tomatoes in the first experiment.

Table 3 – Results on non-linear analysis of translucency development in cut tomato slices as a function of storage temperature.

	Mean		Individual	
	estimate	s.e.	estimate	s.e.
tr_0	0	fixed	0	fixed
tr_{max}	4	fixed	4	fixed
kt_{ref}	0.8304	0.0986	0.7739	0.097
Eat	60.4	19	62.1	19.9
R^2	97.2		79	
Nobs	15		90	
T_{ref}	5		5	

3.2.2. Effect of maturity stage

In the first analysis estimating all initial and final values of ca for both backgrounds (based on Eq. 5), it was observed that the differences between the initial and final values on the white and the black background were roughly constant, especially when taking the standard errors of estimates into consideration. Exception is made for a small deviation for Green value on black background (see Table 4). If this were true, it would imply that the differences between backgrounds of each colour aspect could be estimated in common for all stages of maturity. This is not unlikely, since the difference between black and white backgrounds is defined by the physical difference in the relative proportion of light scattering and light absorption, while the tissue structure remains virtually the same. In the further analyses, this information was used, and only the difference estimated for all three stages of maturity were considered.

Table 4 – Difference in initial and final condition between white and black background for mean values (top) and individual values (bottom). Experiment 1.

	W-B Mean		
	R	G	B
ca_{0I}	34.21	21.88	13.55
ca_{0II}	39.37	16.74	13.99
ca_{0III}	37.12	10.39	11.77
ca_{finI}	41.75	20.71	14.47
ca_{finII}	51.58	14.62	13.66
ca_{finIII}	53.92	16.04	12.32
	W-B Individual		
	R	**G**	**B**
ca_{0I}	34.21	21.87	13.56
ca_{0II}	39.26	16.69	13.94
ca_{0III}	37.13	10.38	11.78
ca_{finI}	41.75	20.78	14.48
ca_{finII}	51.56	14.71	13.64
ca_{finIII}	53.91	16.06	12.38

Considering the fact that the translucency effects on RGB values is a physical one, the rate of development of translucency should be the same, irrespective of the measuring method (visual evaluation or video image analysis) and scale (grade 0-4 or RGB values) applied. Taking this into consideration, the value obtained for the rate constant k_t in the visual translucency as well as the values for the sensitivity st (Table 2) were used in the analysis of RGB behaviour based on Eq. 7.

The parameter estimates and the fitness of the model obtained in both analyses (Eq. 5 versus Eq.7) were virtually the same (data of first analysis not shown), indicating that this assumption was a reasonable one and that the first model was over parameterised.

. **Table 5 – Results on non-linear regression analysis based on Eq. 7.**

	Mean					
	R		**G**		**B**	
	estimate	s.e.	estimate	s.e.	estimate	s.e.
ca_{0I}	180.241	0.857	152.65	1.44	74.883	0.689
ca_{0II}	179.848	0.842	124.82	1.43	69.412	0.639
ca_{0III}	170.161	0.897	97.86	1.41	60.704	0.647
Ca_{finI}	162.34	3.99	114.11	3.85	61.22	2.76
Ca_{finII}	167.42	2.04	97.84	2.23	59.49	1.45
Ca_{finIII}	157.93	1.4	81.05	1.59	52.676	0.998
ca_{0_dif}	37.265	0.846	17.72	1.31	13.093	0.548
ca_{fin_dif}	50.1	1.8	18.43	1.96	12.91	1.27
st_I	0.0778	0.0195	0.0778	0.0195	0.0778	0.0195
st_{II}	0.2059	0.0453	0.2059	0.0453	0.2059	0.0453
st_{III}	1	fixed	1	fixed	1	fixed
k_c	-0.03009	0.00584	0.0552	0.0105	-0.05174	0.00841
k_t	0.897	0.201	0.897	0.201	0.897	0.201
R^2_{adj}	97.7		97.5		94.7	
N_{obs}	84		84		84	
	Individual					
	R		**G**		**B**	
	estimate	s.e.	estimate	s.e.	estimate	s.e.
ca_{0I}	180.2	0.705	152.67	1.34	74.881	0.521
ca_{0II}	179.739	0.702	124.95	1.36	69.43	0.489
ca_{0III}	170.126	0.737	97.87	1.3	60.699	0.488
ca_{finI}	162.32	3.28	114.18	3.55	61.17	2.08
ca_{finII}	167.42	1.68	97.78	2.07	59.49	1.09
ca_{finIII}	157.93	1.15	81.06	1.47	52.667	0.752
ca_{0_dif}	37.232	0.699	17.73	1.22	13.067	0.414
ca_{fin_dif}	50.09	1.48	18.43	1.81	12.895	0.956
st_I	0.0778	0.0203	0.0778	0.0203	0.0778	0.0203
st_{II}	0.2059	0.0472	0.2059	0.0472	0.2059	0.0472
st_{III}	1	fixed	1	fixed	1	fixed
k_c	-0.03035	0.00483	0.05552	0.00978	-0.05149	0.00635
k_t	0.897	0.21	0.897	0.21	0.897	0.21
R^2_{adj}	91.2		88.3		83.8	
N_{obs}	500		500		500	

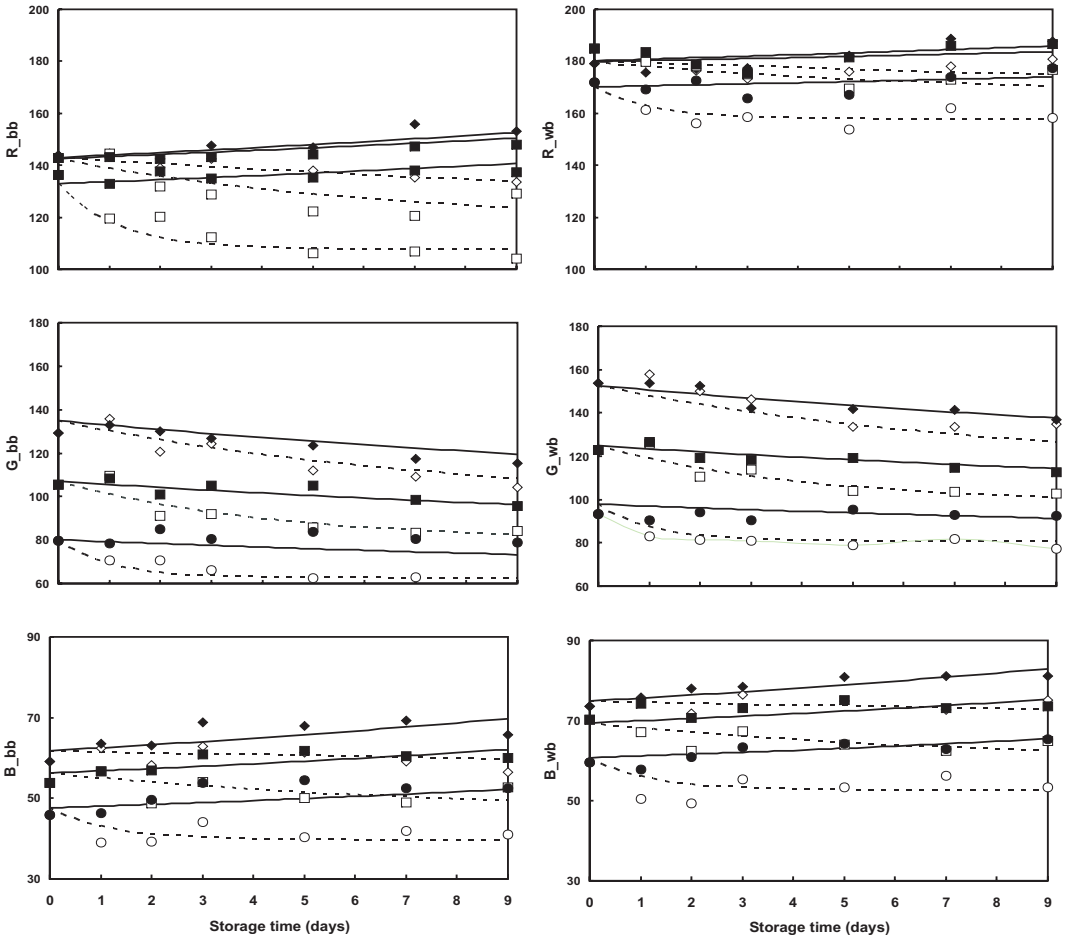

Figure 3 – Red (R), Green (G) and Blue (B) values on black (bb) and white (wb) background of intact (black symbols) and cut (white symbols) tomato fruit harvest at successive maturity stages I (◆ ◇), II (■ □) and III (● ○) and stored at 5°C. Symbols represent measured values and lines represent values simulated according to Eq.7 and the parameters values in Table 5.

In Table 5 the results of the non-linear regression analysis are shown for all three aspects (R, G and B) for the mean values as well as for the individual values. The terms ca_0 and ca_{fin} represents the value of the colour aspect in the white background. The correspondent value in the black background can be obtained subtracting from these values respectively the ca_{0_dif} and the ca_{fin_dif}, that is, the difference between white and black background for the initial and final value of each particular colour aspect. The fact that a common difference between backgrounds could be estimated for all three stages is clear evidence that the development of translucency is not being expressed in the difference between backgrounds, as first hypothesized. When it would have been the case, ca_{fin_dif} would depend on the maturity stage.

The progressive maturation of the tomatoes at successive stages of maturity can be observed in the decreasing values of ca_{0I}, ca_{0II} and ca_{0III} for the white background and ca_{0I}, ca_{0II} and ca_{0III} minus ca_{0_dif} for the black background, for all three colour aspects. In contrast to previous findings (Lana *et al*., 2004), also the end values (ca_{fin}) did exhibit a similar behaviour, that is, they were lower the more mature the fruit (Table 5).

Along storage, a decrease was observed in all colour aspects on both backgrounds for all maturity stages (Fig.3). The change in all colour aspects due to changes in pigments composition or concentration (k_c) was consistently estimated around zero. Sometimes a small negative value was found (e.g. for R and B). This result is consistent with the effect of the low temperature applied in these experiments (5 °C) on the degradation of chlorophyll (Pandrangi and LaBorde, 2004) and production of lycopene (Dumas *et al*., 2003) the two main processes expected to contribute to changes in colour of tomato fruit. The value for k_t, is considerably larger than the estimated values for k_c (Table 5). According to these results, the changes in the RGB colour aspects during storage at this low temperature are mainly due to the development of translucency and not to maturation. The higher susceptibility to translucency the riper the fruit (condition of the fruit when processed) is expressed in the values of the sensitivity st. That means that the more ripened the fruit, the higher the actual rate constant (k_t*st) irrespective of the background.

The range over which each colour aspect does change (the difference between ca_{fin} and ca_0) is specific for each particular colour aspect (Table 6). Within a same background the R and B range seems to be independent of the stage of maturity. For the aspect G, however, the range decreases with maturation.The scatter plot over the two backgrounds for the three colour aspects is shown in Fig. 4. From this Figure, it is not only clear that the fit is indeed high, but also another interesting aspect is revealed, that has a very high bearing on the meaning and application of different ranges as discussed above. For the aspect B, a very small range can be observed, while all data point measured on white and black background are overlapping one another. For the aspect G, a large range shows up, but still the data point on white and black backgrounds are highly overlapped. For the colour aspect R, the range is somewhat smaller than for G, but a clear distinction is found for the black and the white background. That would corroborate the findings of the classical statistical analysis (Lana *et al*., in press) that reported that translucency was to be found in the R aspect solely. In the present analysis

Table 6 – Difference between initial value and end value (range of change) for each colour aspect and each maturity stage, based on the data in Table 5, mean values.

	R	G	B
		White	
I	17.90	38.54	13.66
II	12.43	26.98	9.92
III	12.23	16.81	8.03
		Black	
I	30.74	39.25	13.48
II	25.26	27.69	9.74
III	25.07	17.52	7.85

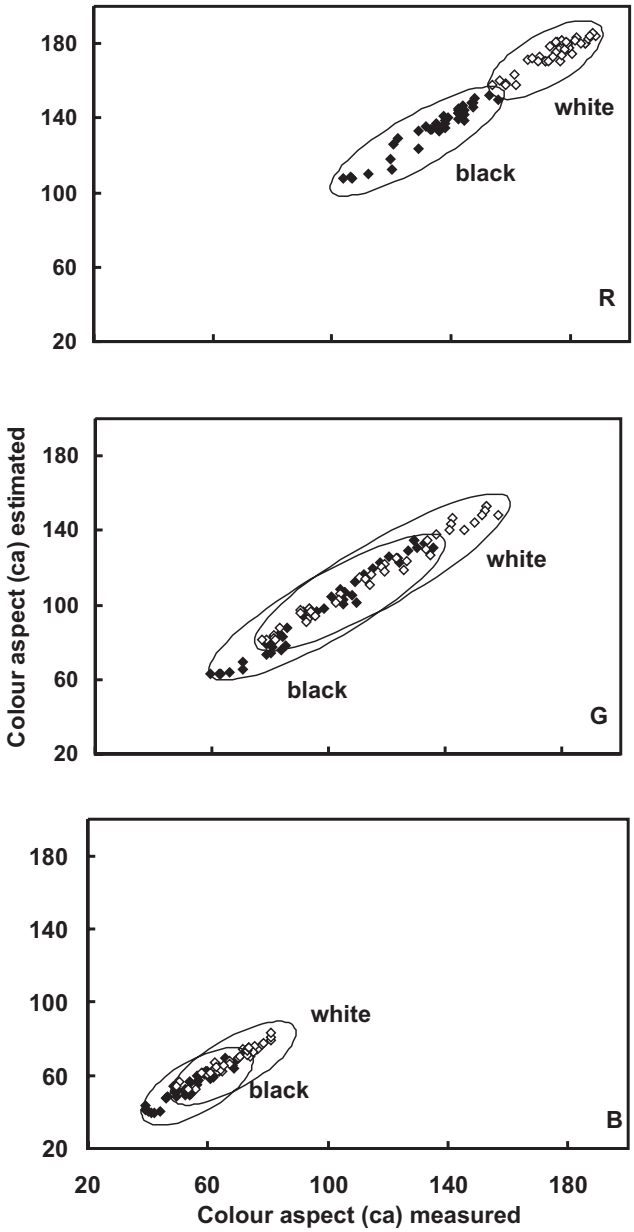

Figure 4 – Scatter plot of R (red), G (green) and B (blue) values over white and black background – Experiment 1.

based on a combined complex model, basically the information is that translucency pops up in all three aspects. Extracting this information would be however, limited to the R aspect only. The scatter plot over cut (translucent) and intact (non-translucent) fruit confirms this information (Fig.5). The B and G values for cut and intact fruit overlap each other while the R value despite a small overlap, is clearly different for intact fruits.

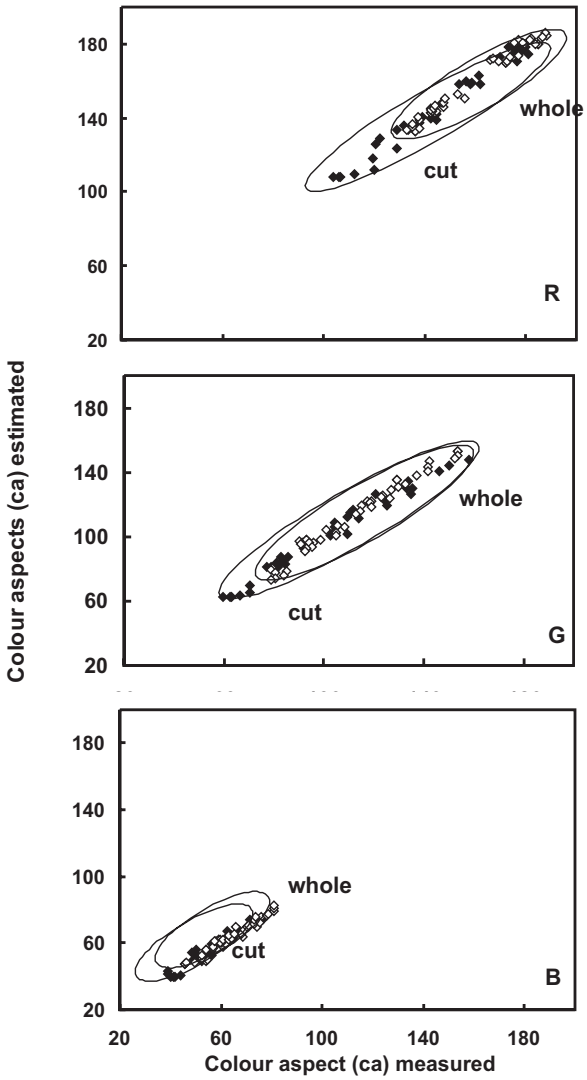

Figure 5 – Scatter plot of R (red), G (green) and B (blue) values over cut and intact tomato fruits – Experiment 1.

3.2.3. Temperature effects

The effect of temperature was assessed applying the same model used before (Eq. 7) together with Eq. 4. The results are shown in Table 7. As observed in the first experiment the changes in the RGB were basically due to the development of translucency. The values of k_c were around zero, whereas k_t was significantly higher for all colour aspects (Table 7).

The estimated differences between black and white backgrounds, for both the initial condition ($ca_{0\_dif)}$ as for the end value (ca_{f_dif}), were estimated in common for all temperatures (Table 7) as in Experiment 1. Again, these differences were considerably larger for the R aspect than for G and B.

Table 7 – Results of non-linear regression analysis to include temperature effects, based on Eq. 4 and 7.

	Mean					
	R		G		B	
	estimate	s.e.	estimate	s.e.		
ca_0	231.751	0.982	129.63	1.2	83.411	0.584
ca_{fin}	212.8	1.33	100.48	1.42	65.067	0.728
ca_{0_dif}	50.86	1.39	18.35	1.51	12.873	0.697
ca_{fin_dif}	77.96	1.89	23.94	1.63	13.863	0.836
kc_{ref}	0.0082	0.0132	0.0653	0.018	0.0289	0.0128
kt_{ref}	0.732	0.117	0.839	0.166	0.854	0.137
Eat	77.7	22.9	48.2	26.8	61	22.8
Eac	109	132	107	24.8	127.4	37.4
R^2_{adj}	98.8		95.1		96.8	
Nobs	60		60		60	
T_{ref}	5		5		5	
	Individual					
	R		G		B	
	estimate	s.e.	estimate	s.e.	estimate	s.e.
ca_0	231.751	0.867	129.63	1.19	83.411	0.51
ca_{fin}	212.8	1.18	100.48	1.41	65.067	0.636
ca_{0_dif}	50.86	1.23	18.35	1.51	12.873	0.609
ca_{fin_dif}	77.96	1.67	23.94	1.63	13.863	0.73
kc_{ref}	0.0082	0.0117	0.0653	0.0179	0.0289	0.0112
kt_{ref}	0.732	0.104	0.839	0.165	0.854	0.12
Eat	77.7	20.2	48.2	26.7	61	20
Eac	109	117	107	24.7	127.4	32.6
R^2_{adj}	94.6		76.2		86.6	
Nobs	360		360		360	
T_{ref}	5		5		5	

Both estimated activation energies were fairly reliable, except for the activation energy for the R aspect on the colour reaction (Eac). For the development of translucency the value for the activation energies were rather low. This indicates that the process of translucency development is rather independent of temperature. The activation energies for the colouring reaction have a low but not unusual value. Because only light-red tomatoes were used in this experiment not many changes in RGB due to maturation are expected and an accurate estimation of the temperature dependence of this process could therefore not be obtained. The results are graphically represented in Fig. 6. The scatter plot over both backgrounds is shown in Fig. 7. As observed in Experiment 1 the differences between backgrounds (and as well between cut and intact fruits) are expressed mainly in the R aspect.

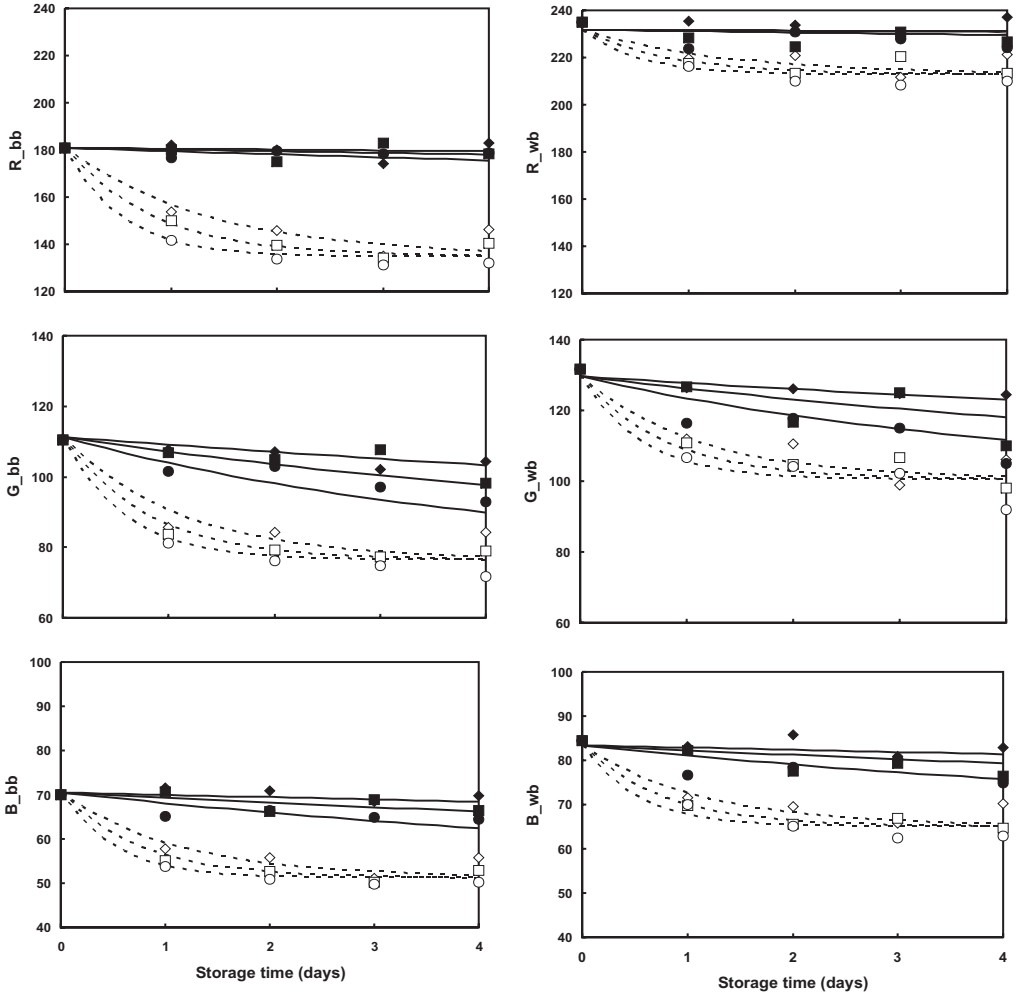

Figure 6 – Red (R), Green (G) and Blue (B) values on black (bb) and white (wb) background of intact (black symbols) and cut (white symbols) tomato fruit stored at 5 ^{o}C (◆ ◇), 9 ^{o}C (■ □) or 13 ^{o}C (● ○). Symbols represent measured values and lines represent values simulated according to Eq.7 and the parameters values in Table 7.

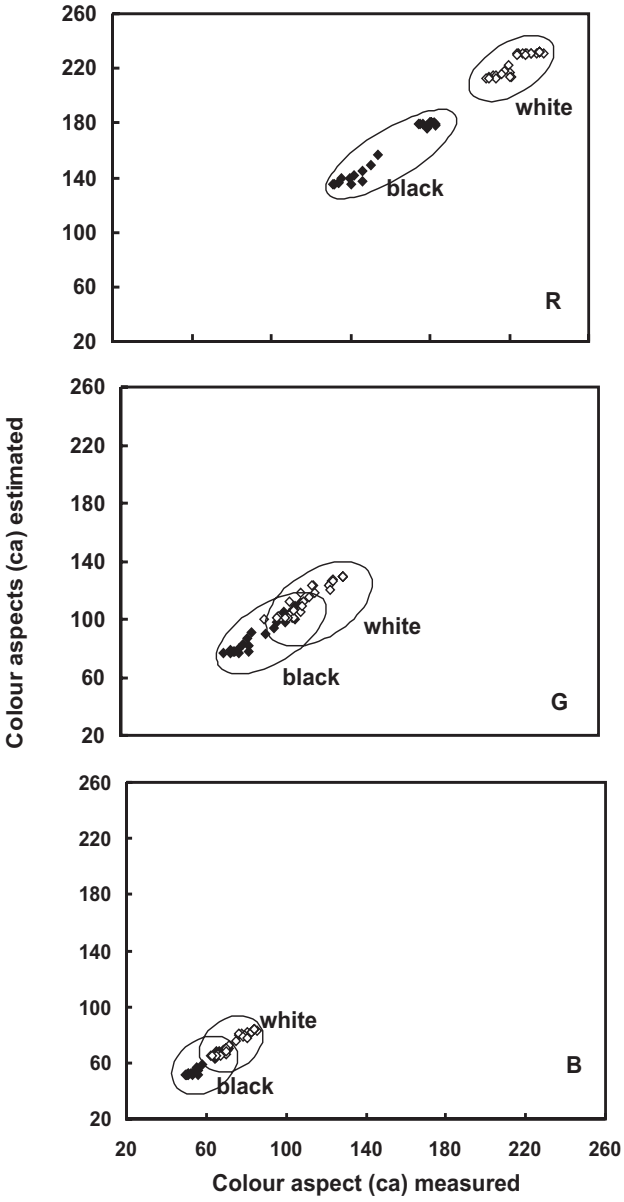

Figure 7 – Scatter plot of R (red), G (green) and B (blue) values over white and black background – Experiment 2.

4. Conclusions

The decrease in the values of R, G and B pixels during storage of cut tomatoes happens because the more translucent the sample the higher the proportion of light that is transmitted through the sample, absorbed by the black background, and consequently, not reflected back. Although all colour aspects share the same information regarding translucency development, the Red colour aspect is the one most pronouncedly affected by translucency and its value decreases significantly in translucent samples when they are placed on a black background. This happens because the Red component probably constitutes the component less absorbed by the tomato itself, being mainly reflected. When the sample is translucent it will eventually be absorbed by the background when it is black or reflected back when it is white. The fact that the B and G component also decreased when the sample is placed on a black background indicates that they are also affected by translucency, although in a lower extent compared with the Red aspect.

Both translucency and maturation results in decrease of the RGB values. It implies that decrease of RGB values during storage could be the result of both processes occurring simultaneously. The measurement on a split white and black background was done to separate both processes, so that changes in optical properties due to maturation would be expressed on the white background and due to translucency development on the black background. However, the physical changes that happens in the tissue and results in translucency can also results in changes in colour expressed in the white background that are not due to maturation but to a higher absorption of light due to a reduction in scattering. A more “complete picture “ on how the interaction of both processes is expressed in the changes of the RGB aspects remains to be elucidated.

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CHAPTER 8

EFFECTS OF CUTTING AND MATURITY ON LYCOPENE CONCENTRATION OF FRESH-CUT TOMATOES DURING STORAGE AT DIFFERENT TEMPERATURES

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Abstract

To investigate the changes in lycopene concentration of fresh-cut tomato during storage, tomato fruit at different stages of maturity were cut into 7 mm slices and stored at temperatures varying from 2°C to 16°C. To assess the effect of cutting, intact fruit were stored in an additional experiment at 5°C. Cutting did not change the accumulation of lycopene in fruit stored at 5°C, compared to intact fruit. The lycopene concentration of the tomato slices stored at different temperatures showed net increases of lycopene concentration for all maturity stages tested at temperatures of 8°C and higher. At lower temperatures no increases or small decreases were observed. The lycopene concentration was considered to be the net result of production and degradation of lycopene. Kinetic models for different pathways were tested. The reaction rate constant for lycopene formation at 8°C was estimated at $2.16 \pm 0.24 \cdot 10^{-2}$ µg/mg.day. The estimated activation energy for the lycopene formation (92.4 ± 7.3 kJ/mol) indicates that this process is highly dependent on temperature. The degradation rate constant was estimated at $2.99 \pm 0.61 \cdot 10^{-2}$ (1/day) and, over the temperature range studied, the degradation rate was not dependent on temperature.

Keywords: minimally processed, carotenoids, mathematical modelling, *Lycopersicon esculentum*

1. Introduction

Data on the concentration and retention of bioactive compounds in minimally processed fruit and vegetables are sparse (Lindley, 1998). Fresh cut tissues are exposed to oxidative stress that can cause membrane damage and alter the composition and concentration of antioxidant compounds, including carotenoids (Chen and Djuric, 2001). Carotenoids are known to be susceptible to oxidation in the presence of light, oxygen and low pH, (Shi and Maguer, 2000) all conditions likely to occur when the fruit or vegetable tissue is cut. Cutting has been shown to induce an increase in the activity of lipoxygenase (Karakurt and Huber, 2003) that, in turn, can promote the co-oxidation of carotenoids (Biacs and Daood, 2000). Because the enzymatic systems are functional in fresh-cut fruit, the biosynthesis of pigments associated with maturation continues to occur during storage (Mencarelli and Saltveit, 1988; Campbell *et al.*, 1990). Thus, it is expected that both synthesis of lycopene due to maturation and degradation of lycopene due to oxidation occurs during storage of fresh-cut tomato.

The objective of this research was to investigate the changes in lycopene concentration of fresh-cut tomato during storage. The processes of lycopene production and degradation are expected to depend on the stage of maturity of the fruit at processing and on the storage temperature.

2. Materials and Methods

2.1. Harvesting, processing and storage

2.1.1. Experiment 1.

Tomatoes (cv. Durinta) grown in the same greenhouse in Wageningen (The Netherlands) were harvested in a single day in October 2002 according to five colour stages, corresponding to the

following grades of the tomato colour scale (ref. The Greenery): I = grade 3-4; II = grade 5; III= grade 7; IV = grade 9; V= grade 11. After harvest, the fruit were sanitised with sodium hypochlorite solution (100 ppm) and sliced in 7-mm thick transversal slices. The third and fourth slice from the stem end were taken and placed in a covered plastic petri dish and stored at 2°C, 5°C, 8°C, 12°C, and 16°C. For each maturity x temperature x storage time combination, 3 petri dishes with 2 slices from a same fruit were analysed separately.

2.1.2. Experiment 2.

Tomatoes (cv. Belissimo) grown in the same greenhouse in Made (The Netherlands) were harvested in a single day in April 2003 in the following colour stages: I, II and IV. After harvest, the fruit were sanitised and cut as described for Experiment 1 and stored at 2°C, 5°C, 8°C, 12°C, and 16°C. The first and the last slice were discarded while the others were grouped in order to obtain one batch for each colour. For each maturity x temperature x storage time combination, 5 petri dishes with 3 slices taken at random were analysed separately.

2.1.3. Experiment 3.

Tomatoes (cv. Belissimo) were harvested at Made (The Netherlands) in May 2003 in the same colour stages described for Experiment 2. Groups of 6 fruit similar in colour, shape and size were grouped. Three were stored intact and three were sliced in 7-mm thick transversal slices. The first and last slices from the stem end were discarded while the following four were stacked in the same position they had in the fruit. For each maturity x temperature x storage time combination, 5 replicates corresponding to a tray with 3 fruit were analysed separately. Both sliced and intact tomatoes were stored at 5°C.

2.2. Sample Preparation

In all experiments slices from fruit at stages IV and V were analysed daily irrespective of temperature. Slices stored at 12°C and 16°C were analysed daily irrespective of stage of maturity. All the other combinations were analysed every other day. After removal from the storage chambers, the slices were frozen immediately with liquid nitrogen and kept at –20°C until freeze-drying. Until analysis, the freeze-dried and powdered samples were stored in the dark, under nitrogen atmosphere at 4°C (Experiment 1) or at –80°C (Experiments 2 and 3). In Experiments 1 and 3 the whole slice was analysed, while in Experiment 2 only the pericarp was analysed.

2.3. Lycopene Extraction and Analysis

In all the three experiments, the lycopene extraction was based on the methods described by (Epler *et al*., 1993), (Konings and Roomans, 1997) and (García-Plazaola and Becerril, 1999). The lycopene

concentration was measured by High Performance Liquid Chromatography (HPLC) with an Alltima C18, 3 μm, 100 mm x 4.6 mm (Alltech) column at 25°C. The mobile phase used was 75% methanol + 0.05 M ammonium acetate + 0.05% TEA and 25% ethyl acetate + 0.05% TEA. The flow rate 1 ml/min and detection of lycopene was at 470 nm.

2.4. Modelling

The lycopene concentration of the tomato slices was considered to be the net result of both production and degradation of lycopene according to the following three investigated reaction pathways:

$$\xrightarrow{k_1} L \xrightarrow{k_2} X \qquad \text{(A)}$$

$$S \xrightarrow{k'_1} L \xrightarrow{k_2} X \qquad \text{(B)}$$

$$\begin{array}{l} S \xrightarrow{k''_1} L \xrightarrow{k_2} X \\ \quad\;\; E \\ \quad\;\; \uparrow_{k_3} \end{array} \qquad \text{(C)}$$

Where:

k_1	reaction rate constant of lycopene formation
k_2	reaction rate constant of lycopene degradation
k_3	reaction rate constant of enzyme system formation
L	lycopene
X	lycopene degradation products
S	substrate for lycopene
E	enzyme system for lycopene formation

From the reaction pathways, sets of differential equations can be derived that describe the concentration of lycopene in time:

For pathway A:

$$\frac{\mathrm{d}L}{\mathrm{d}t} = k_1 - k_2 L \qquad (1)$$

For pathway B:

$$\frac{\mathrm{d}S}{\mathrm{d}t} = -k'_1 \cdot S \qquad (2)$$

$$\frac{dL}{dt} = k'_1 S - k_2 L \quad (3)$$

For pathway C:

$$\frac{dE}{dt} = k_3 \quad (4)$$

$$\frac{dS}{dt} = -k''_1 E \cdot S \quad (5)$$

$$\frac{dL}{dt} = k''_1 E \cdot S - k_2 L \quad (6)$$

k_1 rate constant formation of lycopene
[k_1: µg/mg.day; k'_1: 1/day; k''_1: mg/(µg day)]

k_2 rate constant degradation of lycopene [1/day]

k_3 rate constant formation of enzyme system [µg/mg.day]

L lycopene concentration [µg/mg]

S substrate concentration [µg/mg]

E enzyme system concentration [µg/mg]

t time [day]

The conversion of *S* into *L* is on an equimolar basis. Since the actual substrate(s) for lycopene have not been taken into consideration, the units are expressed in µg and not in moles.

Reactions are assumed to be temperature dependent as described by a rearranged Arrhenius equation:

$$k = k_{\text{ref}} \exp\left(\left(\frac{E_a}{R}\right)\left(\frac{1}{T_{\text{ref}}} - \frac{1}{T}\right)\right) \quad (2)$$

k_{ref} rate constant at reference temperature ($k_{1,ref}$: [µg/mg.day], $k_{2,ref}$: [1/day])

T_{ref} reference temperature [K]

E_a activation energy [J/mole]

R gas constant [J/(mole K)]

The reference temperature was set to 281 K (8°C), the median storage temperature that was applied.

Fitting of the data to the model was done using the software Athena Visual Workbench (www.athenavisual.com). The lycopene concentrations measured for the different experimental settings

(stage of maturity at harvest, cultivars and tissue – pericarp or whole slice) were treated as different responses to allow for their different initial concentrations of lycopene. The data from all temperatures were fitted simultaneously to the model using the determinant criterion (Stewart *et al*., 1992) to estimate the parameters ($L_{t=0}$, $S_{t=0}$, $E_{t=0}$, $k_{1,ref}$, $E_{a,1}$, $k_{2,ref}$, $E_{a,2}$, $k_{3,ref}$ and $E_{a,3}$) together with their confidence intervals.

3. Results and Discussion

3.1. Raw Data

Cutting did not change the accumulation of lycopene in fruit stored at 5ºC, compared to intact fruit (Fig. 1). After 9 days storage, the lycopene concentration of fruit at stage I and II hardly changed compared to the initial concentration of both intact and sliced fruit. The same was observed for stage IV after 5 days storage, although in this case a slight decrease was observed in intact fruit.

Despite the high variability in the lycopene data, there was a clear tendency for the lycopene concentration to increase with time at 8°C and higher (Fig. 2). This trend was more pronounced for the less mature stages (I-II). There was a tendency for the lycopene concentration of red fruit (stages IV and V) to decrease at temperature lower than 8°C. This does not eliminate the possibility that lycopene degradation occurs at other stages of maturity, since the concentration is a result of both synthesis and degradation.

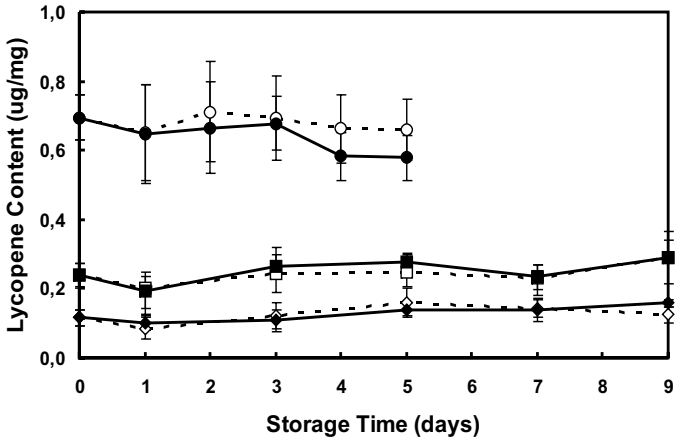

Figure 1 – Changes in lycopene content of sliced (dotted line, white symbols) and intact fruits (solid line, black symbols), harvested at successive maturity stages I (◆ ◇), II (■ □) and III (● ○) and stored at 5 ºC.

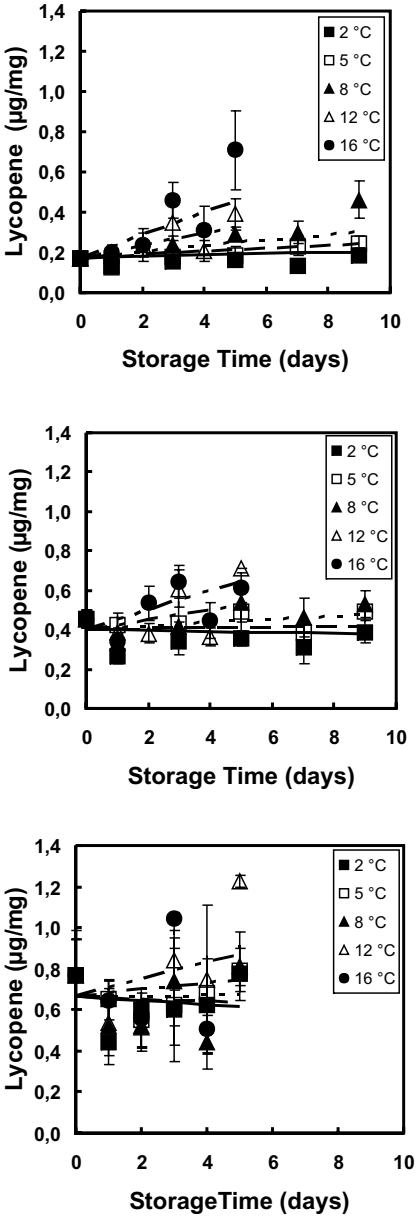

Figure 2 – Data and models fits of changes in lycopene content of fresh-cut tomato during storage at different temperatures as indicated in the legends. Points are means ± SD (n=5). Fitlines were obtained according to the kinetic model based upon pathway A. Tomato fruits were processed at stages of maturity A: stage I; B: stage II and C: stage IV.

Alternatively, lycopene could be protected by other antioxidants that are preferentially oxidised. Vitamin C and vitamin E have been shown to reduce the degradation of lycopene in the presence of lipoxygenase, suggesting that regeneration of lycopene by ascorbic acid occurs during the course of co-oxidation (Biacs and Daood, 2000). The stability of lycopene in the food matrix in conditions where pure lycopene should be easily oxidised was reviewed by Nguyen and Schwartz (1998) and Shi and Maguer (2000).

3.2. Modeling Lycopene production and degradation during storage

Simulations of the three proposed pathways (A, B and C), with hypothetical values for the rate constants, resulted in different patterns of lycopene accumulation during storage (Fig. 3). For pathway A, the simulated lycopene concentration increased with time but the apparent rate decreased at higher concentrations because of the increasing importance of the degradation process at higher concentrations. This effect was even more obvious in pathway B, due to the additional effect of decreasing concentrations of lycopene substrate after long storage time. In pathway C, there was a lag-phase in the formation of lycopene because the activity of the enzyme system is low in the beginning and only increases after some time during storage. In this case, the apparent formation rate also decreased with time due to the reduced concentration of substrate and the increasing importance of the degradation process at higher concentrations of lycopene.

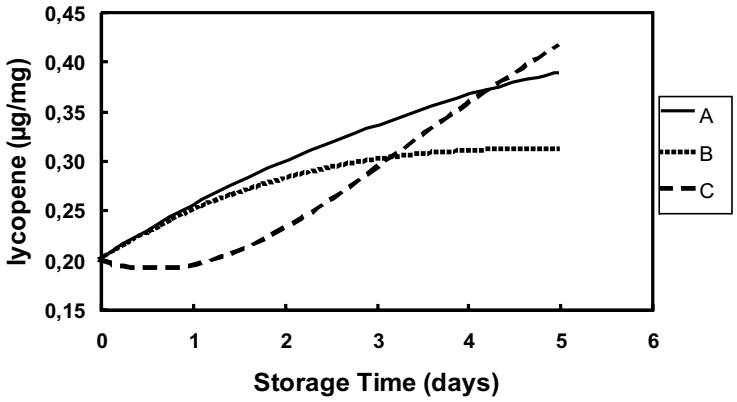

Figure 3 – Simulation of lycopene profiles in fresh-cut tomato slices during storage, using pathways A, B and C (see text for details on pathways).

Because of the observed high variability in the lycopene concentration of the tomato slices during storage it was not possible to select the most suitable pathway based on our data. In the present study, all pathway models were able to fit the data. However, because of the increasing number of parameters required when going from pathway A to B and then to C, the accuracy of the

parameter estimation was very low for model B and C. Therefore, it was decided to use the simplest pathway A in order to obtain kinetic parameters for lycopene formation and degradation as a function of temperature. In Fig. 2, the fits of the kinetic model based on pathway A to the data of Experiment 2 are shown. Similar graphs were obtained for Experiment 1.

The reaction rate constant for lycopene formation at 8 °C was $2.16 \pm 0.24 \cdot 10^{-2}$ µg/mg.day. The estimated activation energy for the lycopene formation (92.4 ± 7.3 kJ/mol) indicated that this process was highly dependent on temperature. In the temperature range investigated, the formation rate was approximately 4 times faster with 10°C temperature increase. The degradation rate had an estimated rate constant of $2.99 \pm 0.61 \cdot 10^{-2}$ (1/day). The confidence interval around the estimated value was about 20%, which is double the confidence interval for the formation rate constant. For the degradation reaction no temperature dependency could be observed ($E_a = 0$). For a chemical reaction this is unexpected, but it might be explained by the fact that this reaction is oxygen dependent. Because oxygen is less soluble at higher temperatures the overall reaction rate will not increase as much as if it was a constant, and in fact it does not seem to increase at all. For the degradation reaction of lycopene during storage of tomato pulp in air a very low temperature dependency has been reported (Sharma and Le Maguer, 1996). These authors found an increase of degradation rates of only 35% with 10°C temperature rise in the range of 20 to 25°C. To compare the actual degradation and formation rates, the degradation rate constant has to be multiplied by the actual lycopene concentration which starts at about 0.1 µg/mg. This always results in a net synthesis of lycopene at the early maturity stages. Since the degradation rate is not dependent on temperature, at temperatures higher than 8°C the formation rate will become increasingly dominant.

The data for cv. Durinta and cv. Belissimo were analyzed separately to investigate a possible cultivar effect on the rate constants. The degradation rate constants for both cultivars were very similar and well within their confidence intervals. There was a tendency for a 50% higher rate constant for lycopene formation for cv. Belissimo, but this difference was within the overlap of both confidence intervals so no definite conclusion about possible cultivar effect on formation could be drawn from our data. Difference in lycopene formation capacity in different cultivars is well known in literature. (Dumas *et al.*, 2003) reported difference in lycopene concentration of one to four folds between different cultivars.

Acknowledgements

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CHAPTER 9

EFFECTS OF CUTTING AND MATURITY ON ANTIOXIDANT ACTIVITY OF FRESH-CUT TOMATOES

Published as: M.M. Lana;L.M.M. Tijskens. Effects of cutting and maturity on antioxidant activity of fresh–cut tomatoes. Food Chemistry, in press.

Abstract

To investigate the changes in total antioxidant activity of fresh-cut tomato during storage, tomato fruits harvested at three different stages of maturity were cut into 7-mm thick slices and stored at 5 ºC. Intact fruits were stored in the same conditions as a control. The antioxidant activity was evaluated as the capacity to scavenge the radical $ABTS^{\cdot+}$ in both hydrophilic (HAA) and lipophilic (LAA) extracts. Cutting resulted in a decrease in the HAA compared to the control fruits and did not influence significantly the LAA. Changes in LAA during storage were described by a simple exponential model developing towards an asymptotic end value. The HAA also decreased exponentially in the beginning of the storage time but increased again afterwards. For both hydrophilic and lipophilic antioxidant activity the riper the fruit the higher was the antioxidant activity. Since no relevant interaction was found between time of storage and stage of maturity, the major factor determining the level of antioxidants in tomatoes, seems to be the initial level of antioxidant activity at the moment of harvest. The levels of HAA did not differ significantly due to maturation during storage while the LAA increased with maturation. These results indicate a potential effect of processing to decrease the antioxidant activity *in vivo*. This would represent a decrease in the value of cut tomatoes as a source of hydrophilic antioxidants in the diet compared with the fruit stored intact. In another experiment, realized at the same conditions, the total antioxidant activity was evaluated using a lipid peroxidation inhibition assay. Antioxidant activity could be measured in methanol extracts, but not in THF extracts. Aqueous extracts showed pro-oxidant activity. No effect of cutting or storage time was observed.

*Keywords***:** minimally processed; fresh-cut tomato; radical scavenging capacity; lipid peroxidation inhibition; *Lycopersicum esculentum***;** modelling

1. Introduction

The consumption of a diet rich in fresh fruits and vegetables has been associated with a number of health benefits including the prevention of chronic diseases (Klerk *et al.*, 1998; WHO, 2003). This beneficial effect is believed to be due, at least partially, to the action of antioxidant compounds, which reduce oxidative damage in the body. While the prescription of supplements containing antioxidants has resulted in contradictory results upon human health, the results from epidemiological studies comparing populations with different diets show a clear trend in reduction of chronic diseases when there is an increase in the consumption of fruits and vegetables (Klerk *et al*., 1998). Because of that, campaigns to increase the consumption of these products have been launched in many countries (http://www.5aday.com/html/international/intmembers.php).

The fresh-cut industry claims their product is a convenient and health alternative to fulfil the dietary needs for fresh food and many fast food companies are diversifying their menu in order to offer a range of ready-to-eat salads to their clients. However the many changes that happen in fruits and vegetables during harvesting, handling and processing can affect antioxidant status (Lindley, 1998). Data on content and retention of bioactive compounds in minimally processed fruits and vegetables are sparse (Lindley, 1998). Fresh cut tissues are primarily submitted to oxidative stress presumably causing membrane damage and altering the composition and content of antioxidant compounds resulting in changes in the total antioxidant activity of the tissue.

Decrease in the antioxidant activity after processing was reported for fresh-cut spinach (Gil

et al., 1999) and fresh-cut mandarin (Piga *et al.*, 2002). In both cases the antioxidant activity was measured as DPPH (2,2-diphenyl-1-picrylhydrazyl)-radical scavenging activity of methanol extracts. Wounding caused an increase in the antioxidant activity of Iceberg and Romaine lettuce measured as DPPH-radical scavenging activity (methanol extracts), or as FRAP -ferric-reducing antioxidant power assay (both methanol and phosphate buffer extracts) (Kang & Saltveit, 2002). The DPPH-radical scavenging activity of fresh-cut cactus fruits did not change during storage at 4°C for up to 9 days (Piga *et al.*, 2003). No reports on the antioxidant activity of fresh-cut tomatoes are known.

The content and composition of antioxidant compounds change during the maturation of tomato fruits and this is reflected in changes in the antioxidant activity (Jimenez *et al*., 2002; Cano *et al.*, 2003). If minimal processing operations result in changes in antioxidant activity, it is expected this will depend on the stage of ripeness of the fruit since the chemical composition of the fruit will be different.

One of the greatest difficulties in assessing the antioxidant activity in food products is the choice of the assay to be used. Antioxidants can act by different mechanisms namely inhibiting the generation of reactive species, directly scavenging them or raising the levels of endogenous antioxidant defences by up-regulating gene expression (Halliwell, 2002). Radical scavenging methods are the most widely used because they are sensitive and easy to perform. However the results must be interpreted cautiously since the ability of antioxidants to scavenge an artificial radical may not reflect the antioxidant activity *in vivo* nor its action against physiologically relevant radicals (Frankel & Meyer, 2000). Nevertheless, *in vitro* tests can indicate whether a given compound or food extract has potential to act as an antioxidant *in vivo* or alternatively that a direct antioxidant action in unlikely (Halliwell, 2002). The antioxidant activity of food or plant extracts can also be evaluated using biological model systems that mimic an *in vivo* situation, using biological membranes (Sluis *et al.*, 2000) and substrates present *in vivo* (Chang *et al.*, 2000).

The objective of the present research was to investigate the changes in total antioxidant activity of fresh-cut tomato during storage, using two methods. One that measures the scavenging of the radical cation ABTS-2,2'-azinobis (3-ethyl-benzothiazoline-6-sulphonate) and the other that measures the inhibition of ascorbate/iron induced lipid peroxidation of rat liver microsomes. The ABTS assay was chosen among the radical-scavenging assays because it allows to assess both hydrophilic and lipophilic extracts and because it has been successfully used to measure the antioxidant activity in fresh tomato and tomato products (Arnao *et al.*, 2001; Cano *et al*., 2003). The second method that uses rat liver microsomes as oxidizable substrate has the advantage of being close to an *in vivo* situation where both an aqueous and a lipid phase are present. This method has been used to measure antioxidant activity of methanol extracts of apple fruit, apple juice, polyphenol standards (Sluis *et al*., 2000; Sluis *et al.*, 2001) and methanol extracts of tomato products (Boxtel, 2002). In the present study this method was used to measure also aqueous and lipophilic extracts of tomato.

2. Materials and methods

2.1. Chemicals

Ascorbic acid and iron (III) sulphate heptahydrate were obtained from Merck. Thiobarbituric acid (TBA) was obtained from BDH chemicals. 2,2-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) as the crystallized diammonium salt was obtained from Biochemika. Horseradish peroxidase type VI (HRP), Trolox and trichloroacetic acid (TCA) were obtained from Sigma. All other chemicals were of analytical or HPLC grade purity.

2.2. Spectrophotometric measurements

Spectrophotometric measurements were recorded with a SLT spectrophotometer with a microtiterplate reader (lipid peroxidation assay) or with a USB 2000 Fiber Optic Spectrometer (ABTS assay), both interfaced on line with a PC-computer.

2.3. Experiment 1

2.3.1. Harvesting and processing

Tomato fruit (*Lycopersicon esculentum* cv. Belissimo) were harvest at Made (The Netherlands) in a single day in May 2003, in three colour stages, named here as I, II and III that corresponded respectively to stages 3, 5 and 9 in the tomato colour scale (kleur stadia-tomaten) of The Greenery (http://www.thegreenery.com).

After harvest, the fruits were washed in tap water, immersed for 60 s in sodium hypochlorite solution (1mg/l, pH 6.8) and then rinsed in tap water. Fruits similar in colour, shape and size were grouped in groups of 6. Three were stored intact and three were sliced in 7-mm thick transversal slices. The first and last slices from the stem end were discarded while the following four were stacked in the same position they had in the fruit in a white polystyrene tray (138 mm x 138 mm x 25 mm) and covered with a plastic film (Magnetron). For each maturity x temperature x storage time combination, 5 replicates, corresponding to a tray with 3 fruits were analysed separately. Both sliced and intact tomatoes were stored at 5 ± 0.5 °C. After slicing, but before cooling, 5 replicates from each maturity stage were analysed. Fruits from stages I and II were analysed every two days and from stage III every day in a total of 6 evaluations per combination. Temperature data were recorded by an 8 channel thermocouple with a personal computer interface.

2.3.2. Sample preparation

At the indicated intervals, the slices were removed from storage. One quarter of each slice was immediately frozen in liquid nitrogen. Intact fruits were cut into slices as described before, and

immediately frozen in liquid nitrogen. Samples were freeze-dried, grounded and stored at -80 °C until analysed as described below.

2.3.3. Extraction Procedure

During the whole extraction procedure the samples were handled in darkness or low light conditions. To an exact amount between 0.1 and 0.2 grams of tomato powder 3 ml of water was added. The samples were stirred in darkness for 30 minutes and centrifuged for 10 minutes at 7000 rpm (Hettich-Universal 16R). About 1 ml of the supernatant was transferred to Eppendorf tubes and centrifuged again for 10 minutes at 16400 rpm at 10 °C (Eppendorf centrifuge 5417R). The supernatant was used in the assay (water extract). The same procedure was repeated after the addition of 3 ml methanol to the remaining pellet (methanol extract), followed by the extraction with 10 ml of tetrahydrofuran –THF (THF extract). The THF extract was diluted 4 times with methanol to avoid damage to the plastic microtiter plates used in the antioxidant activity measurement. The obtained extracts were kept on ice in darkness until assayed (few hours).

2.3.4. Antioxidant Assay

The rat liver microsomes were thawed at room temperature, diluted at least 5 times with Tris-HCl/KCl buffer (50 mM, PH 7.4) and centrifuged for 1 hour at 28.000 rpm at 4 ºC. The pellet was resuspended in Tris-HCl/KCl buffer (50 mM, PH 7.4) to obtain a final concentration of 18.8 mg/ml protein. The 48-wells plates containing 210 µl (for water and methanol extracts) or 240 µl (for the THF extract) of diluted microsome per well were pre-incubated for 5 minutes at 37 ºC. After incubation 60 µl (water and methanol extracts) or 30 µl (THF extract) of the test sample was added. The lipid peroxidation was induced by the addition of 15 ìl ascorbic acid (4mM) and 15 ìl $FeSO_4$ (0.2 mM). After incubation for 60 minutes at 37 ºC, the reaction was stopped by the addition of 0.5 ml TBA/TCA-HCl (16.8% w/v TCA in 0.125N HCL) followed by incubation for 15 minutes at 80 ºC and centrifugation for 15 minutes at 2500 rpm. A 250 ìl aliquot of each sample was transferred to 96-wells plates and the absorption was measured at 540 nm (colour) and 620 nm (turbidity correction) with microtiterplate reader.

Control reactions were prepared by adding the solvent (water, methanol or THF:methanol (1:3)) instead of the tomato extract. Trolox (700 µM) was used as a positive control and controls without the microsomes were used to account for the interference of pigments from tomato in the assay.

2.3.5. Calculations

The antioxidant activity was expressed as IC_{50}: the concentration of the tomato extract at which the oxidation was inhibited in 50% in a relation to a blank. This value was determined from the inhibition versus concentration curves by a fitting procedure described by (Sluis *et al*., 2000).

2.4. Experiment 2

2.4.1. Harvesting, processing and storage

Tomatoes (cv. Belissimo) grown in a greenhouse in Made (The Netherlands) were harvested in a single day in April 2004 in the following colour stages: I, II and IV corresponding to the following grades of the tomato colour scale of The Greenery): I = grade 3-4; II = grade 5; III= grade 9. Selection of the fruits followed by sanitisation, processing, packaging and storage were done as described for Experiment 1.The first measurement was done one day after processing and then every other day in a total of 6 evaluations

2.4.2. Extraction

At the indicated intervals, the slices were removed from storage. Intact fruits were prepared in a manner similar to that described for cut fruits. Extraction was based on the procedure described by (Arnao *et al*., 2001). The tomato slices were homogenised using a domestic homogeniser Tristar for about 20s. An exact weight of the homogenate (around 1g) was immediately mixed with 2 ml of Na-phosphate buffer (pH 7.5) and 5 ml of ethyl acetate and crushed in a homogeniser Heidolph Diax 900 for 1 min. The homogenate was centrifuged at 6000 rpm for 5 minutes at 5 ^{o}C (Hettich-Universal 16R). About 1 ml of each phase was pipetted, transferred to an Eppendorf tube and centrifuged at 16.400 rpm for 5 min at 5^{o}C (Eppendorf centrifuge 5417R). The hydrophilic extract was analysed immediately. The lipophilic extract was stored at –80 ^{o}C overnight and analysed in the next morning.

2.4.3. *Antioxidant Activity Assay*

Antioxidant activity was measured using a modified version of the ABTS/HRP decolouration method as described in (Arnao *et al*., 2001). For the hydrophilic antioxidant activity (HAA), the reaction mixture contained 2 mM ABTS, 20 nM HPR, 60 ìM H_2O_2, in 50 mM Na-phosphate buffer (pH 7.5) in a total volume of 2 ml. The reaction was monitored at 730 nm for 1 minute to check whether the absorbance was stable. Then, 60 µl of the aqueous extract was added to the reaction medium and the decrease in absorbance, which is proportional to the amount of $ABTS^{\cdot+}$ quenched, was determined at intervals of 1 minute, for 5 minutes. For lipophilic antioxidant activity (LAA) the reaction medium contained 0,7 mM ABTS, 200 nM HRP and 60 ìM H_2O_2, in pure ethanol acidified with phosphoric acid (0.6 ìl/ml) in a total volume of 2 ml. The absorbance decrease was determined from the difference between the A_{730} values before and 5 min after sample addition. Antioxidant activity was calculated as moles of $ABTS^{\cdot+}$ quenched by 1 mol of Trolox, based on the stœchiometric relationship that 1 mol of ABTS quenches 2 mol of Trolox (Arnao *et al*., 2001). In both cases, the antioxidant activity was expressed as Trolox equivalents per 100 g of tomato fresh weigh. Total antioxidant activity (TAA) was obtained by the sum of HAA and LAA.

2.5. Internal package Atmosphere

To assure that the cut and intact fruits were stored in the same atmospheric conditions a very permeable film, (recommended for use in microwave cooking) was used. In preliminary tests this film protected the fruits and slices against dehydration without changing significantly the internal atmosphere in the package. After 1 week storage at 5ºC, the O_2 concentration changed from 19.70 ± 0.21% to 21.37 ± 0.22 % in the package containing intact fruits and from 19.71% ± 0.13 to 21.26 ± 0.46 % in the package containing sliced fruits. The CO_2 concentration changed from 0.083 ± 0.010% to 0.065 ± 0.010% in the package containing intact fruits and from 0.065% ± 0.004 to 0.091 ± 0.01 in the package containing sliced fruits.

2.6. Statistical Analysis

Data were first analysed by Analysis of Variance using PROC GLM from SAS (Statistical Analysis System) software considering stage of maturity, treatment (processing or not) and storage time as sources of variance. Later a non-linear regression analysis was conducted in order to study the changes on time, taking into consideration those factors and interactions that were significant in the analysis of variance. The model was developed as described in the next section and calibrated using the nonlinear regression procedure in GENSTAT (Rothamsted, UK).

2.7. Model development

The changes in the antioxidant activity (AA) of fresh-cut tomato slices was described and analysed with a simple exponential model (first order kinetics) developing towards an asymptotic end value. The AA was considered to be built up by a variable part that changes according to a first order mechanism and a fixed part that is invariable or is in equilibrium at the circumstances under study.

This resulted in the basic first order model as described in Eq. 1:

$$AA = (AA_0 - AA_{fix}) \cdot e^{-k \cdot t} + AA_{fix} \qquad \text{Eq. 1}$$

Where:

AA= antioxidant activity at time t after harvest

AA_0= initial antioxidant activity at harvest

AA_{fix}= invariable part of the antioxidant activity

k= reaction rate constant (at storage temperatures)

t= time (in days), counting from the moment of harvest.

In the present experiment, the tomato fruit were harvested from the same greenhouse at the same growing conditions but at different stages of ripeness. Unlike the development of colour (Lana

et al., 2004) and firmness (Lana *et al.*,2005), the level of antioxidants apparently starts to decay only after harvest, especially after processing. The hydrophilic antioxidant activity increased again at the later stages of storage. A linear increase was therefore added to the simple exponential model (Eq. 2). The increase in activity at the later stages of storage was dependent on the treatment. A separate value of k_l was therefore allowed for both treatments. To accommodate for the differences in the general level of antioxidant activity between the treatments (cut-whole) and the stage of development (breaker-pink-red) the asymptotic end value was made dependent on these factors.

$$AA = (AA_0 - AA_{fix}) \cdot e^{-k \cdot t} + AA_{fix} + \Delta AA_{stage} + \Delta AA_{treat} + k_{l,treat} \cdot t \qquad \text{Eq. 2}$$

Where:

$\ddot{A}AA_{stage}$= shift in AA depending on the stage of ripeness

$\ddot{A}AA_{treat}$= shift in AA depending on the treatment

$k_{l,treat}$= rate constant for the linear increase in AA (at storage temperatures), depending on the treatment applied

2.7.1. Data analysis

Based on equation 2, a non-linear regression analysis was performed (Genstat Rothamsted, UK). The data averaged over the 5 replicates were analysed without further transformation using maturity stage, treatment (cut or whole fruit) and time simultaneously as explaining variables (multi-variate non-linear regression analysis). The kinetic parameter (k), the invariable part of AA_{fix} and the initial value of AA_{var} were estimated in common for all the slices. All other parameters were estimated separately for each stage of maturity of treatment.

3. Results and discussion

3.1. Inhibition of Lipid Peroxidation

The water extract showed pro-oxidant activity in the lipid peroxidation assay without a clear effect of sample concentration. The THF extracts showed very flat responses curves and no accurate calculation of IC_{50} could be performed. Therefore, the results presented in Fig. 1 are those obtained with methanol extracts without a previous water extraction.

The antioxidant activity of tomato extracts, expressed as IC_{50} (Fig. 1) was affected by the maturity stage of the fruit (Pr > F= 0.0107) and storage time (Pr > F= 0.0160) but did not differ between cut and intact fruits (Pr > F= 0.8649). No interaction between these factors was significant. The averaged antioxidant activity increased from stage I to II and decreased again at stage III (Table 1). The significant effect of time was probably related with the shorter storage time for fruits at stage III

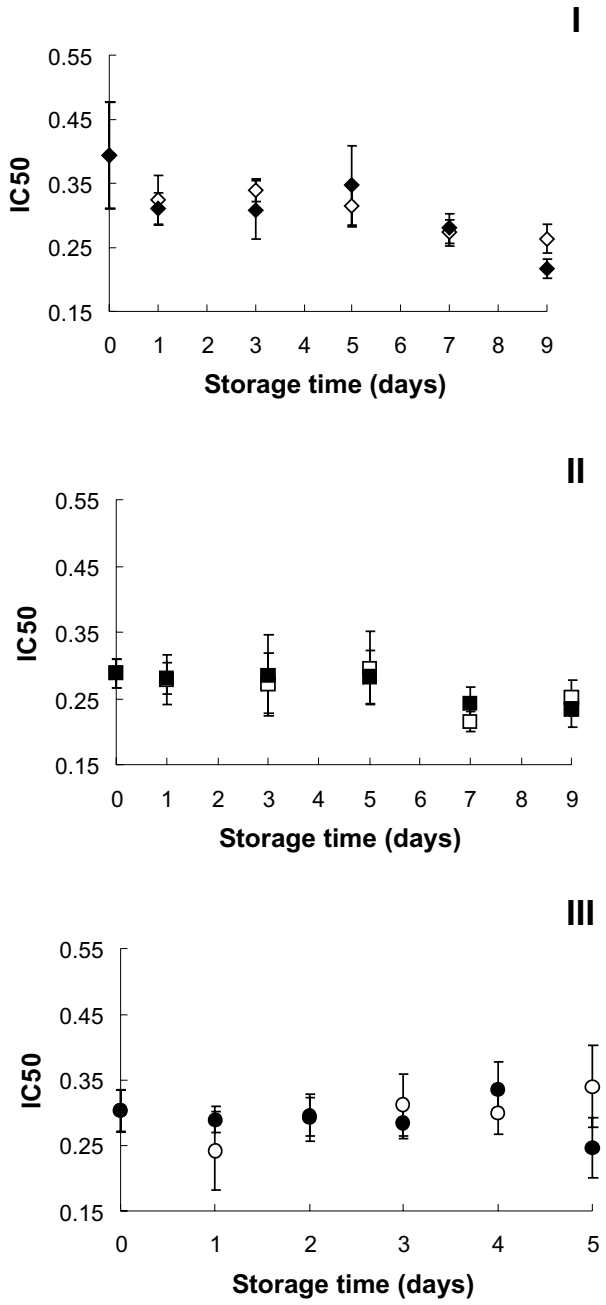

Figure 1 –Antioxidant activity of methanol extracts of sliced tomato harvested at stages I, II and III as indicated. The antioxidant activity is expressed as IC_{50}, which is the concentration in µg tomato dry weight / ml extractor that causes 50% inhibition of lipid peroxidation compared to the control. Open symbols refers to intact fruits and closed symbols to cut fruits. The values are the means of 5 replicates ± SE.

(5 days) compared to stages I and II (9 days). When the time interval was considered as number of sampling dates (6 for all stages), and not as days after processing, the effect of time was considerably smaller (Pr > F = 0.0613). But even in the first analysis the effect of stage was higher than that of storage time, with F values of 4.69 and 2.57 respectively.

Despite of the significant effects indicated by the ANOVA it was impossible to adjust any model to the data. The variation was quite large (Fig. 1) and no clear trend could be established especially in relation to the effects of storage time and stage of maturity.

Table 1 – Antioxidant activity of tomato methanol extracts expressed as IC_{50}, which is the concentration in µg tomato dry weight / ml extractor that causes 50% inhibition of lipid peroxidation compared to the control. The values are the means of 5 replicates, 2 treatments and 6 storage times.

Stage of Maturity	IC50
II	0.288 [a]
III	0.304 [a b]
I	0.393 [b]

Means followed by the different letters are significantly different at P=F < 0.05 by LSD test.

A pro-oxidant action of hydrophilic extracts from tomato samples was reported by Lavelli *et al.*(2000) who used a copper catalysed linoleic acid oxidation assay. However, aqueous extracts of fresh tomatoes showed antioxidant action in a copper catalysed phosphatidylcholine lyposome oxidation assay but no activity when the same extracts were used in a linoleic acid emulsion oxidation induced by endoperoxide (Takeoka *et al*., 2001). The pro-oxidant action reported by (Lavelli *et al*., 2000) was considered to be due to the presence of ascorbic acid and its action activating metal-ion catalysed reactions, what probably also happened in the conditions of the present assay where ascorbic acid is used as oxidative inducer together with iron sulphate.

The lack of antioxidant activity in the THF_extract in the lipid peroxidation assay was highly unexpected since this extract contains lycopene that is known to be an efficient scavenger of peroxyl radicals (Woodall *et al*., 1997). However, Chen & Djuric (2001) questioned whether carotenoids can act as antioxidants in biological membranes and suggested that although carotenoids are sensitive to degradation by free radicals they do not protect against lipid peroxidation. The importance of the lipid substrate should also be taken into consideration as depicted from the results of Takeoka *et al*. (2001). The lycopene-containing hexane extract of fresh-tomatoes presented greater antioxidant activity than the aqueous and methanol extracts when phosphatidylcholine liposome was used as substrate for oxidation but about half the activity of methanol extracts when linoleic acid was used as substrate.

Another possibility is that the dilution of the samples in methanol used here resulted in a concentration of antioxidant compounds too low to be effective in the assay. Wilborts (2003) reported problems when measuring the antioxidant activity of lycopene and â-carotene solutions using the same assay as used here. Lycopene, at concentration high enough to exhibit activity, was only soluble in pure THF, which dissolved the plastic wells in which the assay was performed. When diluted in THF-methanol the concentration of these compounds was insufficient to show activity in the assay. When hexane and ethyl acetate were used in the place of THF in the present work, no activity could be measured either, even when the hexane extract was not diluted (data not shown).

3.2. $ABTS^+$ Scavenging Capacity

3.2.1. Analysis of Variance

The results obtained for antioxidant activity using the ABTS assay are presented in Figs. 2-3. Minimal processing decreased the TAA (data not shown) and HAA ($Pr > F = < 0.0001$) but had no effect on the LAA ($Pr > F = 0.1660$). It indicates that the observed decrease in total antioxidant activity (TAA) during storage was mainly due to the decrease in HAA.

Changes in LAA during storage were dependent on the stage of maturity of the fruit while the changes in HAA depended both on processing and on fruit maturity stage (Table 2). A traditional approach to study the changes in antioxidant activity on time would involve the decomposition of the interactions stage*time and treat*time for HAA and stage*time for LAA, that is the study of the effect of treatment and stage of maturity for each sampling date. Using this approach and given the fact that the sampling time is different in the individual series, makes it difficult to obtain a more general picture of the behaviour in time and how it depends on the other factors under study (in the present case stage of maturity and processing). Instead, the data were analysed using non-linear regression.

3.2.2. Modelling

The first regression analysis was conducted for each treatment (cut and intact) separately (Eq. 1). Later, the effect of treatment was incorporated (Eq. 2) so that the combined analysis included simultaneously the effect of time, treatment and stage. Non-linear regression is an iterative procedure and therefore strongly relies on good initial values for the parameters to be estimated. These values were obtained in a sequence of analyses, each step increasing in complexity in relation to the previous one, while building up the number of reliable estimates. Even in the final analysis (see results Table 3) not every parameter could be estimated simultaneously. This is the case, for example, of AA_{var} for LAA. The reason is that in the time range where a rapid decay occurs, too few measuring points (about 3) were taken. That made it impossible to estimate the initial value and the asymptotic end value in the same analysis.

The antioxidant activity of both the hydrophilic and lipophilic extract showed the same exponential decay starting after processing, with a reference rate constant of 0.949 ± 0.091 for LAA

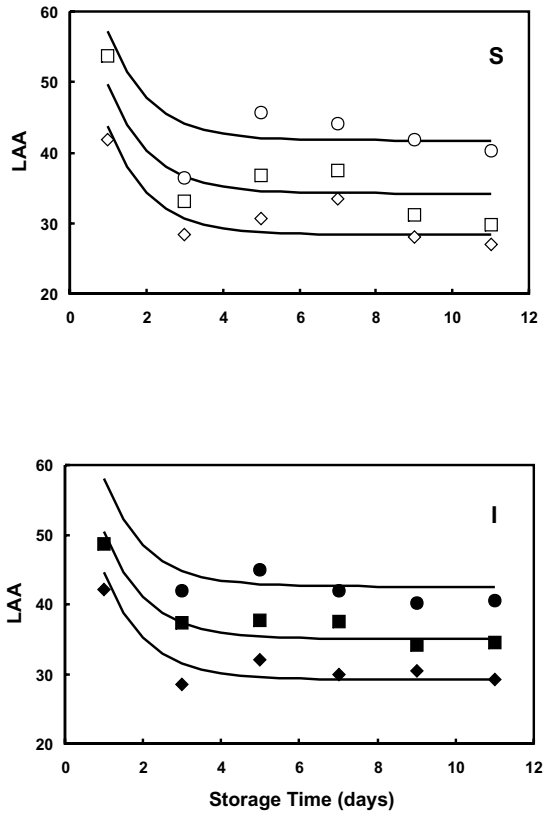

Figure 2 – Lipophilic antioxidant activity of tomato fruits harvested at stages I (◆), II (■) or III (●) and stored sliced (S) (open symbols) or intact (I) (closed symbols) at 5°C. Points are measured data (means of 5 replicates) and lines are simulated values according to Eq. 1.

and 0.281 ± 0.065 for HAA. Later on, the HAA increased with storage time, while the LAA remained practically unchanged. The estimated values for the rate constant of increase ($k_{l,treat}$) where consequently significantly positive for HAA (Table 3) while close to zero for LAA (not shown). Changes in LAA therefore could well be explained by Eq. 1, without the incorporation of the linear increase. The scatterplot for LAA (Fig. 4) and for HAA (Fig. 5) indicates a good fit of the model to the data.

The pattern of change of HAA during storage was the same whether the fruits were intact or cut, but the antioxidant activity was systematically lower for cut fruits (negative value for $ÄAA_{cut}$ when $ÄAA_{whole}$ was fixed to zero). A similar pattern of change was reported for fresh-cut mandarin where the antioxidant activity decreased in the beginning of the storage period and later increased (Piga *et al*., 2002). This was considered to be due to changes in the antioxidant activity of polyphenols, which when undergoing enzymatic or chemical oxidation exhibit increased antioxidant efficiency when present in an intermediary state. The rate constant for this linear increase depended on treatment

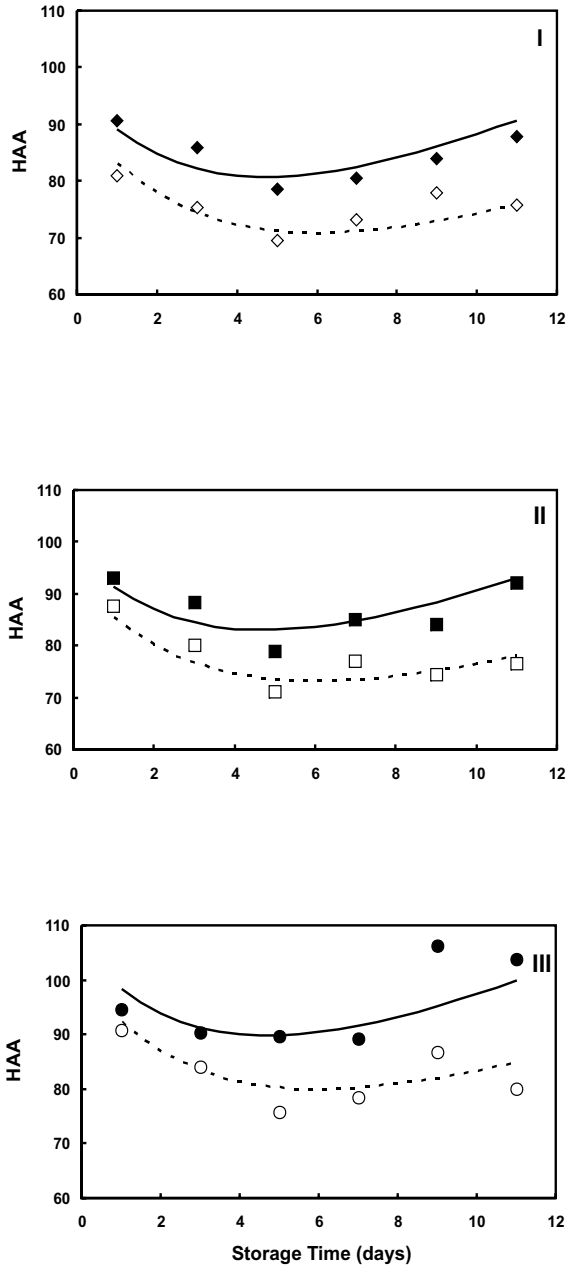

Figure 3 – Hydrophilic antioxidant activity of tomato fruits harvested at stages I, II or III and stored sliced (open symbols and dotted line) or intact (closed symbols and solid lines) at 5°C. Points are measured data (means of 5 replicates) and lines are simulated values according to Eq. 1.

Table 2 – Results of analysis of variance for the lipophilic (LAA) and hydrophilic (HAA) antioxidant activity of sliced tomato.

Extract	Source of Variation	F value	Pr > F
LAA	Stage of Maturity	177.76	< 0.0001
	Treatment	1.94	0.1660
	Storage Time	90.87	< 0.0001
	Stage * Treatment	0.19	0.8299
	Stage * Time	2.37	0.0125
	Treatment * Time	1.75	0.1263
	Stage * Treatment * Time	1.19	0.3006
HAA	Stage of Maturity	49.27	< 0.0001
	Treatment	174.14	< 0.0001
	Storage Time	20.15	< 0.0001
	Stage * Treatment	2.67	0.0732
	Stage * Time	2.79	0.0035
	Treatment * Time	3.73	0.0034
	Stage * Treatment * Time	1.48	0.1514

(Table 3) and was higher for intact fruits. This indicates that the significance of the interaction treatment* time was mainly related with differences in the magnitude of the linear increase, since the reaction rate for the exponential decay could be estimated in common for both cut and intact fruit.

Significant changes in LAA were observed only in the first three days of storage but could not be ascribed to cutting since the same phenomenon occurred in intact fruits. The estimated value of $ÄAA_{cut,}$ although negative, was rather small and could not be reliably estimated (Table 3). The reasons for this decrease in LAA could not be determined in the present work. Probably the physiological response to the stress induced by harvesting consumed a major part of the available antioxidant activity. Reports on the lipophilic antioxidant activity as a response to cutting are unknown by the authors.

The radical scavenging capacity increased with maturity for both fractions lipophilic and hydrophilic. The increase in LAA with maturity was also reported by (Cano *et al*., 2003), and related to changes in lycopene content. Contrary to those authors a significant increase in HAA with maturity was found in the present work. The higher values for the estimates $ÄAA_{pink}$ and $ÄAA_{red}$ for LAA, indicate that the effect of stage of maturity was more pronounced in LAA than HAA confirming the information obtained from the ANOVA where F values of 177.76 and 49.27 were obtained respectively for LAA and HAA.

The same pattern of changes during storage was observed for all stages. That means that differences between fruits at different stages during storage were mainly due to differences in the initial antioxidant activity when the fruits were processed.

Table 3 - Result of statistical non-linear regression based on Eq.2 for HAA (hydrophilic antioxidant activity) and on Eq. 2 without a linear increase ($k_{l,treat}$ =0) for LAA (lipophilic antioxidant activity).

	LAA		HAA	
Parameter	Estimate	s.e.	Estimate	s.e.
$\Delta A_{breaker}$	0	Fixed	0	Fixed
ΔA_{pink}	5.87	1.26	2.34	1.51
ΔA_{red}	13.38	1.26	9.15	1.51
K	0.949	0.091	0.281	0.066
AA_{var}	40.0	Fixed	40.09	6.55
AA_{fix}	29.16	1.09	55.83	3.69
ΔA_{whole}	0	Fixed	0	Fixed
ΔA_{cut}	-0.81	1.03	-5.06	2.49
$k_{l,whole}$	-		3.000	0.282
$k_{l,cut}$	-		2.106	0.282
N obs	36		36	
R^2_{adj}	87.5		80.7	

3.3. Relation between both assays

The effect of cutting, fruit maturity stage and storage time on the antioxidant activity of tomato extracts was not the same in the two different antioxidant assays used in the present work. Although different extractors were used in each assay, part of the compounds extracted by the Na-P buffer should be present in the water and methanol extracts used in the lipid peroxidation assay. Similarly, part of the compounds extracted by ethyl acetate should be present in methanol and THF extracts.

Contrasting results between radical scavenging methods and *in vitro* lipid peroxidation methods were discussed in an extensive review by Frankel *et al*. (2000) and reported by Garcia-Alonso *et al*. (2004). This apparently contradictory behaviour occurs because the effectiveness of antioxidants depends on the test system used (Frankel *et al*., 2000). The lipid peroxidation method includes other mechanisms of antioxidant action besides the radical scavenging, the only mechanism evaluated in the $ABTS^+$ assay. On the other hand, partitioning and interfacial properties are not taken into consideration in the ABTS assay, while they represent an important factor in biological assays.

3.4. Significance of the decrease in HAA

The present results indicate that processing can induce a reduction of radical scavenging capacity, which is only one of the mechanisms by which antioxidants can prevent oxidative damage. The fact that an artificial radical was used should be kept in mind while extrapolating the results to an *in vivo* situation.

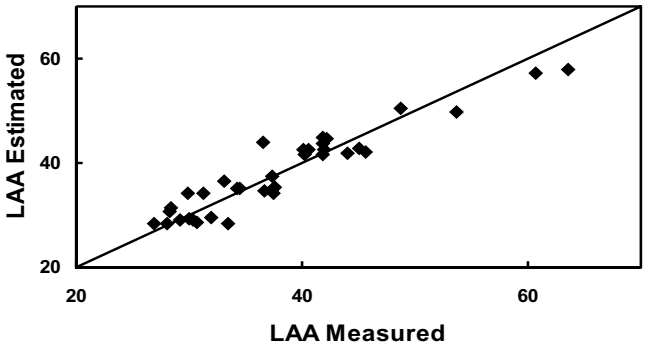

Figure 4 – Scatter plot for mean data of lipophilic antioxidant activity.

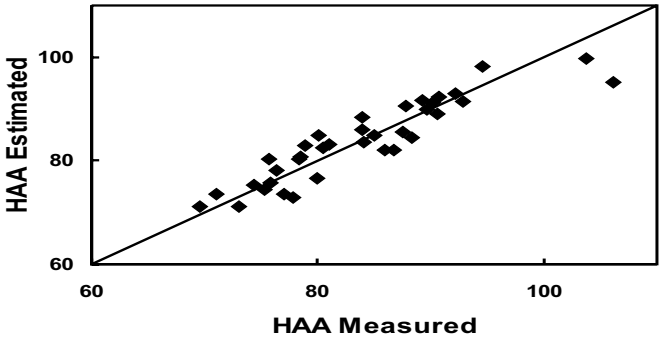

Figure 5 – Scatter plot for mean data of hydrophilic antioxidant activity.

Although a minor decrease in antioxidant activity was measured as a response to processing, at the moment it is not possible to know the impact of this decrease in the value of the food as source of antioxidant in the diet. In spite of the agreement about the health benefits of antioxidants, there is no dietary prescription of recommended daily consumption as it is the case for nutrients such as vitamins and proteins.

4. Conclusions

The processing of tomato fruit into transversal slices induced a decrease in the radical scavenging capacity of hydrophilic extracts towards the ABTS radical. This indicates a potential effect of processing to decrease the antioxidant activity *in vivo*. This would represent a decrease in the value of cut tomatoes as a source of antioxidants in the diet compared with the fruit stored intact. The observed increase in the hydrophilic antioxidant activity in the end of the storage period indicates that some repair or recycling mechanism is operative. This same mechanism is present in intact fruits

since they showed the same pattern of change as sliced fruits. Nevertheless, even with this later increase, the level of antioxidant activity in cut fruits did hardly reach the level present in intact fruits.

The radical scavenging capacity of lipophilic extracts decreased sharply in the first two days of storage. Since no significant effect of cutting was observed, this decrease in activity is more likely to be related with stress caused by harvesting or low temperature storage.

For both hydrophilic and lipophilic antioxidant activity the riper the fruit the higher was the antioxidant activity. Since no relevant interaction was found between time of storage and stage of maturity, the major factor determining the level of antioxidants in tomatoes, seems to be the initial level of antioxidant activity at the moment of harvest.

The information obtained from analysis of variance could effectively be used in developing dynamic models. Although the behaviour of antioxidant level in whole and cut tomatoes seems to be rather complex, the dynamics of change could be modelled in a surprisingly simple way.

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CHAPTER 10

SUMMARY AND GENERAL DISCUSSION

1. Summary of the Main Results

1. 1. Changes in quality attributes

To study the changes in the quality of fresh-cut tomato during storage this thesis has focused on three main groups of quality attributes. Changes in quality were then studied as related to firmness (related with organoleptical quality), colour and translucency (related with visual quality) and antioxidant content and activity (related with nutritional and nutraceutical quality). Classical statistical techniques together with kinetic modelling were used to study how the changes in these attributes are influenced by the stage of maturity of the fruit at harvest, the storage temperature and the storage time.

In short, the post cutting life based on appearance was shorter than that based on textural and nutritional value. The greatest range of changes happened in the first 2 days of storage for all attributes. After that, the changes were of low magnitude and developed towards an end value, exception made for hydrophilic antioxidant activity.

A significant decrease in firmness during storage was observed but this was not a limiting attribute in relation to quality retention and shelf-life (**Chapter 2**) as reported for other fresh-cut products as papaya (Karakurt and Huber, 2003), pears (Soliva-Fortuny *et al*., 2002) and kiwi (Varoquaux *et al*., 1990). Different to what was reported for those products the increase in translucency was not associated with accelerated softening. Rather than that, the development of translucency possibly related with the filling of the intercellular space with liquids, made the tissue more firm similar to a tissue with high turgor. This inverse relation between translucency and firmness change was also observed in minimally processed melon (Aguayo *et al*., 2004).

Changes in colour due to (post harvest) maturation were of low magnitude and were not affected by cutting when the fruits were stored at low temperature. The results presented in Chapters 4 and 7 showed that cutting did not accelerate ripening when the processed product was kept at low temperature (5^{o}C). This was confirmed by the results reported in **Chapter 8**, where cutting did not change the accumulation of lycopene (the main pigment responsible for the red colour of tomato) compared to intact fruit. Besides that, at temperature equal to or lower than 5^{o}C no increase or a small decrease in lycopene concentration was observed in cut tomato. The small increase in redness reported for tomatoes sliced at red stage was more likely due to an effect of translucency (**Chapters 5** and **7**). Changes in the refractive index and homogeneity of the tissue related to watersoaking result in changes in the perceived colour due to a reduction in light scattering and concomitant increase in light absorption by the pigments already present in the tissue (Hunter and Harold, 1987). The redder colour of translucent slices can give an impression of over-ripeness, usually associated with loss of quality and excessive softening, even when both processes do not occur.

The main consequence of cutting was the development of translucency. The development of translucency seems to be a common problem in fresh-cut fruits (O'Connor-Shaw *et al*., 1994; Artes *et*

al., 1999; Soliva-Fortuny *et al*., 2002) and has been ascribed as a consequence of membrane damage resulting from the enhanced activity of cell wall and membrane hydrolases as a response to wounding. If this were the case with tomato slices, extensive softening would be expected to be associated with it, what was not the case as discussed previously. This together with the findings in **Chapter 5** and **6** gives a strong indication that an enzymatic process involving cell wall and membrane degradation, as described for papaya (Karakurt and Huber, 2003) and pears (Soliva-Fortuny *et al*., 2002) although it can not be excluded, is unlikely to play a major role in the process of development of translucency in cut tomatoes. The inhibition in translucency development by rinsing and drying the cut surface suggests, however, that compounds released from the cells during cutting may have a role in promoting translucency. The reason why removing the locular gel inhibits the development of translucency, remains to be elucidated. Two possibilities were raised: a purely physical process where liquid from the locular gel is infiltrated in the intercellular space or the action of chemical compounds present in this tissue that acts upon the integrity of the cut issue and promotes cell leakage.

The results described in **Chapters 4** to **6** suggest that the development of translucency in the tomato pericarp is a direct consequence of wound injury and not a symptom of chilling injury as first reported. The evidences for that includes: (1): translucency developed under temperature not chilling injurious for tomato fruit; (2) there was a tendency to an increase in the intensity of translucency at higher temperature; (3) the susceptibility to translucency increased with ripening, the opposite of what is observed to sensibility to chilling injury (Bergevin *et al*., 1993).

Cutting induced a decrease in the antioxidant activity of hydrophilic extracts (**Chapter 9**). This indicates a potential effect of processing to decrease the antioxidant activity in vivo, what would represent a decrease in the value of cut tomatoes as a source of hydrophilic antioxidants in the diet as compared with intact fruits. The hydrophilic antioxidant activity increased later on what indicates a possible regeneration system working for both intact and cut fruits. Even with this later increase, the level of antioxidant activity in cut fruits remained lower as compared to intact fruits along storage. A sharp decrease in the antioxidant activity of lipophilic extracts was observed in the first two days of storage but this could not be due to cutting since the same decrease occurred in intact fruits. Also the lycopene content, considered the main lipophilic antioxidant present in tomato fruit, was not significantly affected by cutting when the fruits were stored at 5 ^{o}C, what confirms the results obtained in the ABTS assay (**Chapter 8**). At higher temperature there was a net increase in the lycopene content of tomato slices during storage. Since in this case no control (intact fruits) were evaluated it is not known how it much differs from the maturation-associated accumulation of lycopene occurring in intact fruits.

1.2. Effects of stage of maturity at harvest and of storage temperature

The changes in all attributes measured in the present thesis (colour, translucency, firmness, lycopene content and antioxidant activity) were very much dependent on the maturity of the fruit at harvest. The stage of maturity determined the initial values as much as the rate of decrease (or

increase) of each attribute along storage. The effect of temperature although present was far lower than the effect of stage of maturity.

The firmness decay during storage was mainly affected by the stage of maturity and it was more pronounced the less mature the fruit (**Chapter 2**). Major differences in firmness were observed between fruits harvested at different stages of maturity. Within a same stage of maturity the firmness decreased only slightly during storage especially when the slices were kept at low temperature (2-8 ^{o}C). At higher temperatures (12-16^{o}C) the decrease was more pronounced but still very small. Interesting to note the maturity stages, used in these studies, were not far apart since immature and too ripened fruits were not used. Still, the main difference in firmness was found in the initial values and not in the difference between initial and final values.

Changes in the RGB colour aspects were also very much dependent on the stage of maturity at harvest (**Chapters 3-5** and **7**). In **Chapter 3**, it was shown that the RGB values of cut fruits decreased consistently from less to more mature stages and as observed for the firmness. The changes in colour aspects were more pronounced the less mature the fruit. All aspects seemed to decrease faster at higher temperatures (8 to 16 ^{o}C) but due to the high variation in the data for successive measuring days, the effect of temperature was rather unclear.

In two other experiments (**Chapters 4**, **5** and **7**) the changes in colour aspects due to ripening (change from green to red colour) could be distinguished from those due to the development of translucency. In the first experiment (**Chapters 4** and **7**), slices together with intact control fruits obtained from fruits at three maturity stages were stored at only one low temperature (5 ^{o}C). Again, the changes in RGB values were very dependent on the stage of maturity of the fruit at harvest. All colour aspects decreased during storage in cut fruits while hardly changing in intact fruits. The changes in RGB values due to ripening happened in both cut and intact fruits. They were of low magnitude and more pronounced the less mature the fruit. The development of translucency on the other hand, only happened in cut fruits. It was responsible for most of the decrease in RGB values and was more pronounced the more mature the fruit. In the second experiment (Chapters 5 and 7) slices obtained from ripe fruits were stored at three different temperatures. Again, all RGB values decreased during storage for cut fruits, while remaining practically the same for intact fruits. Theses changes were basically due to the development of translucency. A small but significant effect of temperature was observed for changes in RGB due to maturation, while those due to translucency development were rather independent of temperature.

The effect of maturity stage and storage temperature in the development of translucency measured using Video Image Analysis (VIA) was confirmed in **Chapter 6** where the translucency was evaluated using Kubelka-Munk analysis. An increase in the K/S (absorption/scattering coefficients) ratio, which indicates an increase in translucency, was observed for cut fruits only.

The net accumulation of lycopene during storage depended both on the stage of maturity of the fruit at harvest and on the storage temperature (**Chapter 8**). There was a tendency for the lycopene concentration to increase at temperatures higher than 8 ^{o}C especially for less mature stages, while

more mature fruits (light red and red stage) showed a tendency for decrease in lycopene content at temperature lower than 8 °C. The estimated activation energy showed that the process of lycopene formation was highly dependent on temperature while for the degradation reaction no temperature dependence was observed. As a consequence of that, at temperatures higher than 8 °C the formation of lycopene becomes increasingly dominant over the degradation.

Both the hydrophilic and lipophilic antioxidant activity increased with maturation (**Chapter 9**). There was no relevant interaction between time of storage and maturity stage meaning that the same pattern of change was observed for fruits harvested at different stages. This means that the major factor determining the level of antioxidants in tomatoes, at any time during storage, was the initial level of antioxidant activity at harvest. The influence of maturity stage at harvest in the antioxidant activity was more pronounced for the lipophilic fraction than for the hydrophilic one. Increase in the lipophilic antioxidant activity with maturation is in part likely to be due to an increase in lycopene content (**Chapter 8**). In this case, the temperature effect could not be evaluated since fruits were stored only at 5 °C.

2. General Conclusions

Based on the knowledge available for intact fruits, the quality of cut tomatoes was first evaluated in terms of firmness and colour of the pericarp. The starting hypothesis was that wound injury associated with the slicing of the fruits would result in accelerated changes in these two attributes, thereby considerably reducing the shelf life as compared to the intact fruit. At the same time, it was expected that cutting would induce a depletion in the level of antioxidants in the tomato tissue, what could eventually reduce its value as a source of antioxidants in the diet.

The results presented in the previous chapters indicate however that the low temperature (around 5°C), together with the short time of storage, inhibited all the processes that would be otherwise accelerated by cutting. The changes in firmness, colour (due to maturation), lycopene content and antioxidant activity were of small magnitude and for some of these attributes not significantly different from intact fruits. The main effect of cutting was the induction of translucency in the tomato pericarp.

Since there were no major changes in time (exception made for translucency development), the main factor determining the levels of each attribute during storage was the stage of maturity of the fruit at harvest. The changes within each stage as a function of time and temperature were less important then the initial differences between stages.

Using mathematical models based on simplified kinetic mechanisms it was possible to integrate all available information and describe the dynamics of changes of each evaluated quality attribute. The incorporation of the biological shift factor into the description of changes in firmness and in the RGB colour aspects made it possible to analyse successive maturity stages as belonging to a sequence on the same curve. It means that all stages of maturity at all temperatures of storage followed the same exponential behaviour towards a common end value, regardless whether they were at the plant,

harvested or cut.

The changes in antioxidant activity and translucency were described by basically the same simple exponential model (first order kinetics) developing towards an asymptotic end value (exception made to the hydrophilic antioxidant activity that presented a later linear increase, instead of an asymptotic end value, following the initial exponential decay). In both cases the biological shift factor could not be applied since both processes only occurred after cutting and the differences between stages could be not be shifted along the biological time (X) axis but were expressed only in the differences in the initial levels (Y axis). The major problem, however, that prevented an integral approach applying the biological shift factor, is the fact the properties studied decreased towards an end value different for each stage of maturity. A realistic mechanism for this phenomenon has still to be formulated.

The exponential decay towards an end value indicates that most of the changes in quality of cut tomato occur in the first days after cutting. Except for HAA, they attain a sort of steady state after that. Applying standard measures to decrease quality decay like low temperature and MA packaging only affects the rate constants of the processes. Far better result would be possible if one could influence the range of change, i.e. the end value, of all these quality attributes.

Since translucency was the main change in appearance and no ready measuring techniques were available, major efforts were spent to develop a technique to quantify it. Based on studies for measuring the opacity power of colorant layers the video image analysis (VIA) previously used was slightly modified by imaging the slices over a double background (one half white, one half black). The same basic model of exponential decay towards an end value was used to describe the changes in RGB values, this time incorporating the effects of storage time, storage temperature, stage of maturity of the fruit at harvest and background.

The same conclusions about the effect of stage of maturity and temperature were obtained when the translucency was quantified using spectrophotometry, through Kubelka-Munk analysis. Although translucency could be assessed by both techniques, the VIA presented a series of advantages as compared to the Kubelka-Munk analysis. Translucency could be expressed numerically (as a particular value for colour aspects) making it easier to describe mathematically the effect of different factors in its development and to apply statistical analysis. In the case of Kubelka-Munk analysis the information on translucency is contained in spectra in the range 400-700 nm, which seriously hampers regression analysis. Very translucent samples could not be evaluated by the Kubelka-Munk analysis, while they did not represent a problem in VIA. When using a double background the processes of colour change and of translucency development could be separated and a better understanding of the processes contributing to changes in total appearance was achieved. Additionally, VIA is a non-destructive technique that can be used with non-homogenous tissue and with the same image it is possible to assess different optical properties all of the same sample.

The development of translucency was better expressed as decrease in the colour aspects values themselves (RGB on a black background) than in differences between values obtained on both backgrounds. However, both maturation (change from green to red colour) and translucency

development (change from opacity to translucency) result in a decrease in RGB values. How the interaction between both processes is expressed in the changes in the RGB values was not fully understood and requires further investigation. In the present studies, this interaction was minimal due to inhibition of the coloration process by the low temperature of storage.

Although the information about colour and translucency is somehow in both backgrounds the information of translucency was better expressed in the black background. This indicates that the reported decrease in RGB values when the tomato slice was placed on a dark grey background (**Chapter 3**), probably refers to a great deal to the development of translucency rather than to maturation and ripening. Why the end value was constant for all stages of maturity in one experiment (**Chapter 3**) while variable with the stages in another one (**Chapter 7**) is at the moment not clear. More dedicated research is needed to clarify this.

Another point that remains to be elucidated is in which colour aspect the translucency of samples with other colour than red (e.g. immature tomatoes, cucumber, kiwi, melon) is expressed. The correlation between the decrease in Red value on black background and the increase in translucency was higher for red fruits. When fruits at three maturity stages were used there was a small overlapping between the R in black and the R in white background values, what did not happen when only fully ripe fruits were used. The correlation between the differences in black and white background was also higher for red fruits. In reality, for red fruits the difference between both backgrounds was almost as discriminant for translucency as the Red value on black background. This indicates that for less mature fruits the development of translucency was also expressed in other colour aspects besides the Red. When this is known, it will probably be possible to use the difference in both backgrounds for that (those) colour (s) aspect (s) to quantify translucency for fruits of any colour.This also implies that for other fruits like melon (yellow) or kiwifruit (green) the translucency will be probably expressed in other colour aspect than Red.

The nature of the process, or rather the nature of the actors in this process, leading to the water-soaking of the pericarp, and consequently the appearance of translucency, remains to be elucidated. The removal of the locular gel as much as the removal of the cell contents in the cut surface greatly inhibited the development of translucency. However, it is not clear whether or not membrane breakdown and consequent cell leakage contribute to the development of translucency. This signifies that the origin of the soaking liquid is not clear (locular gel, cell contents of cut cell, or cell content of damaged cells). This origin, however, will define for the major part the kind of action (e.g. drying, enzyme blocking, less cutting damage) that should be taken to prevent development of translucency in a particular product as well as the optimal maturity for cutting particular fruits.

As observed for many other cut fruits, determining the best stage of maturity for cutting is a conflict of interests. The ripe fruits present better organoleptical properties in relation to taste, aroma and initial colour and higher contents of antioxidant compounds. But they deteriorate more rapid in quality and get translucent more rapidly and to a higher intensity. For a short period (less than 1 week) the low temperature is efficient to prevent most of the deleterious changes in quality (softening, over-

ripening, loss of antioxidants) but not to prevent water-soaking of the pericarp and translucency development.

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SUMMARY

Fresh-cut or minimally processed vegetables are those which have been trimmed and/or peeled and/or cut into 100% usable product and still maintain freshness. Minimal or light processing operations refers to abrasion, trimming, peeling, cutting, sectioning, slicing, shredding, and coring of fruit and vegetables. The main appeal of this kind of product is to combine the convenience of a ready-to-use product with the healthiness and lack of chemical additives of a fresh product. In terms of research, it constitutes a very interesting subject that combines knowledge in the fields of Plant Physiology and Food Science. Fresh-cut products differ from intact fruit and vegetables in terms of their physiology, handling and storage requirements. Contrary to other processing methods (freezing, canning and drying for example) the minimal processing operations reduce the shelf life in relation to the raw material (intact product).

This thesis examines the changes that occur in fresh-cut tomato fruits during storage. The main objective is to describe the dynamics of changes on time of selected quality attributes considered important to determine the keeping quality and acceptability of the product. These quality attributes include firmness, colour, translucency, lycopene content and total antioxidant activity. The second objective is to describe how the stage of maturity of the fruit at harvest and the storage temperature influence these changes.

The firmness of the tomato pericarp decreased exponentially during storage. Within a maturity stage, the firmness decreased only slightly during storage and major difference was found in the initial firmness at the different stages of maturity. The changes in the RGB (Red,Green,Blue) colour aspects of the pericarp also decreased exponentially during storage. The system of incorporating a biological shift factor is described in both cases. The development of translucency in the pericarp was found to be more important than changes in colour or firmness. A new methodology to evaluate changes in colour and translucency simultaneously using video image analysis is presented. A range of variables, both directly measured and derived from those, was examined to determine which one expresses more clearly changes in colour and translucency. The development of translucency was highly determined by the stage of maturity of the fruit at harvest and rather independent of temperature. Using a spectrophotometric technique (Kubelka-Munk analysis) to evaluate the development of translucency

these findings were confirmed. The model on RGB development was extended to describe the changes in the RGB colour aspects resulting from two different processes namely changes in colour due to the production and/or degradation of pigments and development of translucency due to watersoaking of the pericarp. It incorporates in a single equation the effects of time, temperature, stage of maturity and background over which the tomato slices were imaged, while taking previous results into consideration. Both translucency and maturation resulted in a decrease of the RGB values. The Red colour aspect measured on a black background was the one most affected by translucency and its value decreased significantly in translucent samples while remained practically constant in non-translucent samples.

Changes in lycopene content were studied using kinetic modelling, considering that lycopene production and degradation happen simultaneously during refrigerated storage. Cutting did not change the lycopene accumulation when the slices were kept at low temperature, while lycopene production predominated over degradation at temperature higher than 8 ^{o}C. The antioxidant activity of fresh-cut and intact tomato fruits was assessed during storage at low temperature. The results from the lipid peroxidation inhibition assay were inconclusive. The results from the radical scavenging assay indicate a reduction of the hydrophilic antioxidant activity induced by cutting while the lipophilic antioxidant activity was not affected. The antioxidant activity of both fractions increased with maturation.

In short, the post cutting life based on appearance was shorter than that based on textural and nutritional value. The greatest range of changes happened in the first 2 days of storage for all quality attributes. The stage of maturity determined the initial values as much as the rate of decrease (or increase) along storage of each attribute. The effect of temperature although present was far lower than the effect of stage of maturity. For a short period (less than 1 week) the low temperature was efficient to prevent most of the deleterious changes in quality (softening, over-ripening, loss of antioxidants) but not to prevent water-soaking of the pericarp and translucency development.

SAMENVATTING

Vers gesneden groenten en fruit zijn ontdaan van oneetbare delen, gepeld en gesneden. Ze zijn 100% bruikbaar terwijl ze toch vers en houdbaar zijn. De international gebruikte term "minimally processed" heeft betrekking op één of meer behandeling die het product gekregen heeft zoals: wrijven, trimmen, pellen, snijden, versnipperen, ontdoen van de kern of klokhuis voor zowel groente als fruit.

Het voordeel van dit type product is het gemak van dit product, kant-en-klaar voor gebruik, dat het gezonde imago en de afwezigheid van chemische toevoegingen deelt met het verse product. Voor onderzoek is het een interessant onderwerp op de scheidingslijn van plantenfysiologen en voedsel wetenschap. Ten opzichte van niet gesneden producten verschillen de wel gesneden producten in hun fysiologisch gedrag, maar vooral in de eisen die gesteld worden aan opslag en handling. In tegenstelling tot andere conserveringsmethoden, zoals steriliseren, invriezen en drogen, vermindert het proces zelf de levensduur van dit soort producten.

Dit proefschrift behandelt de veranderingen die gedurende de bewaring optreden in vers gesneden tomaten. De dynamiek in de tijd van deze veranderingen van een aantal attributen, geselecteerd als belangrijk bij het bepalen van de houdbaarheid en aanvaardbaarheid, wordt geanalyseerd en beschreven. De bestudeerde kwaliteitsattributen zijn stevigheid, kleur, glazigheid, lycopeen gehalte en totale antioxidant activiteit. Een bijkomend doel van dit proefschrift is het bestuderen en beschrijven van de effecten van bewaartemperatuur en rijpheid bij oogst op deze dynamiek.

De stevigheid van het pericarp weefsel van tomaten neemt exponentieel af gedurende de bewaring. Binnen één enkel rijpheidsstadium is de afname minimaal. Het grootste effect is de beginstevigheid voor alle stadia van rijpheid bij oogst. De kleuraspecten RGB (Rood, Groen, Blauw) nemen ook exponentieel af in de tijd. Het gebruik van de biologische tijdsverschuiving wordt geïntroduceerd om al deze effecten gelijktijdig te beschrijven. De ontwikkeling van glazigheid blijkt veel belangrijker te zijn dan de kleurverandering.

Een nieuwe methode met behulp van video analyse, gemeten tegen een witte én een zwarte achtergrond, wordt voorgesteld om de veranderingen in kleur en glazigheid gelijktijdig te beoordelen. Van een groot aantal variabelen, zowel direct gemeten als daaruit afgeleid, wordt bepaald welke variabele het beste aansluit bij de waargenomen verandering in kleur en glazigheid. Het ontwikkelen van glazigheid

wordt hoofdzakelijk bepaald door de rijpheid bij oogst en grotendeels onafhankelijk van de temperatuur.

Tevens wordt een spectrofotometrische techniek gebruik (ook tegen een witte én een zwarte achtergrond), waarvan de gegevens geanalyseerd worden volgens de Kubelka-Munk techniek. Hierbij is het bepalen van de verhouding tussen verstrooiing en reflectie van het licht het belangrijkste doel. De resultaten uit dit experiment onderschrijven geheel de voorgaande conclusies.

Het model voor de ontwikkeling van RGB wordt uitgebreid naar de twee belangrijkste processen namelijk de verandering in kleur componenten door productie of degradatie en de ontwikkeling van glazigheid. In één enkele vergelijking worden de invloeden van bewaarduur, temperatuur, rijpheid en de twee achtergronden samengevoegd, waarbij rekening is gehouden met de voorgaande resultaten. Zowel glazigheid als rijping verminderen de waarden voor RGB. Het kleuraspect R wordt het meest beïnvloed door glazigheid, en zijn waarde is significant lager in glazige monsters, gemeten op een zwarte achtergrond dan in niet-glazige monsters of wanneer gemeten op een witte achtergrond.

De belangrijkste kleur component voor tomaten is lycopeen. De verandering in lycopeen concentratie wordt bestudeerd, geanalyseerd en gemodelleerd gedurende koude bewaring, er rekening mee houdend dat zowel aanmaak als afbraak gelijktijdig optreden. De conclusie van dit onderzoek is dat bij lage temperatuur het snijden van de tomaten geen invloed heeft op de lycopeen ophoping. De productie van lycopeen is gevoeliger voor temperatuur dan zijn afbraak, en de productie bij temperaturen boven de 8 ^{o}C is groter dan de afbraak.

De activiteit van antioxidanten in gesneden en hele tomaten wordt bepaald gedurende koude bewaring. De resultaten van de experimenten ten aanzien van de inhibitie van lipide peroxidatie zijn niet dusdanig dat eenduidige conclusies kunnen getrokken worden. Wanneer de werking radicaal scavengers direct wordt gemeten, zijn wel duidelijke conclusies te trekken: hydrofiele antioxidant activiteit wordt door het snijden verminderd terwijl de lipofiele activiteit niet veranderd. De antioxidant activiteit van beide fracties neemt toe met de rijpheid.

Als algemene conclusie kan gesteld worden dat de levensduur van gesneden tomaten gebaseerd op uiterlijk (kleur en glazigheid) korter is dan wanneer men het baseert op stevigheid en voedingswaarde. Voor alle kwaliteitsattributen treden de grootste veranderingen op in de eerste twee dagen na het snijden. De rijpheid bij oogst bepaalt niet allen de beginwaarde van de meeste attributen, maar ook de snelheid van af- of toenemen gedurende de bewaring. Temperatuur heeft een effect op alle processen, maar mede door de lage temperaturen van wezenlijk kleiner invloed dan de rijpheid bij oogst. Opslag bij lage temperatuur is voldoende efficiënt om gedurende een korte periode (minder dan 1 week) kwaliteitsverlies tegen te gaan (stevigheid, verder rijpen, verlies van antioxidanten), maar is bij lange na niet voldoende om de ontwikkeling van glazigheid te voorkomen.

RESUMO

Hortaliças e frutas minimimamente processadas são produtos submetidos às operações de abrasão, descascamento, remoção de partes não comestíveis e seccionamento em partes menores. Este tipo de produto é particularmente atrativo por combinar as características de conveniência do produto processado, pronto para ser consumido, com o valor nutricional e ausência de aditivos químicos do produto fresco. Em termos de pesquisa, este tema apresenta novos desafios ao situar-se na interseção das áreas de Ciência dos Alimentos e Fisiologia Vegetal. Os produtos minimamente processados diferem dos produtos intactos do qual originam-se quanto à sua fisiologia e quanto às exigências de manuseio e armazenamento. Diferentemente dos demais métodos de processamento (congelamento, pasteurização e desidratação, por exemplo) as operações de processamento mínimo reduzem a vida de prateleira em relação ao produto não processado que lhe deu origem.

A presente tese descreveu as mudanças que ocorrem em tomate minimamente processado durante o armazenamento. O principal objetivo consistiu em descrever as alterações sofridas no tempo por atributos de qualidade considerados importantes (firmeza, cor, translucência, teor de licopeno e atividade antioxidante) para determinar a aceitabilidade e vida de prateleira de tomate fatiado, e também como essas mudanças são influenciadas pelo estágio de maturação do fruto e pela temperatura de armazenamento.

Esta pesquisa demonstrou que a firmeza do pericarpo de tomate e os valores dos aspectos de cor V V A (Vermelho, Verde e Azul) decresceram exponecialmente durante o armazenamento. E que para um mesmo estágio de maturação houve pequena alteração no tempo enquanto as maiores diferenças foram observadas em relação à firmeza inicial de frutos colhidos em diferentes estágios de maturação, sendo utilizado, em ambos os casos, o conceito de fator de conversão biológica (biological shift factor). O desenvolvimento de translucência no pericarpo mostrou-se mais importante que alterações de cor ou de firmeza. Em vista disso, uma nova metodologia para a avaliação de translucência utilizando análise de imagens digitais foi desenvolvida, onde uma série de variáveis foi avaliada para se determinar aquela com maior poder discriminante para expressar alterações de cor e translucência. Foi demonstrado que, o desenvolvimento de translucência é largamente determinado pelo estágio de maturação do fruto na colheita e praticamente independente da temperatura (resultados confirmados por meio da

técnica de espectrofometria (análise de Kubelka-Munk)). O modelo matemático utilizado para descrever as alterações nos aspectos de cor V V A foi ampliado para descrever as alteraçoes resultantes das alterações de cor devido à produção ou degradação de pigmentos e o desenvolvimento de translucência devido ao encharcamento do pericarpo. Esse modelo incorporou em uma única equação os efeitos de tempo, temperatura, estágio de maturação, e cor do fundo utilizado na obtenção das imagens, levando em consideraçãos resultados obtidos anteriormente. Ambos os processso resultaram em decréscimo dos valores de V V A. O aspecto vermelho (V) medido sobre fundo preto foi o aspecto de cor mais influenciado pelo desenvolvimento de translucência e seu valor decresceu significativamente durante o armazenameto de tomate fatiado (translucente) permanecendo praticamente conatante para tomate intacto (opaque).

As alterações do teor de licopeno em tomate fatiado foram estudadas através de modelos cinéticos, considerando que os processos de produção e degradação ocorrem simultaneamente durante o armazenamento. Conclui-se que, o fatiamento não alterou a acumulação de licopeno quando as fatias foram mantidas à baixa temperatura e a produção de licopeno predominou sobre a degradação quando a temperatura foi superior a 8^{o}C. A atividade antioxidante total de frutos de tomate inteiros e fatiados foi avaliada durante o armazenamento à baixa temperatura, sendo os resultados, empregando o método de inibição da peroxidação de lipídeos, inconcluso. Os resultados do método de seqüestro de radicais livres indicaram uma redução da atividade antioxidante em extratos hidrofílicos enquanto em extratos lipofílicos não foi observado efeito do processamento.

Conclui-se que a vida de prateleira de tomate fatiado baseada na aparência do produto foi mais curta do que aquela baseada em textura e valor nutricional. As maiores mudança foram observadas nos primeiros dois dias após o processamento para todos os atributos de qualidade avaliados. O estágio de maturação dos frutos determinou não só o valor inicial mas também a taxa de redução (ou aumento) ao longo do armazenamento de cada atributo avaliado. O efeito de temperatura apesar de significante foi consideravelmente menor do que o efeito do estágio de maturação. A curto prazo (período inferior a uma semana) baixa temperatura foi eficiente em prevenir muitas das alterações indesejáveis de qualidade (amolecimento, amadurecimento, perdas de antioxidantes) mas não inibiu o devenvolvimento de translucência e aparência de encharcamento do pericarpo.

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