MATURITY AND TEMPERATURE INFLUENCE ON LYCOPENE DISTRIBUTION DURING FILTRATION PROCESSING OF RED-FLESHED WATERMELONS

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Lycopene is a carotenoid pigment responsible for the color of red fleshed watermelons, as well as for other fruits and vegetables such as tomatoes, guava, papaya, mango, red grapefruit, autumn olive and more (Johnson, 2002; Wilberg and Rodrigez-Amaya, 1995). In humans it can be found in blood plasma, adipose and adrenal tissues (Stahl and Sies, 1996), and it has also been detected in ocular tissues (Khachik *et al*., 2002).

The major source of lycopene in today's nutraceutical market is tomatoes and tomato based products (Edwards *et al.*, 2003; Johnson, 2002), with more than 85% of our dietary lycopene coming from tomato-based products (Bramley, 2000). Red fleshed watermelons are another rich source of lycopene; some cultivars contain more lycopene per wet basis than tomatoes (Perkins-Veazie *et al.,* 2001). Currently watermelons are used almost exclusively for fresh and minimally processed markets, while there is no market for nutraceutical use.

1.1 Properties of Lycopene

1.1.1 Chemical, Structural and Biological

Lycopene has an acyclic carbon chain with 11 conjugated double bonds ($C_{40}H_{56}$) (fig.1) and a chemical structure similar to tetraterpenes ($C_{40}H_{64}$), since its skeleton contains eight isoprenic groups (Chasse *et al.*, 2001; Stahl and Sies, 1996). In fruits and vegetables it is found naturally in the all-*trans* (or E) form (Schierle *et al*., 1997), while in processed tomato products and human serum both trans and mono-cis isomers (5-, 9-, 13- and 15-cis) of lycopene are present (Lee and Chen 2002). The same authors tested under illumination (2000-3000 lux for 1-144h) and heat (50^{O}C, 100^{O}C and 150^{O}C at various times) a standard of trans- lycopene, which contained several cis isomers, such as 5-cis-, 9-cis, 13-cis-, 15-cis-lycopene, and possibly one more mono-cis and four di-cis isomers. They concluded that during illumination isomerization and degradation of lycopene and its cis- isomers may proceed simultaneously, and that all-trans lycopene might be isomerized to form mono-cis- or di-cis-lycopene. Heating at 50^{O}C and 100^{O}C initially showed isomerization of the mono-cis isomers to the di-cis-, however degradation was more prevalent as heating time proceeded. At 150^{O}C a large decrease was observed for the concentration of all trans-lycopene, and no lycopene was detected after 10 minutes.

Along with β-carotene, lycopene belongs to the group of carotenes, which are very fat soluble (Johnson, 2002). Consequently lycopene is insoluble in water and very soluble in organic solvents such as hexane, benzene, chloroform and methylene chloride (Vasapollo *et al.,* 2003). Carotenoids such as *α-carotene, β-*

carotene and cryptoxanthin have provitamin A activity, but lycopene does not (Bramley, 2000; Rao and Agarwal, 2000; Stahl and Sies, 1996), as it lacks the *β*-ionone ring structure (Setiawan et al, 2001; Clinton, 1998).

Lycopene is a photo-sensitive compound and degrades when exposed to light, oxygen and heat (Lee and Chen, 2002; Wright and Kader, 1997; Sharma and Maguer, 1996). In many studies it has been suggested that the action of lycopene is limited by its bioavailability in human tissue. Lycopene was more bioavailable from tomato paste than from raw tomatoes, possibly due to isomerization caused by thermal processing steps (Edwards *et al*., 2003; Johnson, 2002; Giovannucci, 2002). When different varieties of tomatoes were compared (Cherry-CA, Roma and on the Vine), Cherry-CA had the highest lycopene content on a wet weight basis and Roma had the highest on a dry weight basis (Tawfik, 2002). In the same study four tomato products were studied (paste, puree, juice and ketchup) and lycopene content was highest in the paste form followed by puree and ketchup. Juice had the lowest content on a wet weight basis, but on a dry weight basis ketchup contained the least amount of lycopene.

Lycopene has been found in a number of human tissues, such as liver, lung, breast, cervix, skin, cilliary body and retinal pigment epithelium (Khachik *et al*., 2002). It is also located in high concentrations in the adrenal, testes (Stahl and Sies, 1996) as well as in human serum and prostate tissue (Rao and Agarwal, 2000).

1.1.2 Antioxidant

Many of the beneficial effects of carotenoids in human health are attributed to lycopene and its antioxidant properties as a strong singlet oxygen ($^1O_2$) quencher. Among the carotenoids, lycopene has the highest Trolox-equivalent antioxidant capacity (Bramley, 2000) and it can also reduce the levels of oxidation of DNA and LDL (Low Density Lipoproteins), preventing initiation of specific types of cancer, heart problems and mutagenesis (Tawfik, 2002; Heber and Lu, 2002; Chasse *et al*., 2001; Rao and Agarwal, 2000). The effectiveness of lycopene in preventing prostate cancer, the most common cancer in American men, has been documented (Giovannucci, 2002). It may also be active in prevention of cardiovascular disease and cataracts (Setiawan *et al.*, 2001). Lycopene may also promote increased cell-to-cell communication and modulate immune responses (Dwyer and Wang, 2003). The cell-to-cell communication activity is thought to reduce tumor proliferation by upregulating the expression of the connexin 43 gene (Heber and Lu, 2002).

Several studies illustrate this antioxidant activity against specific radicals. Yaping et al. (2002) found that lycopene can rapidly react with the trichloromethyl peroxyl radical $CCl_3O_2\cdot$, which may explain why CCl_4^- damaged rats survived when fed lycopene. Lycopene can also scavenge nitrogen dioxide (NO2·), thiyl (RS·) and sulphonyl ($RSO_2\cdot$) radicals, as well as reactive oxygen species (ROS) (Rao and Agarwal, 2000). When compared to other carotenoids, lycopene is a stronger oxygen quencher (Setiawan, 2001; Miller *et al*., 1996; Krinsky *et al*.,

1990); followed by α-tocopherol, α-carotene, β-cryptoxanthin, zeaxanthin, β-carotene and finally lutein (Heber and Lu, 2002). Miller *et al*., (1996) report that lycopene was also a stronger scavenger for the $ABTS^{\cdot+}$ radical cation.

1. 2 Stability of Lycopene during Storage

In a study by Sharma and Maguer (1996) lycopene content of tomato pulp reportedly decreased under different heating treatments. Other factors like acids, sugars, air, and light also increased lycopene degradation. During storage, freeze-dried samples were more susceptible to lycopene loss when compared to oven dried samples between 25 and $75^{O}C$. They concluded that lycopene degradation kinetics during heating fitted a pseudo first order model reaction at $100^{O}C$; $L = L_O \times [\exp(-K_t)]$, where L= amount of lycopene at time t (mg/100g Total Solids), L_O= initial amount of lycopene (mg/100 g TS), K= apparent reaction rate constant (min^{-1}) and t= time of heating (min).

Lee and Chen (2002) studied the effects of heating and illumination on lycopene stability. The heat treatments were 50, 100, and $150^{O}C$. Heating times ranged from 20 to 120 minutes for 50 and $100^{O}C$, while the heating time shortened from 2 to 10 minutes for $150^{O}C$, because of excessive lycopene loss under high temperature. At $50^{O}C$ isomerization from trans to cis form was favored during the first 9 hours, and followed by degradation. At higher temperatures of 100 and $150^{O}C$, degradation always occurred instead of isomerization. Activation energy was found to be 61kJ/mol. Illumination intensity

ranged from 2000-3000 lux for 6 days at 25^{O}C, and caused isomerization before degradation. The degradation and isomerization processes of lycopene fitted a first-order model.

As already mentioned, most lycopene (97%) in fresh tomatoes is found in all-trans isomers. Isomerization to cis-isomers (mainly 5-cis-, 9-cis- and 15-cis-) takes place during exposure to high temperature as a consequence of processing. The cis-isomers were more bioavailable than trans- isomers, causing enhanced lycopene bioavailability in processed tomato products as compared to fresh tomato fruits (Shi and Le Maguer, 2000).

In a study of Su *et al*. (1999), stability of carotenoids in human plasma was tested under fluorescent light for up to 72h (0, 4, 24, 48, 72), extracted and then stored in darkness at -20^{O}C, 4C and room temperature (19-22^{O}C), for up to 48h (0, 4, 24, 48). Most of the carotenoids in human plasma were stable under fluorescent light. Greater variability in measurement of most analytes was observed at the room temperature storage than at 4^{O}C and -20^{O}C, but with small range, having little biological significance. Lycopene showed a non significant decrease with time at room temperature conditions. Craft *et al*. (1988) also observed a slight decrease of lycopene in plasma at room temperature in the first four hours, when stability of individual carotenoids was tested for up to 24h.

Fish and Davis (2003) investigated the frozen storage effects on lycopene stability in watermelon puree and cubes. Over one year storage, 30-40% losses were observed under storage at -20^{O}C, and 5-10% losses at -80^{O}C. During the initial freeze-thaw a lycopene degradation of 4-6% was observed. Lycopene in

pureed flesh was more stable compared to watermelon cubes at -20^{O}C; where no difference was observed in lycopene content of cubes or puree held at -80^{O}C.

Stability of fresh-cut watermelon was also evaluated in 5cm flesh cubes from a seeded ('Summer Flavor 800') and a seedless ('Sugar Shack') cultivar (Perkins-Veazie and Collins, 2004). Cubes were placed into unvented polystyrene containers, stored for 2, 7 and 10 days at 2^{O}C, and evaluated for juice leakage, carotenoid composition, color changes, soluble solids content, and titrable acidity. Results showed that juice leakage was about 13% for 'Summer Flavor 800' and 11% for 'Sugar Shack' after 10 days of storage. Lycopene content loss were 6 and 11% after 7 days at 2^{O}C, for 'Summer Flavor 800' and 'Sugar Shack' respectively, while β-Carotene and cis-lycopene contents did not change. CIE *L** and chroma values suggested a loss in color saturation and increased lightening of watermelon flesh after 10 days storage. No chilling injury symptoms were found, but a slight decrease in soluble solids content was observed. Levels of atmospheric CO_2 and O_2 were also monitored, were CO_2 increased and O_2 decreased linearly after 10 days storage, creating a modified atmosphere of 10kPa of both CO_2 and O_2.

A carrot variety, *Daucus carota* L. var. *Kintoki,* contains 9 mg lycopene per 100g wet weight and can be considered as another potential source of lycopene (Mayer-Miebach and Spieß, 2003). Blanching at high temperature (90^{O}C) and under oxygen-free conditions provided high lycopene availability and stability in carrot products from this variety.

Grapefruit (*Citrus paradisi Macfad*) juice concentrates had a more intense color fade when they were stored for 12 months at -23^{O}C in plastic containers compared to metal cans, with estimated shelf life for lycopene of 18 months and 26.1 months respectively (Lee and Coates, 2002). In addition, more than 20% loss of lycopene and about 7% loss of *β*-carotene was observed for the same storage conditions.

When fresh cut persimmons (*Diospyros kaki* L.) were held in controlled atmosphere of 2% O_2 at 5C for eight days, *β*-cryptoxanthin content decreased while lycopene increased (Wright and Kader, 1997).

1.3 Instrumentation - Extraction

Fish *et al*. (2002) modified the method of Sadler *et al*. (1990) for extracting lycopene from food sources that utilized reduced volumes of organic solvents. The conventional method uses 25ml of 95% ethanol, 25ml acetone with 0.05% (w/v) BHT (Butylated HydroxyToluene) added, and 50ml hexane per sample. Fish *et al*. (2002) reduced the volumes of these solvents 5 times to volumes of 5, 5 and 10ml respectively, with also reduced sample sizes to 0.4 or 0.6g. Precision of this method was very close to the conventional method (average STD error per triplicate was 1.22± 0.84% $_{S.D.}$).

A study by Vasapollo *et al*. (2003) proposed a supercritical CO_2 lycopene extraction from tomato, using vegetable oil as co-solvent. The co-solvent was reported to increase lycopene extraction and stabilize it. The highest amount recovered with this procedure was 60% of the total lycopene. The optimum

parameters for extraction of lycopene were found to be: pre-treatment of raw material (dry, grind, screen), pressure 450bar (≈6,526.7psi), temperature 65-70C, CO_2 flow rate 18-20 kg/h, 1 mm particle size, and 10% (w/w) vegetable oil used as co-solvent in the extractions. Hazelnut oil with its lower acidity avoided lycopene loss during extraction and resulted in higher extraction yields compared to other vegetable oils like almond, peanut, and sunflower seed oil. The presence of the co-solvent was believed to promote transportation and solubility of lycopene from solid to supercritical phase.

Arias *et al.* (2000) reported that the color classification of tomato ripening stages using a chroma meter camera correlated with objective HPLC lycopene content. They suggested that lycopene content in tomatoes could be determined non-destructively with a portable chroma meter camera.

Tawfik (2002) used a 5µm C_{30} stationary phase column, with a flow rate of 1ml/min and a linear slope of 40%-50% methanol and methyl *t*-butyl ether mobile phases for 35 minutes. He separated lycopene isomers (cis-, all-trans- and 5-cis) from three raw tomato varieties (Cherry-CA, Roma and On the Vine) and different tomato products (tomato paste, puree, juice and ketchup). Lycopene content was highest in tomato paste, then puree and depending on fresh or dry weight basis, juice or ketchup had the lowest content respectively.

In 2004 Pól et al. developed a method that could eliminate lycopene losses during analysis. A supercritical fluid extraction system (SFE), with CO_2 as an extraction medium, was connected on-line with a High Performance Liquid Chromatography system (HPLC), using a single monolithic column to trap and

separate the analytes. Lycopene was determined in several fruits and food products and due to the on-line structure of the system, lycopene was not influenced by air or oxygen. This procedure proved to be fast (about 35min for the whole lycopene determination process), sensitive (the limit of detection for the HPLC was 0.5ng for the trans-lycopene) and repeatable. A range of temperatures for extraction was tested from 40-140^{O}C, with maximum extraction yield at 80-100^{O}C. Optimum temperature of 90^{O}C was suggested for lycopene extraction.

Setiawan *et al*. (2001), studied carotenoid content of Indonesian fruits. They found that Salak (*Salacca edulis*) and guava (*Psidium guajava*) are excellent sources of provitamin A carotenoids (140+ µg retinol eguivalents/100g wet weight), while mango (*Mangifera indica*), red watermelon (*Citrulus vulgaris*) and papaya (*Carica papaya*) were good sources as well (70+ µg retinol eguivalents/100g wet weight).

A lycopene market is needed for stabilization and expansion of the watermelon industry and to provide a value added product. The long term goal of this research is to develop and evaluate processing steps for watermelons as an alternative source of lycopene for the nutraceutical market and as a good colorant source. The goal of this project was to use a novel filtration procedure with centrifugal precipitation processing to evaluate cultivar and maturity differences in lycopene content and purity.

1.4 Objectives

- To evaluate filtration processing and extraction steps necessary for purification of lycopene
- To determine maturity influence on lycopene distribution during filtration processing of red-fleshed watermelons
- To evaluate temperature effect on lycopene segregation during processing

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CHAPTER II

Maturity and Temperature Influence on Lycopene Distribution during Filtration Processing of Red-fleshed Watermelons

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Additional index words. Citrullus lanatus, antioxidant, lycopene, filtration processing

Abstract.

Lycopene from ground watermelon (*Citrullus lanatus*) flesh can be segregated between filtrate and filter cake by coarse filtration. Low speed centrifugation of the filtrate can further segregate filtrate lycopene between an easily recoverable precipitated high lycopene pellet (approximately 600-800µg•gm^{-1}) and a serum. Lycopene in watermelon flesh increases steadily during maturation and remains constant, or slightly decreases in overripe melons. This study was conducted to document the effect of watermelon maturity on lycopene segregation during filtration/centrifugal processing. Flesh from seedless watermelon cultivars 'Hazera 6007', 'Hazera SW1', 'Sugar Shack', 'Hazera 6008', 'Hazera 5109' and 'Sugar Time' was ground and filtered through two layers of Miracloth. Filter cakes were rinsed with water and filtrates were centrifuged at 3500g to precipitate lycopene. Centrifugal recovery of lycopene from filtrates was about the same for undermature and mature melons (50-70%), but was much lower for overripe watermelons (35-45%). Lycopene centrifugal recovery from overripe filtrates was

improved up to 85% if ground flesh was heated to 60^{O}C or 85^{O}C prior to filtration. Lycopene from preheated flesh (60^{O}C or 85^{O}C) shifted segregation into the filter cake for all three ripeness stages and increased the total recoverable lycopene from ground flesh by 5-10% more than the no heat procedure. This was especially beneficial for overripe melons. When heat treated filter cakes were rinsed and refiltered to reduce the high sugar content of the filter cake, lycopene was redistributed from the filter cake to the filtrate. These results show that heating watermelon puree can be used to improve lycopene recovery into the filter cake, especially in overripe watermelons.

Introduction

Lycopene is one of the most potent antioxidants in today's nutraceutical market, believed to prevent the initiation of certain types of cancer, especially prostate cancer (Giovannucci, 1999). Lycopene has also shown promising results in prevention of alcohol-induced liver injury by prevention of severe oxidative stress induced by arachidonic acid in liver cells (Tapiero et al., 2004).

The major dietary source of lycopene in the U.S. is tomatoes (USDA, 2004) with average concentration of 3mg/100g of fresh weight (Perkins-Veazie et al, 2001). Lycopene is also found in watermelon, guava, papaya, mango, red grapefruit and autumn olive (Johnson, 2002). Several watermelon cultivars contain more lycopene on a fresh basis than tomatoes (on average 5-6.5mg/100g of fresh weight) (Perkins-Veazie *et al*, 2001). Bőhm et al. (2003) reported rosehip (*Rosa canina* L.) as another potential source of lycopene (12.9-

35.2 mg/100 g). Lycopene bioavailability has been reported to increase with heat and/or homogenization from tomato products, probably due to break down of the cell walls, allowing carotenoid release (Stahl and Sies, 1996). Lycopene in tomato products is typically found 80% or greater in the trans- form, whereas in plasma lycopene and other carotenoids are found generally >50% in the cis- form. Isomerization of trans- to the cis- form is hypothesized to occur during digestion, but only after trans- lycopene is released from the food matrix (Boileau et al., 2002). In the studies of Nguen and Schwarz (1998) and Schierle et al. (1997), only small increase of lycopene isomerization to the cis- form was reported during thermal processing.

During storage of fresh cut watermelon cubes at $2^{O}C$, loss of 6-11% lycopene was observed after seven days, probably due to oxidation, without any isomerization (Perkins-Veazie and Collins, 2004).

In a study of Fish and Davis (2003), stability of watermelon puree and flesh chunks was evaluated under storage conditions at $-20^{O}C$ and $-80^{O}C$ after up to 12 months in storage. Faster rate of lycopene deterioration was observed in chunks than puree at $-20^{O}C$, while at $-80^{O}C$ they behaved similarly. About 10% of lycopene from puree degraded after 30 days of storage at $-20^{O}C$, while less than that degraded over a year's storage at $-80^{O}C$. A lycopene loss of 4-6% was attributed to an initial freeze-thaw cycle of watermelon tissue, while such a loss hasn't been reported in the tomato system. No isomerization of lycopene from all-trans to cis- forms was observed during storage.

Recovering and utilizing lycopene from watermelons would present an untapped secondary market for marketable watermelons, and offers a market for cull watermelons (20-80% of production). A means to concentrate and stabilize lycopene from watermelons is needed to meet the demands of this potential market. In our study we outline a procedure to obtain lycopene from watermelons using a filtration and centrifugal precipitation process, and investigate the maturity and temperature influence on the segregation of lycopene during the various steps of the procedure.

MATERIALS AND METHODS

2.1 Plant Material

Watermelon seeds for 2003 and 2004 plantings were obtained from Sugar Creek Seed Inc, cultivars 'Sugar Shack' and 'Sugar Time', and from Hazera Genetics Ltd, cultivars 'SW1', '6007', '5109' and '6008' ('Extazy'). All cultivars were triploids (seedless). 'XIT 101' (Sugar Creek Seed, Inc.) was used as diploid pollinator in both years.

In 2003 seeds from cultivars 'Sugar Shack', '6007' and 'SW1' were planted in Speedling flats on May 16th, held under high humidity and warm temperature for two days to enhance uniform germination and grown at the horticulture greenhouse facilities in Stillwater, OK. Seedlings were transplanted on the 4th of June (after 17 days) at the Oklahoma State University Vegetable Research Station (OSU VRS) in Bixby and harvested at different maturity stages during August and until early September (first harvest on August 14th, last on September 4th). In both years, seeds of 'XIT 101' were also sown and transplanted in the field in alternating rows (one row of pollinator followed by two rows of triploids) to serve as diploid pollinator. Plants were placed 0.7m apart within the row, and rows were 6m apart. In 2004, seeds from cultivars 'Sugar Time', '6008' and '5109', were planted on May 5th in the Horticulture greenhouse facilities in Stillwater and transplanted on 1st of June at OSU VRS in Bixby (after 28 days). Watermelons were harvested at different maturity stages in late August (the 24th).

2.2 Initial Watermelon Handling

Watermelon maturity stage was initially based on characteristics evaluated on site at the field. In particular, color of the groundspot (surface of the watermelon touching the ground), tendril vitality and sound of fruit by thumping were applied. Several melons were cut to assure validity of our methodology just prior to harvest for these studies.

After harvest, watermelons were transferred to a research lab at Stillwater and held at room temperature (20-25^{O}C) until processing.

2.3 LABORATORY PROCESSING

Individual watermelons were cleaned to remove soil residues, weighed and cut in half through the center of the ground spot from stem to blossem end. Additional observations were recorded using destructive "in lab" observations (^{O}BRIX, flesh color and texture, rind thickness, flesh firmness). Three maturity stages were utilized for segregation processing:

2003 ('Sugar Shack', '6007', 'SW1')

1. Under mature - Green succulent tendril, greenish groundspot, 6-7 ^{O}BRIX, pink to white flesh color, crisp texture; 2. Mature - Dead tendril, yellow groundspot, 7.5-8.5 ^{O}BRIX, red flesh color, crisp texture; and 3. Overripe - Brown, dead tendril, yellow groundspot, 7.5-9 ^{O}BRIX, pale red flesh color with an orange tint at the flesh/rind interface, grainy texture.

2004 ('Sugar Time', '6008', '5109')

Same characteristics as 2003 except the ^{O}BRIX was lower for the 2004 cultivars.

1. Under mature: 5-6.5 ^{O}BRIX; 2. Mature: 5-7 ^{O}BRIX; and 3. Overripe: 7-8 ^{O}BRIX

Flesh was carefully separated from rind for three melons each representing the three maturity stages identified above (nine melons per cultivar), and were placed into individual preweighed tubs. Weight was recorded and rind was discarded. Flesh was chopped in small chunks (<4cm), mixed and nine samples (≈ 1 to 2 g) of raw watermelon were taken for lycopene analysis, placed into prelabeled brown bottles (120ml) and stored in a freezer at -20^{o}C for lycopene quantification procedure. Six samples of ≈1 g flesh were also placed into small preweighed and prelabeled metal weighing boats for moisture content analysis and placed into an oven at 70-100^{O}C. Samples of juice were placed into 2 dram vials (17x60mm screw thread with rubber lined cap; Fisherbrand, Fisher Scientific LLC, Denver, CO), centrifuged for 15min using a Speed Vac centrifuge (Savant RVT4104 Refrigerated Vapor Trap, Savant Instruments Inc., Farmingdale, NY) (approximately 3500g) and duplicate 50 µl samples were obtained and frozen at -20^{o}C for sugar analysis.

2.3.1 2003 FILTRATION PROCESSING

For this study a total of 27 melons were used, representing three melons each per three maturity stages and three cultivars.

Chopped flesh (500g) was proportioned into three prelabeled and preweighed quart grinding jars. Each jar was weighed and then contents were homogenized on ice using an Omnimixer homogenizer with polytron attachment (Post Mounted Homogenizer, Omni-Mixer with interchangeable rotor stator and 20mm diameter x195mm length w/saw teeth polytron-style generator probe; OMNI International, Waterburry, CT) at speed 6 for 2 bursts of 1 min each, recovering 99% of the flesh. With a large mouth Pasteur pipette triplicate samples were withdrawn from puree for lycopene analysis (≈1ml) and placed into preweighed and prelabeled 2 dram vials. Weights of vials were recorded and they were capped and stored at -2^{O}C to await analysis. Duplicate samples for sugar analysis (50µl into 2 dram vials) were also obtained. The remaining contents were then reweighed and filtered through two layers of Miracloth under vacuum, using a Buchner funnel into vacuum flasks until no dripping occurred (approximately 10-15 minutes). Grinding jars were reweighed for any residues.

The filtrates (90% of the flesh weight) were transferred from the vacuum flasks into preweighed and prelabeled 1L plastic bottles. Filtrate weight was obtained for later use in filter cake rinsing. With a calibrated pipette triplicate 1.0ml samples were withdrawn during agitation into 2 dram glass vials from each filtrate for lycopene analysis and duplicate samples were also withdrawn for

sugar analysis (50µl into 2 dram vials). Filtrate was then used for centrifugal processing as described in the next section.

Filter cakes (10% of the flesh weight) were placed with miracloths onto large preweighed and prelabeled plastic weighing boats. Weights were obtained and filter cakes were transferred into another large preweighed weighing boat. Weights of filter cakes and soaked miracloths were obtained, and miracloths were discarded. Triplicate samples (≈0.5g) from each filter cake were stored in prelabeled 120ml brown bottles for lycopene analysis. Equal amount of samples was placed onto preweighed and prelabeled metal weighing boats for moisture analysis.

2.3.1.1 Filtrate Centrifugation Procedure

Sets of 18 preweighed 2dram vials (6 samples from 3 separate filtrates, 2.00ml each) were centrifuged for 15 min in the Speed vac to precipitate lycopene. The supernatant layer (serum) was withdrawn and transferred into separate preweighed and prelabeled 2dram vials using a Pasteur pipette, and the centrifuged vials were weighed to obtain pellet weight. A 50µl subsample was withdrawn from the serum samples with a calibrated pipette, placed into prelabeled 2dram vials and stored at -20^{O}C for sugar analysis. Weights for serums were recorded and vials were capped and stored at -20^{O}C for lycopene analysis. Nine vials containing pellets (3 out of 6 from each filtrate) were capped and stored at -20^{O}C for lycopene analysis.

To the other nine vials, 2ml of deionized water was added and vials were capped and vortexed until mixed. Vials were re-centrifuged for 15 min in the Speed vac, and then serums were transferred into preweighed and prelabeled 2 dram vials and processed as described above. All vials were then capped and stored at -20^{O}C until lycopene analysis.

2.3.1.2 Filter Cake Rinse

The remaining paste-like filter cake fraction, derived from ground flesh filtration and sampled as described previously, was rinsed with deionized water. The volume used was equal to the ratio of filtrate to filter cake for the preceding filtrate, accounting for loss of filter cake weight from sampling. Filter cake and water were magnetically stirred for 15min, and the mixture was filtered as before. Samples for lycopene and sugars were taken as previously described.

Filtration resulted in two new fractions; a set of new filter cakes and new filtrates. Nine samples of 2ml each were placed into 2 dram vials from the new filtrates (3 samples from each of 3 filtrates), centrifuged and separated into serum and pellet as before with no additional water rinse of pellets. The new filter cakes were rinsed once more as before and re-filtered to segregate a third set of filter cakes and filtrates. Samples of lycopene, moisture and sugar were taken at all appropriate steps as mentioned previously. All samples were stored at -20^{O}C until analysis.

2.3.2 2003 HEATING TRIAL

A small trial was conducted to test heat influence on lycopene segregation during processing. Six overripe watermelons from the 'SW1' cultivar were used and treated as two triplicate sets. All melons from both sets were handled as before until just after the grinding step. After the three 500g aliquots were ground, the contents of each quart jar were treated differently. For each watermelon in the first triplicate set, the contents of the first jar were transferred into a 1L fleaker, covered with aluminum foil and heated to $85^{O}C \pm 2^{O}C$ for two minutes before filtration. The contents of the second jar were first filtered and the subsequent filter cake was resuspended in a volume of deionized water equal to the volume of the preceding filtrate and then heated to $85^{O}C \pm 2^{O}C$ for two minutes (heat was applied after the first filtration). The third jar was handled entirely as described previously, at $25^{O}C$ (room temperature). All other treatments were as described in the 2003 study, with three filtrations (two filter cake rinses) and two filtrate centrifugations (1^{st} and 2^{nd} filtrate). The second triplicate set of watermelons was handled the same as the first, except that filter cakes for the first and second jars were rinsed with hot water ($\approx 80^{O}C$), instead of room temperature water, to investigate the effect of rinse water temperature on the segregation pattern of lycopene. The third jar was handled entirely at $25^{O}C$, as before. Samples for lycopene, moisture and sugar were taken at all steps as before.

2.3.3 2004 FILTRATION PROCESSING AND HEATING PROJECT

For this study, a total of 27 fruit, representing three per maturity per cultivar ('Sugar Time, '6008' and '5109'), (Undermature, Mature and Overripe), were processed into red flesh as described earlier.

To further investigate and characterize temperature influence on lycopene segregation during processing, three 500g aliquots were prepared and ground as before, but were treated with stirring in three different ways; heated at 60^{o}C for 5min, heated at 85^{o}C for 2min or held at 25^{o}C for 15min.

Ground flesh from jars 1 and 2 were transferred into preweighed and prelabeled foil wrapped 1L glass fleakers for heat treatments. Ground flesh from jar 3 was transferred into a 1L plastic bottle and stirred at 25^{O}C as before. For heat treatments at 60±2^{O}C or 85±2^{O}C, foil wrapped fleakers were placed onto a hot plate and stirred vigorously. Temperature was monitored with a thermocouple thermometer and once the target temperature was reached, fleaker contents were maintained at ±2^{O}C of the treatment temperature by periodically removing from the hot plate. Immediately after treatment, fleakers were reweighed and sampled. Triplicates of approximately 1gm were obtained with a wide mouth Pasteur pipette into preweighed and prelabeled 2 dram vials for lycopene analysis and weights were recorded. The contents of the fleaker were then reweighed and filtered as before. The non-heated sample was also sampled and filtered as before. All containers were reweighed, after transfer onto miracloth in a buchner funnel for filtration, to account for residual sample.

Filtration resulted again in an aqueous filtrate and a pasty semisolid filter cake. All filtrates and filter cakes were weighed and samples for lycopene (filter cake, ≈0.1-0.5g into 120ml brown bottles; filtrate, 1ml into 2dram vials) and sugars (filtrate only, 50µl into 2 dram vials) were frozen at -20^{o}C to await analysis. Triplicate samples of 0.5g from the filter cakes were also taken for moisture content determination.

Following the first filtration all resultant filter cakes were rinsed once at 25^{O}C, with a volume of water equivalent to the previous filtrate volume and then re-filtered. Sampling was conducted as described before, except that a third filtering was not conducted. Filtrate and filter cake samples were obtained as before for lycopene and sugar analysis.

2.4 Sugar Analysis

Sugars were analyzed following the HPLC procedure of Leskovar *et al.* (2004); 50 µl juice samples were diluted appropriately with dionized water, and 1ml samples were placed into an AS-3500 autosampler. Samples were injected automatically onto a Dionex DX-500 HPLC system (Dionex Corporation, Sunnyvale, CA), overfilling a 50µl injection loop. Sucrose, fructose and glucose were separated with a 4mm x 250mm Carbopac PA-1 column under isocratic conditions of 92% water and 8% 0.5M NaOH for 20 minutes. Peaks were detected with a pulsed electrochemical detector in pulsed amperometry mode and a Peak-Net data station. A flow rate of 1ml per minute was used and after every 30 injections the carbonate buildup was removed using 50% water and

50% 0.5M NaOH rinse, at the same flow rate for 60 minutes. Five minutes prior to injection, the column was allowed to reequilibrate at isocratic conditions. Sugars were identified by co-elution with authentic standards and quantitated by the external standard procedure.

Samples collected during processing were obtained only from liquid fractions and were diluted as described for HPLC analysis to determine sugar content of those liquid fractions (filtrates and serums). We assumed that the solid fractions (filter cakes and pellets) had the same concentration of sugars in their aqueous portion as filtrate serums; moisture content of these fractions was used in combination with the concentration of their paired liquid fraction from processing (e.g. 1^{st} filter cake- 1^{st} filtrate and 2^{nd} pellet- 2^{nd} serum etc.), to estimate sugar content of the solid fractions.

2.5 Lycopene Analysis

Lycopene was quantitated by a modified spectrophotometric procedure of Sadler *et al.* (1990). Tissue samples were thawed at room temperature and ethanol, acetone and hexane were added at 1:1:2 ratios respectively, along with 0.05% BHT (Butylated HydroxyToluene). For smaller sample sizes, the procedure of Fish et al. (2002) for reduced organic volume extraction was used, at the same solvent ratios. Samples were agitated for 10min on a shaker, then dionized water was added (three ml of water for every 10ml hexane used) to help phase separation and shaking was continued for another 5 minutes. Samples

were then allowed to stand for 15min, after which a fraction of the top hexane layer was filtered through Whatman no.1 paper using a 5ml syringe with a Millipore syringe filter (Swinney Stainless, 13mm; Millipore Corp., Bedford, MA).

Lycopene was quantified with a dual beam Shimadzu spectrophotometer at 503nm (Shimadzu UV-160U Visible Recording Spectrophotometer, Shimadzu Scientific Instruments, Houston, TX).

The **µg/g** per sample was calculated by the formula:

$$\frac{(\text{Abs@503nm} / 0.172) \times \text{Organic volume used (ml)}}{\text{Weight of the sample (gm)}} \times 0.5369, \quad (2.5.1)$$

were Abs@503nm is the spectrophotometric absorbance units as given by the instrument; 0.172 is the extinction coefficient of lycopene in hexane; organic volume stands for the volume in ml of the hexane used for extraction; weight of the sample used for extraction is in grams and 0.5369 is the molecular weight of lycopene divided by 1000 (536.9 g/Mole).

For lycopene data analysis we followed a split plot design with cultivar, maturity and temperature as the main plots, and the different fractions of the procedure as subplot. Lycopene concentration on a per fraction basis (µg lycopene per g of fraction) was normalized (µg lycopene per g red flesh) to simplify comparisons between different cultivars and maturity levels.

RESULTS

Lycopene results were normalized to allow comparison among watermelons varying in size (cultivars, maturity stage) and between various fractions from these fruit (filter cakes, filtrates and filtrate pellets). The absolute concentration in µg lycopene/g sample was normalized to µg lycopene/g ground flesh that segregated into that fraction during the procedure. An example of weight yields for fractions during processing is indicated in fig. 2.

Our filtration processing steps outlined in materials and methods caused lycopene to segregate into two fractions, filtrate and a filter cake, with weight yields of 90-95% for the filtrate and 5-10% for the filter cake (Fig. 2). We consider as final utilizable products the filter cakes derived from filtrations, and the pellets precipitated from filtrates after centrifugation (about 2% of filtrate weight). Filtrate supernatant serums were considered waste, since they could not be further purified, or such a procedure would be economically unfavorable.

Watermelon lycopene concentration was influenced by maturity and differed among cultivars. Although cultivars differed in normalized lycopene concentration (‘6007’> ‘SW1’, ‘6008’> ‘5109’ ≥ Sugar Shack, Sugar Time), the maturity influence within cultivars was relatively constant. Undermature melons of all cultivars generally contained less lycopene than mature and overripe melons (Fig. 3).

In 2003 we focused on evaluating lycopene segregation as affected by watermelons maturity during filtration processing at 25^{O}C. Segregation of

lycopene between filtrate and filter cake was strongly influenced by maturity. Lycopene segregated more into the filtrate especially as maturity increased, reaching nearly 90% for overripe melons (Fig. 4). Although lycopene was much higher in terms of absolute concentration in the filter cakes, the filtrates contained more total lycopene on a normalized basis (Fig. 5) due to the greater weight of filtrate versus filter cake (Fig. 2). This high filtrate lycopene content, and its high total weight, led us to search for means to purify lycopene from the aqueous, high sugar environment. Precipitation by centrifugation proved to be an effective method.

Filtrates were centrifuged (3500g) to produce a high lycopene pellet (Table 1) and a serum, of about 2-3% and 97-98% weight recovery respectively (Fig. 2). Maturity effect was clear on lycopene recovery into the pellet for the first filtrate, where 55-80% of the lycopene from under-mature and mature melons was precipitated as a pellet (Table 2). One notable exception was for mature '6007' where only 37% precipitated. Although there was a higher segregation of lycopene into the first filtrate fraction from overripe melons (Fig. 4), centrifugal lycopene precipitation was much less, with pellets containing less than 35% of the total filtrate lycopene (Table 2).

One water rinse of the pellets reduced sugar concentration from 6-9% to less than 0.1% (data not shown). Pellet lycopene content was not affected for the cultivar 'Sugar Shack', as well as for overripe 'SW1', and both mature and overripe melons of '6007' (data not shown), but was reduced for all maturity stages of the 2004 cultivars (25^{O}C on table 3).

Rinse of the first filter cake, followed by a second filtration, reduced the segregation of lycopene into filtrate to less than 70% when compared to the first filtration (Fig. 4). Subsequent centrifugation also altered lycopene precipitation, where the supernatant replaced the pellet as the dominant fraction (Table 2). Consequently, lycopene yields decreased between filter cake rinses and subsequent second and third filtrations (second filtration yields shown in Fig. 6).

In 2003, an initial effort to change the segregation pattern and yields of lycopene, especially from overripe melons, led us to conduct the heat trial described in Materials and Methods, where only overripe melons of cultivar 'SW1' were used. Our aim was to identify a method which could change the segregation pattern of lycopene and enhance lycopene content of one of the recoverable fractions (filter cake or filtrate pellet).

Heating showed promising results by retaining lycopene into the filter cake fraction during the first filtration, minimizing the segregation into the filtrate (Table 4). About 90% of lycopene was segregated into the filter cake for heated melons, but only 15% segregated into the filter cakes with no heat application. This effect was only observed for ground flesh heated prior to the first filtration; further heating of the rinsed filter cake after first filtration did not favor lycopene retention in the following filter cake fraction, with less than 50% recovery from filtrates as pellet after centrifugation (Table 4).

After conducting the initial heat trial on the overripe watermelon cultivar of 2003, we documented our initial findings of the heat influence on lycopene segregation during processing in 2004. The 2004 study was based on heat

treatments applied to three watermelon cultivars similar to the 2003 ones, at the same three maturity stages. Heat applications included the control at 25^{O}C and heating of ground flesh to 85^{O}C ±2^{O}C for two minutes, as well as one additional treatment of heating to 60^{O}C ±2^{O}C for five minutes.

The 2004 cultivars could be paired in terms of normalized lycopene concentration with the 2003 cultivars as follows: '6007'-'6008' (35-53 and 28-47 normalized µg/g respectively, range is relative to maturity stage), 'SW1'-'5109' (28-44 and 11-50 normalized µg/g respectively) and 'Sugar Shack-Sugar Time' (20-28 and 18-27 normalized µg/g respectively) (Fig. 3). Comparing the control treatment of 2004 (25^{O}C) with the 2003 procedure, we saw initially the same segregation pattern for lycopene distribution after the 1st filtration (Fig. 7). More lycopene segregated into the filtrate fraction as maturity increased, but after centrifugation less than 45% of the lycopene was recoverable as a precipitated pellet for the overripe melons (25^{O}C in Table 5). In contrast for mature and under mature watermelons, 75-85% of the filtrate lycopene was recovered as a pellet.

Rinsing of the filter cake once with deionized water caused lycopene to segregate equally as filter cake and filtrate after the second filtration (Fig. 7). Centrifugation of the produced filtrate precipitated less than 60% of the lycopene as a pellet for all maturity stages (25^{O}C in Table 5) resulting in a total lycopene recovery from rinsing (2nd filter cake plus 2nd pellet) of less than 60%, similar to the 2003 yield (Fig. 6).

Initial heating of ground flesh at either 60^{O}C or 85^{O}C caused lycopene loss (measured as difference in lycopene content prior to heating versus just after

heating) of approximately 20-25%, regardless of cultivar and maturity stage (Table 6).

When compared to 25^{O}C, both heat treatments increased lycopene segregation into the filter cake fraction after the first filtration, by at least two fold for under mature and mature melons, and four fold for overripe melons (Fig. 7). After centrifugation of the filtrates, an increased segregation of lycopene from overripe melons as a pellet was observed for both heat treatments, while less lycopene was present in the filtrate fraction (Table 5).

Rinsing the filter cake once and a subsequent second filtration, resulted in 50-60% segregation of lycopene as a new filter cake for undermature and mature melons treated at 25^{O}C and 60^{O}C. Heating at 85^{O}C segregated 55-70% of the lycopene into the second filter cake for all maturity stages, with higher segregation observed for overripe melons (Fig. 7).

Centrifugation of the produced second filtrate showed approximately 45-70% loss of lycopene (into the serum fraction) for the 25^{O}C treatment depending on maturity, with 45-50% for undermature and mature, and 70% for overripe melons. Heat influence of both treatments (60^{O}C and 85^{O}C) decreased that loss to about 35% - 45% for all maturity stages (Table 5).

DISCUSSION

Our filtration processing and lycopene precipitation steps provided a means to concentrate watermelon lycopene into two fractions; a filter cake and a centrifugal filtrate pellet. These fractions present a means to collect lycopene from watermelons in less than 10% of the original watermelon weight. Segregation in to these two fractions was maturity dependent, with overripe melons loosing more than 45% of filtrate lycopene to the supernatant serum fraction after centrifugation (Table 2). This excessive lycopene loss was probably caused as a part of senescence, where extended breakdown of chromorplast membranes occur in overripe watermelons, releasing lycopene from its matrix and affecting lower lycopene precipitation.

Heating puree prior to filtration at 60^{O}C and 85^{O}C increased segregation of lycopene by at least two fold into the filter cake fraction, for all maturity stages (Fig. 7). This change in lycopene segregation away from the filtrates was especially useful for the overripe melons where lycopene segregation was four fold into the filter cake fraction, and lycopene recovery from filtrates of unheated purees was poor.

Rinsing the centrifugal pellets from filtrates with water, successfully reduced sugars to less than 0.1%, with an acceptable lycopene loss (25^{O}C in Table 3), or even negligible lycopene loss for the heat treatments (60^{O}C and 85^{O}C in Table 3). On the other hand, rinsing of the first filter cake can not be recommended due to substantial lycopene loss into the subsequent centrifuged filtrate serum fraction (Table 5).

The two products that best serve as a means to concentrate and recover lycopene were the filter cakes and filtrate pellets from a single filtration. Optimum recovery of lycopene following this procedure was maturity dependent without heat application, and highly temperature dependent when watermelons flesh was subjected to heat treatments (table 6).

For the 2003 cultivars, the total amount of lycopene yielded from ground flesh (as a total of normalized µg/g) after one filtration step was about the same for all maturity stages. However, due the different amounts of initial lycopene contained on undermature, mature or overripe melons, the recovery was greater for undermature and mature watermelons than for overripe watermelons (45-50% compared to 25% respectively) (Table 6). Lycopene recovery without heat was best for ripe watermelon with one filtration step, utilizing the first filter cake and first pellet and resulting in 45% recovery.

Following heat treatments of 60^{O}C, or 85^{O}C, total lycopene recovery from puree filtration step (as a total of normalized µg/g) was not significantly different among temperatures (Table 6). Despite the significant retention of lycopene in the 1st filter cake fraction described earlier of heat treatments on lycopene segregation (compared to the 25^{O}C), total lycopene recovery was about the same due to the difference of lycopene content in the utilized fractions. In particular for the total lycopene recovery at 25^{O}C, a lower content filter cake and high content filtrate pellet was produced (similar to 2003), while for the heat treatments a high content filter cake and a lower content filtrate pellet was produced (Table 5). Recoveries yielded about 50-60% of the lycopene content of

ground flesh, which could be 75-80% from heated ground flesh, if heat loss prior to filtration is factored in (2004 in table 6). Consequently, any treatment would yield about the same amount of lycopene after one filtration step, with differences only in the amounts contained in the final products. We recommend use of mature and overripe melons since they contain more lycopene than undermature melons (Fig. 3).

Best lycopene yields could be achieved after only one filtration step, thus it is recommended to utilize the two concentrated fractions produced from ground flesh filtration; the first filter cake and the centrifuged first filtrate pellet. Because lycopene segregation in these two fractions is maturity dependent, but can be altered with heat application of ground flesh, the maturity level of the watermelons should be determined to utilize the best procedure for lycopene recovery. Consequently, for undermature and mature melons, no heat application is necessary, since we can recover about 45-50% of the total initial lycopene as a high content (low weight) pellet and a lower content filter cake. To achieve similar or higher lycopene recoveries from overripe melons (on average 50-60%), heating of the ground flesh is necessary to drive lycopene away from the filtrate. In this case a high content filter cake is used with a lower content filtrate pellet. Using concentrations of the cultivars used in this study, as shown in figure 3, we can estimate lycopene yields using our procedure. For example, a 3000g undermature watermelon (about 1500g flesh) can yield about 8.3-26.3mg lycopene and a mature watermelon can yield about 19.5-38.5mg lycopene depending on the cultivar used, without heat application (50% recovery). Similar

size overripe melons can yield about 25.2-47.7mg lycopene, depending on cultivar, using the heat processing extraction (60% recovery).

This procedure gives a means to potentially utilize two high lycopene fractions; a filter cake and a precipitated pellet. However, stability of these fractions during storage is not yet known and needs to be investigated, for potential marketing and shipping requirements. For instance, a freeze-drying procedure could be tested with the high moisture filter cake, and different oil suspensions (hazelnut oil, sunflower oil etc.) for the highly concentrated pellets. In addition, a larger scale experiment of this procedure would be very helpful to evaluate if similar recoveries of lycopene are applicable when larger quantities of melons are used, and try to engineer equipment for close-to-field application (harvesters, rind-flesh separation, low light-oxygen facilities etc.).

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Table 1. Lycopene concentration of first pellet versus first serum for the different maturity levels of 2003 melons.

Fraction	µg lycopene • gm fraction^{-1}		
	Undermature	**Mature**	**Overripe**
1st Pellet	535 $_{ab}$[z]	643 $_{a}$	376 $_{b}$
1st Serum	2 $_{e}$	7 $_{d}$	19 $_{c}$

[z] Means with different letters denote significance at $P \leq 0.05$ among fractions and maturity stages.

Table 2. Lycopene recovery in normalized µg/g after: A) first filtration, B) first filtrate centrifugation, C) 2nd filtration and D) 2nd filtrate centrifugation of the different cultivars and maturity levels of 2003 melons. Comparison of filter cakes vs. filtrates and pellets vs. serums.

Processing step	Fraction	Normalized µg/g lycopene								
		Undermature			Mature			Overripe		
		'6007'	'SW1'	'SuSh'	'6007'	'SW1'	'SuSh'	'6007'	'SW1'	'SuSh'
A) 1st Filtration	1st Filter Cake	8.8 $_{a}^{y}$	5.2 $_{a}$	2.6 $_{a}$	5.0 $_{a}$	4.6 $_{a}$	4.9 $_{a}$	3.5 $_{a}$	3.5 $_{a}$	2.4 $_{a}$
	1st Filtrate	15.5 $_{bc}$	15.1 $_{bc}$	9.1 $_{c}$	37.8 $_{a}$	21.4 $_{b}$	14.1 $_{bc}$	44.6 $_{a}$	38.3 $_{a}$	18.2 $_{bc}$
		NS	*	NS	*	*	*	*	*	*
B) 1st Filtrate Centrifugation	1st Pellet	12.5 $_{a}$	8.3 $_{a}$	6.0 $_{a}$	14.0 $_{a}$	13.7 $_{a}$	9.6 $_{a}$	10.8 $_{a}$	5.2 $_{a}$	5.8 $_{a}$
	1st Serum	1.1 $_{d}$	1.9 $_{cd}$	0.6 $_{d}$	14.2 $_{abc}$	2.4 $_{cd}$	1.6 $_{d}$	20.4 $_{ab}$	21.0 $_{a}$	8.3 $_{bcd}$
		*	*	*	NS	*	*	*	*	NS
C) 2nd Filtration	2nd Filter Cake	2.5 $_{a}$	1.5 $_{a}$	0.6 $_{a}$	1.2 $_{a}$	1.4 $_{a}$	1.2 $_{a}$	1.5 $_{a}$	0.6 $_{a}$	0.4 $_{a}$
	2nd Filtrate	3.8 $_{a}$	2.4 $_{a}$	1.4 $_{a}$	2.9 $_{a}$	2.5 $_{a}$	2.7 $_{a}$	1.8 $_{a}$	1.9 $_{a}$	1.3 $_{a}$
		NS	NS	NS	NS	NS	NS	NS	NS	NS
D) 2nd Filtrate Centrifugation	2nd Pellet	0.4 $_{a}$	0.5 $_{a}$	0.5 $_{a}$	1.3 $_{a}$	0.6 $_{a}$	1.3 $_{a}$	0.7 $_{a}$	0.5 $_{a}$	0.5 $_{a}$
	2nd Serum	0.9 $_{a}$	0.5 $_{a}$	0.3 $_{a}$	0.9 $_{a}$	0.7 $_{a}$	1.0 $_{a}$	0.8 $_{a}$	0.9 $_{a}$	0.6 $_{a}$
		NS	NS	NS	NS	NS	NS	NS	NS	NS

[y] Means with different letters denote significance at $P \leq 0.05$ between maturity stage and cultivars in the same row. [NS], * Non significant or significant at $P \leq 0.05$ between means in the same column for the same processing step.

Table 3. Temperature effect on first pellet lycopene concentration (normalized µg/g), after rinse with deionized water of 2004 cultivars.

Fraction	Undermature			Mature			Overripe		
	25°C	60°C	85°C	25°C	60°C	85°C	25°C	60°C	85°C
1st Pellet	8.0	2.8	1.7	13.3	4.2	2.4	12.6	5.2	0.8
Rinsed 1st pellet	6.5 *	2.3 NS	1.4 NS	9.5 *	4.1 NS	2.4 NS	8.5 *	4.6 NS	0.8 NS

NS, * Nonsignificant or significant between fractions in the same column at $P \leq 0.05$.

Table 4. Lycopene recovered concentration in normalized µg/g after: A) Heated ground flesh, B) Filtration of heated ground flesh (first Filtration), C) Heated first filter cake (2nd Filtration) and D) 2nd Filtrate centrifugation, on 2003 heat trial.

Processing Step	Fraction	Temperature		
		25C	85C Heat before 1st Filtration	85C Heat after 1st Filtration
A) No Filtration	Ground Melon	42.5 $_{a}$	42.6 $_{a}$	43.0 $_{a}^{x}$
	Heated Ground Flesh		31.5 *	
B) 1st Filtration	1st Filter cake	4.6 $_{b}$	20.1 $_{a}$	5.2 $_{b}$
	1st Filtrate	27.7 $_{a}$ *	2.2 $_{b}$ *	30.1 $_{a}$ *
C) 2nd Filtration	2nd Filter cake	1.2 $_{b}$	11.9 $_{a}$	1.8 $_{b}$
	2nd Filtrate	2.4 NS	7.3 NS	1.1 NS
D) 2nd Filtrate Centrifugation	2nd Pellet	0.7 $_{a}$	2.7 $_{a}$	0.7 $_{a}$
	2nd Serum	1.2 $_{a}$ NS	3.5 $_{a}$ NS	0.7 $_{a}$ NS

[x] Means with different letters denote significance at $P \leq 0.05$ between temperature treatments in the same row. [NS, *] Non significant or significant at $P \leq 0.05$ between means in the same column at the same processing step. Solid filled cells show the fraction where heat was applied.

Table 5. Lycopene recovery in normalized µg/g after: A) first filtration, B) first filtrate centrifugation, C) 2nd filtration and D) 2nd filtrate centrifugation of different maturity and temperature levels of 2004 melons. Comparison of filter cakes vs. filtrates and pellets vs. serums.

Processing step	Fraction	Normalized µg/g lycopene								
		Undermature			Mature			Overripe		
		25°C	60°C	85°C	25°C	60°C	85°C	25°C	60°C	85°C
A) 1st Filtration	1st Filter Cake	5.3 $_{b}$	12.0$_{ab}$	12.8$_{ab}$	6.0 $_{b}$	17.7 $_{a}$	18.9 $_{a}$	4.4 $_{b}$	17.9 $_{a}$	22.1 $_{a}$ [w]
	1st Filtrate	9.5 $_{bc}$	2.4 $_{c}$	2.1 $_{c}$	17.9 $_{b}$	3.8 $_{c}$	2.4 $_{c}$	29.3 $_{a}$	6.1 $_{c}$	0.9 $_{c}$
		NS	*	*	*	*	*	*	*	*
B) 1st Filtrate Centrifugation	1st Pellet	8.0 $_{abc}$	2.8 $_{abc}$	1.7 $_{c}$	13.3 $_{a}$	4.2 $_{abc}$	2.4 $_{bc}$	12.6 $_{ab}$	5.2 $_{abc}$	0.8 $_{c}$
	1st Serum	1.2 $_{a}$	0.5 $_{a}$	0.7 $_{a}$	4.2 $_{a}$	0.7 $_{a}$	0.7 $_{a}$	10.2 $_{a}$	1.1 $_{a}$	0.3 $_{a}$
		*	NS	NS	*	*	NS	NS	*	NS
C) 2nd Filtration	2nd Filter Cake	1.9 $_{ef}$	4.2 $_{cdef}$	5.0 $_{bcde}$	2.1 $_{def}$	6.2 $_{bc}$	8.3 $_{b}$	1.2 $_{f}$	5.4 $_{bcd}$	13.5 $_{a}$
	2nd Filtrate	1.5 $_{a}$	4.2 $_{a}$	3.6 $_{a}$	1.5 $_{a}$	5.3 $_{a}$	4.8 $_{a}$	1.4 $_{a}$	8.4 $_{a}$	4.8 $_{a}$
		NS	NS	NS	NS	NS	*	NS	*	*
D) 2nd Filtrate Centrifugation	2nd Pellet	0.9 $_{a}$	2.9 $_{a}$	2.0 $_{a}$	0.9 $_{a}$	3.3$_{a}$	2.3 $_{a}$	0.6 $_{a}$	3.2 $_{a}$	1.8 $_{a}$
	2nd Serum	0.7 $_{a}$	1.4 $_{a}$	1.7 $_{a}$	0.9 $_{a}$	1.9 $_{a}$	2.3 $_{a}$	1.3 $_{a}$	3.8 $_{a}$	2.3 $_{a}$
		NS	NS	NS	NS	NS	NS	NS	NS	NS

[w] Means with different letters denote significance at $P \leq 0.05$ between maturity stage and temperature in the same row. [NS], * Non significant or significant at $P \leq 0.05$ between means in the same column for the same processing step.

Table 6. Total lycopene recovery from watermelon ground flesh in normalized µg/g after 2003 and 2004 processing. Total lycopene expressed as a summary of the produced filter cakes and pellets depending on the processing termination step. Fractions: A) 1st filter cake + 1st pellet, B) 2nd filter cake + 1st and 2nd pellets and C) 3rd filter cake +1st and 2nd pellets.

Processing step	Fraction	Normalized µg/g lycopene		
		Undermature	**Mature**	**Overripe**
2003	Ground Flesh	27.4 $_{II}$	36.8 $_{I,II}$	43.2 $_{I}^{v}$
1st Filtration	A	14.5 $_{a, I}$	16.7 $_{a, I}^{v}$	10.4 $_{a, I}$
2nd Filtration	B	10.9 $_{b, I}$	14.3 $_{b, I}$	8.7 $_{b, I}$
3rd Filtration	C	9.7 $_{c, I}$	13.2 $_{c, I}$	7.9 $_{b, I}$
		25°C	**60°C**	**85°C**
2004	Ground Flesh	31.2 $_{I}$	33.1 $_{a, I}^{v}$	32.4 $_{a, I}$
	Heated Ground Flesh		26.7 $_{b, I}$	24.5 $_{b, I}$
1st Filtration	A	16.5 $_{a\,b}$	19.7 $_{a}^{u}$	19.6 $_{a}$
2nd Filtration	B	14.2 $_{b}$	12.8 $_{b}$	12.9 $_{b}$

^{v} Means with different Latin numbers denote significance at $P \leq 0.05$ in the same row, and different letters denote significance at $P \leq 0.05$ in the same column. ^{u} Means with different letter denote significance at $P \leq 0.05$ between temperatures and A and B fractions for 2004 melons.

FIGURE LEGENDS

Figure 1. Chemical structure of 5 cis and trans isomers of lycopene.

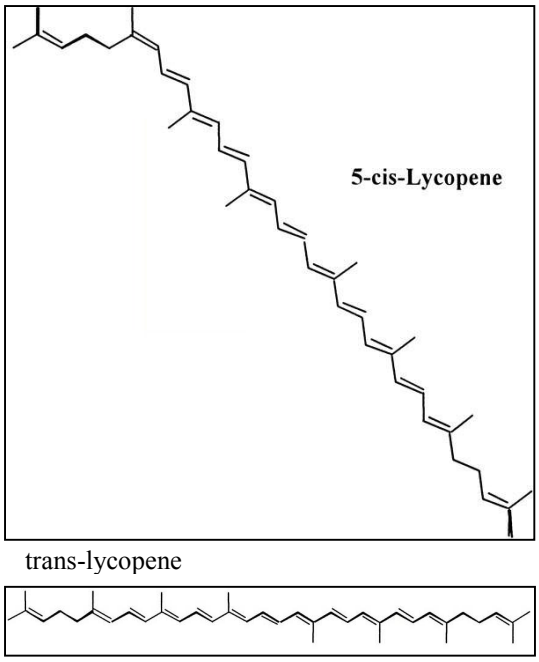


Figure 2. Example of weight distribution (g) of a mature 'SW1' watermelon during the various steps of processing of 2003.

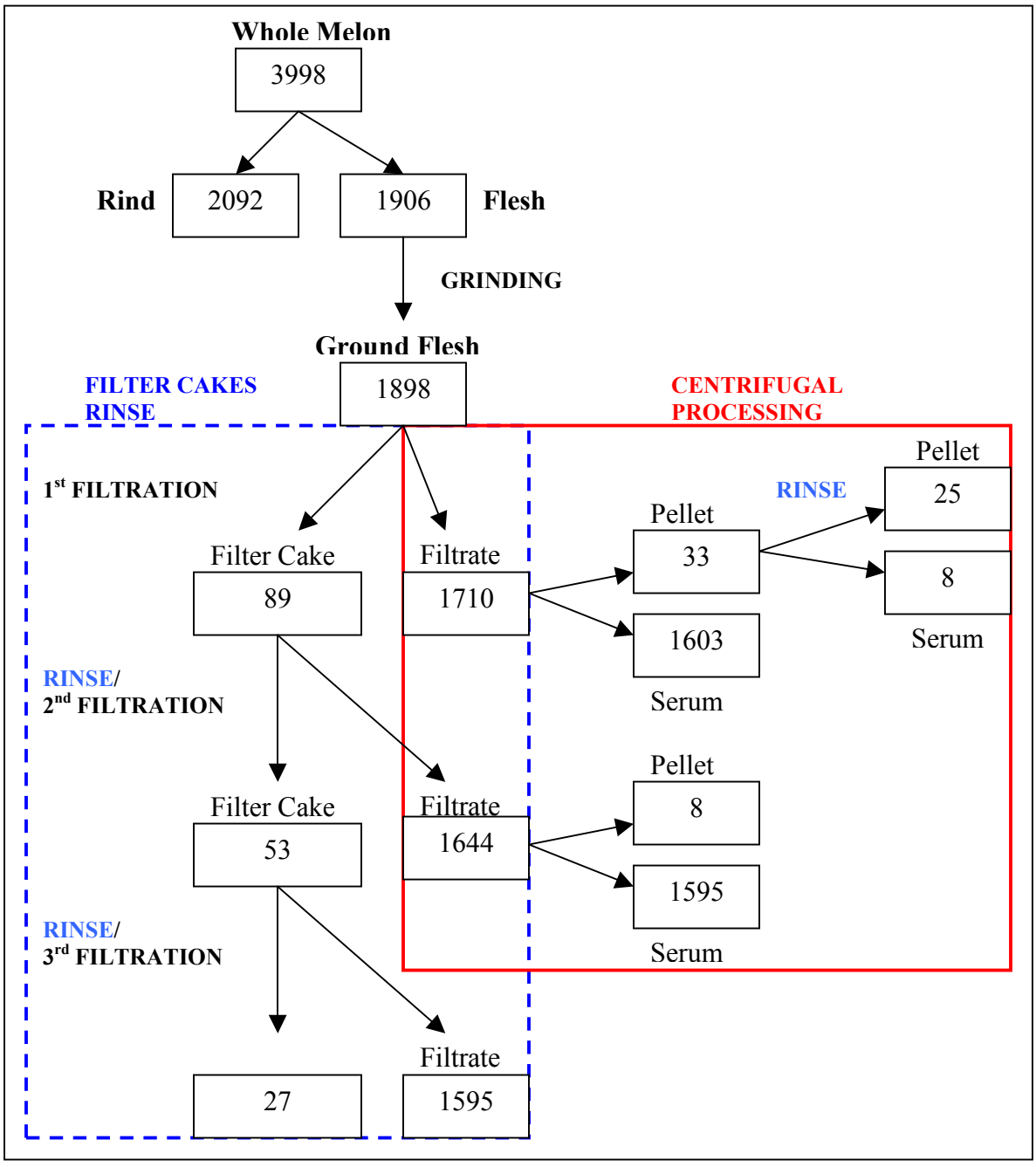


Figure 3. Normalized µg/g concentration of ground flesh for 2003 and 2004 watermelon cultivars. Means with different letters denote significance at $P \leq 0.05$ between cultivars and maturity stages for the same year.

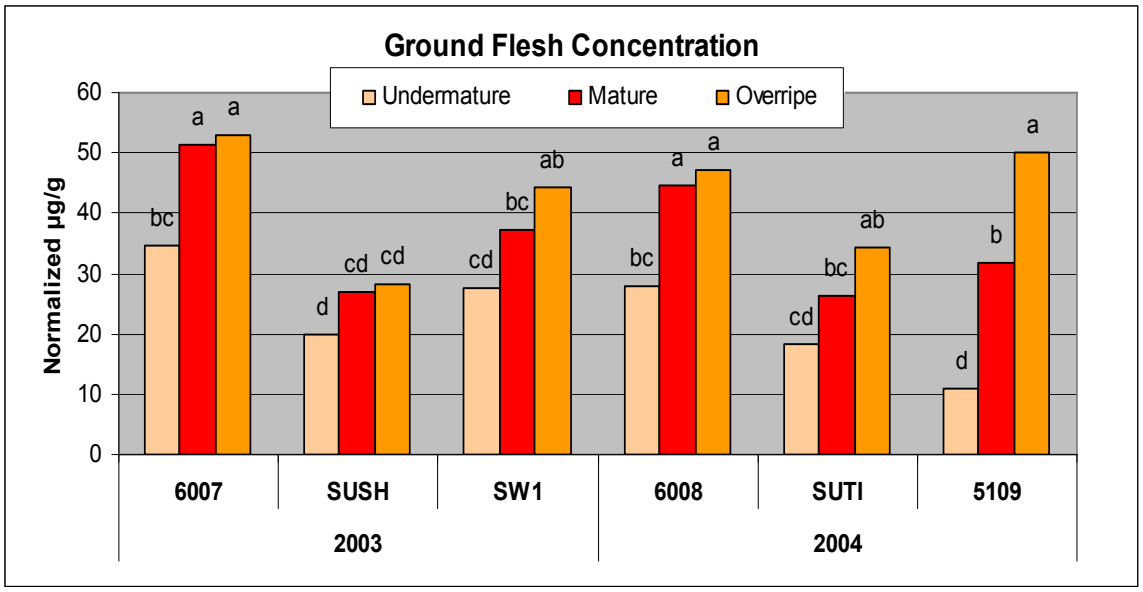


Figure 4. Lycopene % segregation from first and 2nd filtrations, to filter cake and filtrate of 2003 cultivars at undermature, mature and overripe maturity stages. Percent with different letters denotes significance at *P*<0.05 between all cultivars, maturity stages and fractions.

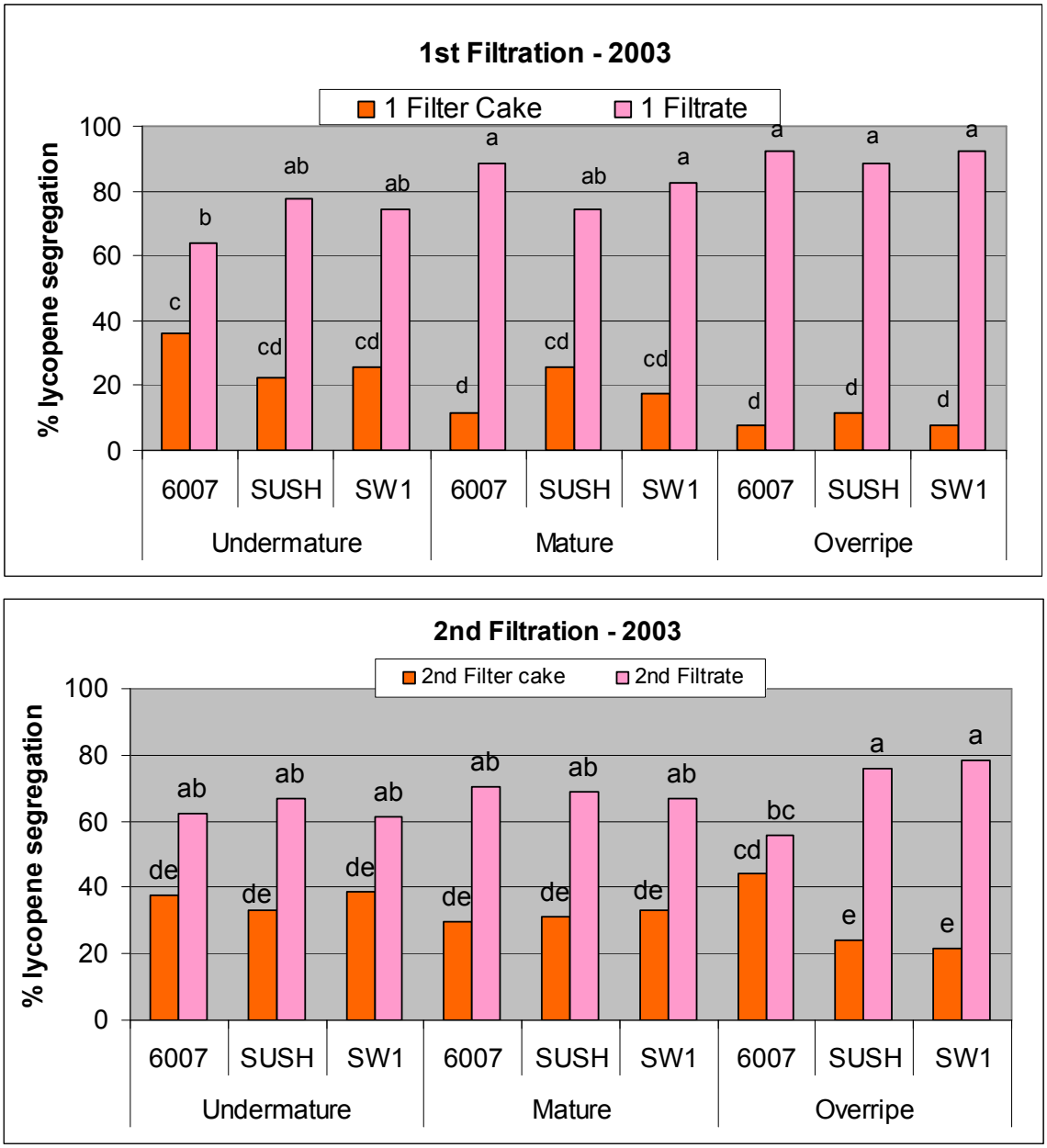


Figure 5. Comparison of absolute µg/g versus normalized µg/g for first filter cake and first filtrate of 2003 cultivars at three maturity stages. Different letters above bars denote significance at P<0.05 between all maturity stages and fractions of the same concentration.

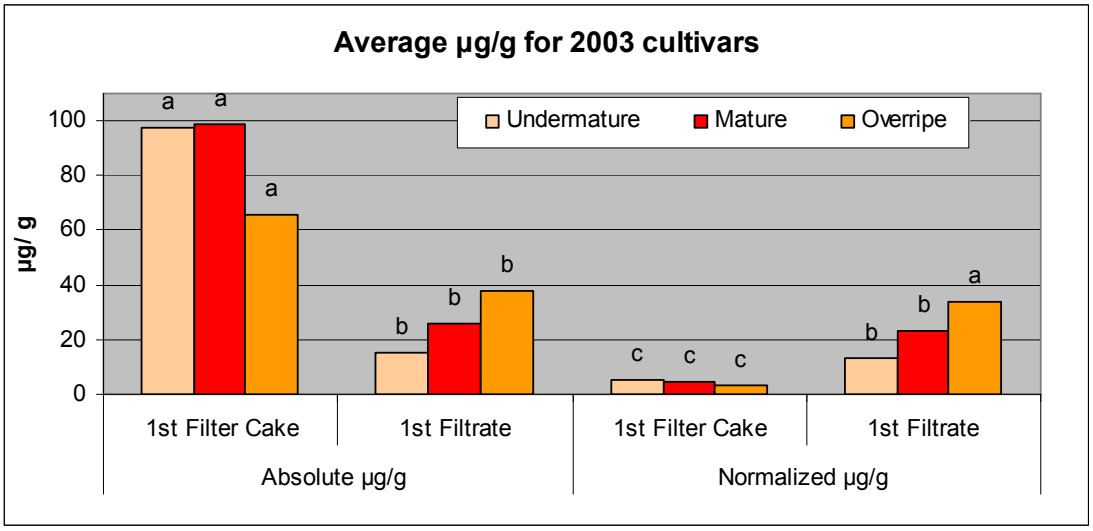


Figure 6. Lycopene recovery (%) as a total of 2nd filter cake plus 2nd pellet after 2nd filtration of 2003 and 2004 cultivars at 25OC.

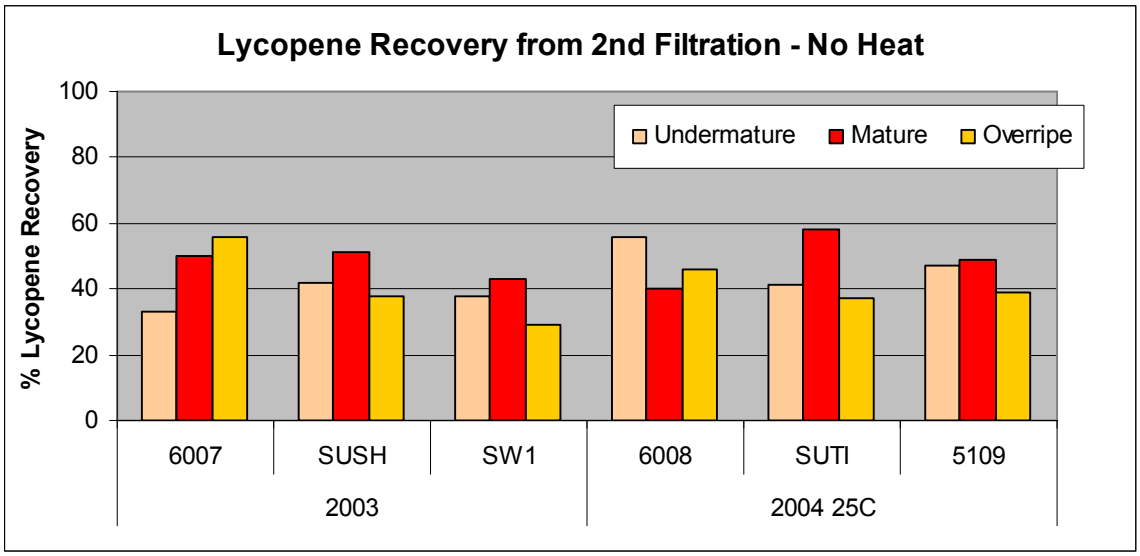
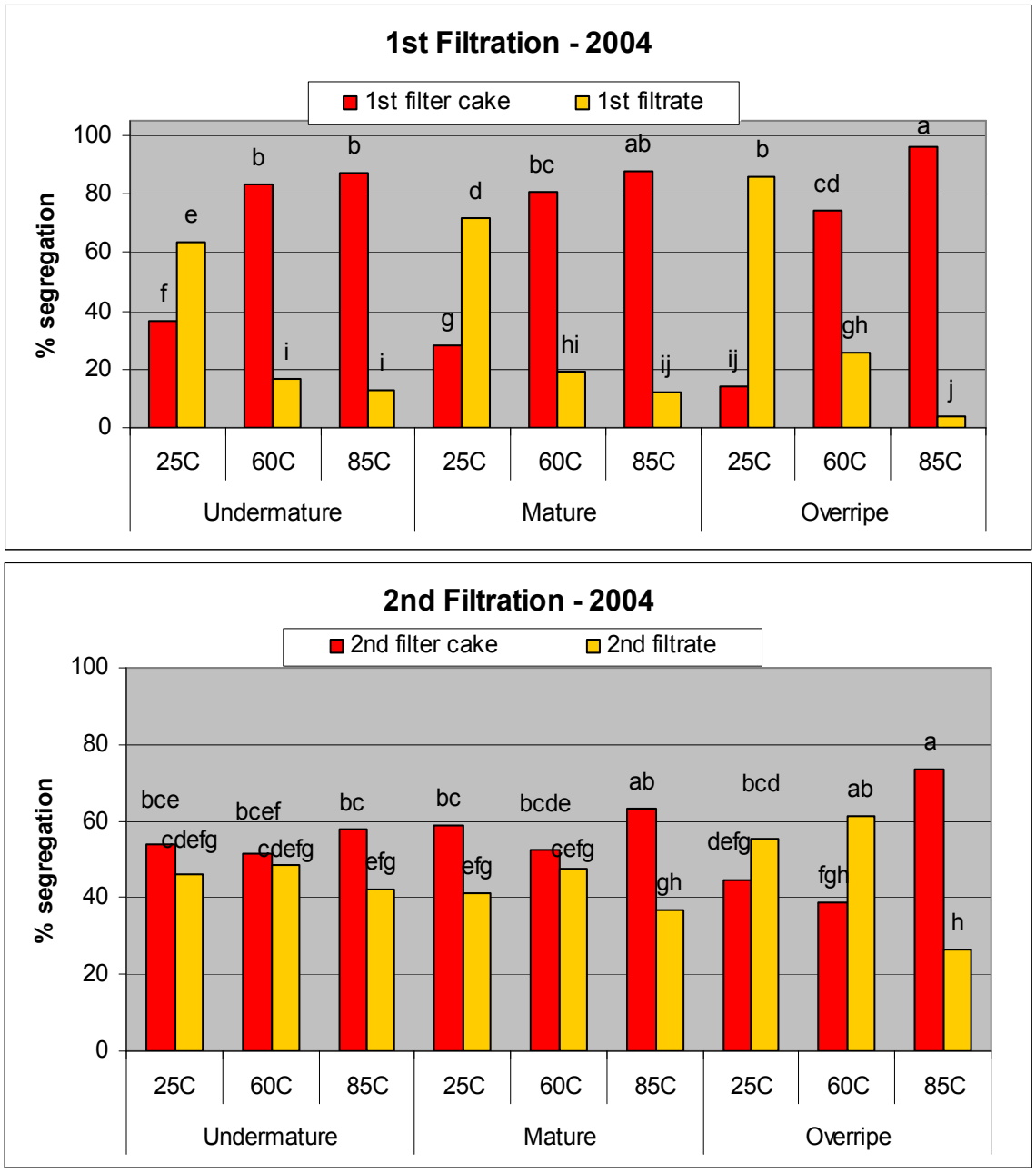


Figure 7. Lycopene segregation (%) in filter cake and filtrate from 1st and 2nd filtrations, as affected from temperature at all maturity stages on 2004 melons. Different letter designations above bars, denote significance at *P*<0.05 between all maturity stages, temperature and fractions.



VITA

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Candidate for the Degree of

Master of Science

Thesis: MATURITY AND TEMPERATURE INFLUENCE ON LYCOPENE DISTRIBUTION DURING FILTRATION PROCESSING OF RED-FLESHED WATERMELONS

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Title of Study: MATURITY AND TEMPERATURE INFLUENCE ON LYCOPENE DISTRIBUTION DURING FILTRATION PROCESSING OF RED-FLESHED WATERMELONS

Pages in Study: 55 Candidate for the Degree of Master of Science

Major Field: Horticulture

Scope and Method of Study: Our objectives were to evaluate filtration processing and extraction steps necessary for purification of lycopene from red-fleshed watermelons. Maturity and temperature influence on lycopene segregation during processing were investigated. Purification of lycopene was evaluated with a filtration procedure on ground flesh and subsequent centrifugation of the produced filtrate. Temperature treatments included $25^{O}C$, $60^{O}C$ and $85^{O}C$, applied on ground flesh before filtration.

Findings and Conclusions: Ground flesh filtration segregated lycopene in two fractions; a filter cake and a filtrate. At $25^{O}C$ segregation was maturity dependent, with overripe melons segregating more in the filtrate. About 75-85% of the filtrate lycopene could be recovered as pellet for undermature and mature melons, while only 55% for overripe. This loss could be partially avoided with heat application on ground flesh, which resulted in lycopene retention to the filter cake fraction. Further rinse of the filter cakes and subsequent filtrations was not recommended, due to excessive lycopene loss.

Advisor's Approval:……..Dr. Niels Maness