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# Compositional changes during ripening of two cultivars of muskmelon fruits

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#### Abstract

Chemical composition changes during ripening of the fruit of the muskmelon (Cucumis melo L.) were evaluated. Two cultivars, Piel de Sapo and Rochet, were studied in five different stages of maturity. Weight, soluble solids, pH, total carbohydrates, proteins and ash increased up to the full-ripe stage of both melon types, while a decrease in moisture occurred in the same period. Neutral Detergent Fiber (NDF) and pectins decreased continuously during the ripening process in the two cultivars of melon. The sugars were isolated, identified, and quantified using HPLC. The results showed that sucrose was the predominant sugar in ripe fruits, while glucose and fructose were higher in immature fruits. Analysis of variance confirmed significant differences  $(p < 0.05)$  between the different stages in each cultivar for every parameter. A two-way ANOVA test showed significant interactions in the behavior of all the compounds between cultivars during ripening.

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Keywords: Muskmelon; Changes ripening; Approximate composition; Soluble sugars

# 1. Introduction

Melon (Cucumis melo L.) is a commercially important crop in many countries. It is cultivated in all the temperate regions of the world due to its good adaptation to soil and climate. Fruits are consumed in the summer period and are popular because the pulp of the fruit is very refreshing and sweet, with a pleasant aroma. Piel de Sapo and Rochet are the two main commercial cultivars harvested between June and September. The fruits are of medium size, greenish color, thick peel with reticular surface, clear and aromatic pulp and their seeds have developed resistance againts Fusarium.

Melon is considered a climateric fruit and so quick changes in chemical composition occur during the maturation stages (Bower, Holford, Latche, & Pech, 2002; Flores, Martínez-Madrid, Sánchez-Hidalgo, & Romojaro, 2001; Hadfield, Rose, & Bennett, 1995; Seymour & McGlasson, 1993). Ripening of the fruit involves a series of complex reactions originating

changes in hormonal levels, respiratory activity, enzymatic activity and cellular organisation. The most easily perceptive changes during the maturation process are those related to color. The texture and taste of the fruits occur mainly at fruit surface. Internal changes are related with pulp softening, due to progressive degradation of cellular cell walls, and taste modifications due to changes in aromatic compounds, organic acids and soluble sugars (Barceló, Nicolás, Sabater, & Sánchez, 1992; Seymour, Taylor, & Tucker, 1993). Changes in soluble sugars are of special importance to the taste of the fruit.

Ripening and fruit quality in melons are assessed by the sugar content. Accumulation of sugars during development is of great importance because of the high correlation that appears to exist between sugar content and fruit quality. There is an initial accumulation of glucose and fructose, which are subsequently converted to sucrose. The relative proportions of the different sugars may account for differences in the taste for equal amounts of total sugars (Mutton, Cullis, & Blakeney, 1981; Seymour et al., 1993; Yamaguchi, Hughes, Yabumoto, & Jennings, 1977).

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The mature stage of the fruit at harvest is of fundamental importance, because sugar content does not increase after this moment. Therefore, if the fruit is not sufficiently mature when harvested, it will not reach an optimum level of ripening, and if it is too ripe, its storage life will decrease. The presence of tasteless melons at the start of the season is caused by attempts to force early ripening that fails to achieve the desired sugar content.

The degree of melon ripening required in each individual market is the single most important commercial factor in relation to quality. Melon maturity is determined on the basis of the sugar content, more routinely as the  $\textdegree$ Brix of the pulp.

Some work has been undertaken to ascertain the changes taking place in various chemical parameters during ripening in different types of melon (Bianco & Pratt, 1977; Chachin & Iwata, 1988; Hubbard, Huber, & Pharr, 1989; McCollum, Huber, & Cantliffe, 1988; Miccolis & Saltveit, 1991; Schaffer, Aloni, & Fogelman, 1987; Wang, Wyllie, & Leach, 1996).

Chemical composition depends on the cultivar, environmental conditions and also on the stage of maturity of the fruit. In the present paper, the composition of two cultivars of muskmelon that are widely consumed (Piel de Sapo and Rochet) was studied at different stages of maturity. The main compositional changes were evaluated. Among them, changes in sugars were of great importance due to their relation with the final taste of the fruit.

## 2. Materials and methods

## 2.1. Plant material

Melon (Cucumis melo L.) cultivars Piel de Sapo and Rochet were grown outdoors using customary culture practices in Talavera (Spain). Fruits were selected according to color, size and lack of blemishes in order to obtain homogeneous samples. The fruits were harvested during the months of July and August approximately every 8 or 9 days. Samples comprised a number of fruits (6–8) in order to limit individual sample variability and make the results for each sample more meaningful. Fruits were picked at five stages of maturity over the period from fruit set to full ripe (Table 1).

### 2.2. Sample preparation

The melon samples were chilled to keep them in optimum condition during transportation to the laboratory, where the fruits were weighed. The peel or rind and seeds were removed, and the pulp was sliced into small portions.





<sup>a</sup> Days after fruit set.

For those parameters that needed to be measured fresh (moisture, pH, and °Brix), analyses were performed immediately. The remaining sample was then frozen and later freeze-dried (Telstar model Cryodos lyophilizer). The freeze-dried pulp was comminuted to an appropriate particle size and stored at  $-20$  °C. Four replications of all the analyses listed below were performed.

#### 2.3. Chemical analysis

Parameters regarded as being the most representative were selected for study to provide the most complete information about the chemical changes in ripening process. These were: unit weight expressed as g; moisture, determined gravimetrically by drying at  $105 \degree C$  (AOAC, 1984);  $pH$ , measured using a pH-meter (Orion-Research model 701 Al Digital Ioanalyzer); titratable acidity, measured according to the AOAC (1990); soluble solids, measured at 20 °C using a digital refractometer (Atago) and expressed as °Brix; *proteins*, analyzed by Kjeldahl procedure (AOAC, 1990); Neutral Detergent Fiber (NDF), carried out by extraction with a neutral detergent solution and subsequent weight of the insoluble residue (Robertson, 1981); pectic substances, obtained from alcohol insoluble material and hydrolyzed with  $H_2SO_4$ , and quantified by colorimetric reaction with  $m$ -phenylphenol (Blumenkrantz & Asboe-Hansen, 1973). Finally the *ash* content was determined by mineralization of samples at 450  $^{\circ}$ C (AOAC, 1990).

# 2.4. HPLC determination of sugars

The sugars in the melon pulp were separated and quantified individually by means of high-performance liquid chromatography (HPLC) according to the method of Mendoza (1996).

### 2.4.1. Analytical procedure

To extract the sugars, 1 g of lyophilized sample was weighed out and homogenized in hot 85% methanol with agitation for 20 min. The mixture was then centrifuged at 3500 rpm for 20 min and the supernatant decanted. The extraction procedure was repeated to maximize yield. The supernatant was evaporated to dryness at 50  $\mathrm{^{\circ}C}$  in a rotary evaporator, and the residue

was redissolved in distilled water. Thus prepared, the samples were filtered through Sep-Pak  $C_{18}$  cartridges (Waters, Milford, MA) and through Millipore membranes with a pore diameter of 0.45  $\mu$ m and were then ready for chromatographic analysis. Sample injection volume was 20 µl.

#### 2.4.2. HPLC equipment and conditions

The chromatographic equipment (Waters, Milford, MA) used for the determination of sugars consisted of an automatic injector (Waters model 717), a pump (Waters model 510), an oven (Waters), a differential refractometer (Waters model 410), and a data processor using the Millenium 2.0 program. The column was an Aminex HPX-87P column (Bio-Rad, Richmond, CA) measuring 30 cm  $\times$  7.8 mm with a particle size of 9 µm. A guard column with the same packing as the column was used (Waters, Guard-Pak<sup>™</sup> Inserts). The mobile phase was bidistilled water with a resistance of 18  $M\Omega$  cm prepared using a Milli-O system (Millipore, Milford, MA) and degassed with helium. The chromatographic conditions were: isocratic elution at a constant flow rate of 0.6 ml/min and 85  $\degree$ C column temperature. For quantification, an external standard was used to prepare calibration curves for each sugar over the range of  $5-100 \mu g/ml$ , yielding resolutions with correlation coefficient values of greater than 0.99.

## 2.4.3. Validation

Recovery trials were performed using the same experimental conditions, adding known concentrations of each sugar to the fruit sample. Recoveries were in all cases on the order of 100%. Reproducibility was validated by performing the method on six consecutive days. Variability was less than 4% in all cases.

#### 2.5. Statistical analysis

Analysis of variance (ANOVA) was applied to the data. Means corresponding to the different stages of evolution were compared using Duncan's multiple range test  $(p < 0.05)$ . Two-way ANOVA (cultivar and time) was applied in order to know the incidence of these factors.

## 3. Results and discussion

During ripening, a fruit passes through a series of changes in color, texture and flavor indicating that compositional changes are taking place. Tables 2 and 3 present the values of the representative parameters over the course of development for the two melon cultivars considered.

During ripening, weight increased considerably in both cultivars in the initial stages of development (the first half of the cycle), then remained fairly constant for 26 days after fruit set (Table 2). The same table shows that the percentage of pulp underwent a substantial increase, whereas the percentage of rind and seeds declined. As expected, the greatest variation took place during the initial stages of development of the fruit.

Melons are among the fruits with the highest sugar content. Brix followed an upward trend. At first this parameter increased only slightly in the Piel de Sapo cultivar and held steady in the Rochet cultivar, but after 18 days from fruit setting to maturity the increase was significant in both cultivars as shown by Duncan's multiple range test. Bianco and Pratt (1977) recorded melons with a soluble solids content of 17%. The minimum recommended sugar level for this fruit is  $8 \text{ }^{\circ}$ Brix. Below that level, the melons are not usually suitable for market, though in some regions, such as Britain and Scandinavia, the fruit does not have to be too ripe, because it is normally eaten as a garnish, and when it is used in desserts the taste is masked by liqueurs and additives (Zapata, Cabrera, Bañón, & Roth, 1989).

The total acidity (TA) in the melon samples increased until midway through development (26 days from fruit set), after which it decreased, dropping back close to the initial values; this decline was more marked in the Rochet cultivar. The variations were significant according to Duncan's multiple range test. The pH in these

Table 2

Changes in weight, pulp, rind, and seeds during the ripening of melon fruits

5 2371<sup>a</sup> 56.2<sup>a</sup> 59.4<sup>a</sup> 4.1<sup>a</sup> Rochet 1 1089<sup>c</sup> 41.3<sup>c</sup> 44.6<sup>b</sup> 14.1<sup>d</sup> 2  $1630<sup>b</sup>$   $41.5<sup>c</sup>$   $44.3<sup>b</sup>$   $12.7<sup>c</sup>$ 3 2066<sup>a</sup> 2066<sup>a</sup> 46.3<sup>b</sup> 44.4<sup>b</sup> 8.8<sup>b</sup> 4 2030<sup>a</sup> 51.0<sup>a</sup> 40.0<sup>a</sup> 40.0<sup>a</sup> 7.2<sup>b</sup> 5 2199<sup>a</sup> 53.3<sup>a</sup> 40.5<sup>a</sup> 5.8<sup>a</sup> 5.8<sup>a</sup>

2 1554c  $1554^{\circ}$  46.2<sup>c</sup> 43.6<sup>b</sup> 9.7<sup>c</sup> 3 2166<sup>b</sup> 52.2<sup>b</sup> 40.9<sup>a</sup> 6.4<sup>b</sup> 4 2161<sup>b</sup> 53.9<sup>b</sup> 40.1<sup>a</sup> 4.4<sup>a</sup>

Cultivar Stage of ripening Weight (g) Pulp (%) Rind (%) Seeds (%) Piel de Sapo 1 955<sup>d</sup> 41.4<sup>d</sup> 46.3<sup>c</sup> 11.6<sup>d</sup>

Means in the same column followed by different letters are significantly different according to Duncan's test  $(p < 0.05)$ .



Moisture (g/100g), °Brix, pH, total acidity (mg/100g citric acid) and index ripening during ripening of melon fruits<sup>a</sup>

Means in the same column followed by different letters are significantly different according to Duncan's test  $(p < 0.05)$ .<br><sup>a</sup> Data are the means of four replications.

cultivars followed a declining trend in the first part of development (to 18 days from fruit set), after which it recovered up to 6.05 in the cv. Piel de Sapo melons and 6.55 in the cv. Rochet melons at maturity. The variations in this latter cultivar were significant (Duncan's multiple range test) for all sampling times (Table 3).

The ripening index (Brix/TA) followed upward trends throughout the sampling period in both cultivars, though values were higher in cv. Rochet than in cv. Piel de Sapo.

Table 4 shows the changes of some chemical compounds during fruit maturation. Proteins of both melon cultivars increased significantly  $(p < 0.05)$ . In Piel de Sapo melon increases were detected 26 days after set and in Rochet melon 18 days after set. Increases up to the final ripening stage in both melon cultivars were 48.5% (Piel de Sapo) and 57.0% (Rochet). Data reported by Rowan, McGlasson, and Pratt (1969), show that there is an increase in melon proteins during maturation. Abu-Goukh and Abu-Sarra (1993) described that proteins of three different mango cultivars increased up to the fullripe stage and then decreased at the over-ripe stage, due to enzymatic activity.

The results for carbohydrate content in the two melon cultivars considered in this study are indicative of a distinctly gradual rising trend. In cv. Piel de Sapo, the calculated increase in sugars was approximately 72.6% and in cv. Rochet approximately 68.0%. Duncan's multiple range test confirmed this trend, with significant differences between the samples at all stages of development.

Ripening of fruits is characterized by softening of the flesh. The loss of the texture is associated with cell wall disassembly (Seymour & Gross, 1996). NDF (cellulose, hemicelluloses and lignin) and pectic substances are compounds of cell wall and their quantification are indicative of the ripening evolution.

The results for NDF were higher for samples of Piel de Sapo (8.7 g/kg) than for Rochet ones (5.6 g/kg). NDF is a fairly stable chemical fraction, though in both melon cultivars, a general slight decrease of NDF content  $(p < 0.05)$  at final stage of ripening was observed. The percentage decrease was 17.2% (Piel de Sapo) and 14.3% (Rochet).

A degradation in pectic substances (expressed as galacturonic acid) was observed in both cultivars. Statistical analysis revealed significant variation between the first and final stages of maturation. In Piel de Sapo no statistical differences were observed between two consecutive sampling moments, while in Rochet significant

Table 4





Means in the same column followed by different letters are significantly different according to Duncan's test  $(p < 0.05)$ .<br><sup>a</sup> Data are the means of four replications.

Table 3

decreases were detected from the beginning until 26 days after set. Although variations of these compounds were not large, they are important because their decrease is related to modifications of texture which make the fruit much more acceptable for human consumption.

The changes during maturation are frequently attributed to the enzymatic degradation of fruits cell wall, like pectinesterase, polygalacturonase and cellulase and modifications of the pectin fraction are some of the most apparent changes that take place in the cell wall during ripening (Marín-Rodríguez, Orchard, & Seymour, 2002; White, 2002).

Total mineral content (ash) increased slightly during the evolution period ( $p < 0.05$ ). Piel de Sapo melons varied significantly until 35 days after fruit set and Rochet cultivar showed significant differences between the first and the last stage of ripening with constant values in the middle period. This is due to the fact that during maturation inorganic ions migrate from different parts of the plant to the region of active growth. Sánchez, Lorente, Río, Valenzuela, and Romero (1991) studied mineral elements in melon and observed that these were transported by xylem and floem from the leaves to the fruits.

Table 5 sets out the changes in the sugar fraction during ripening of the fruits. Both melon cultivars had high content in sugars, consisting of glucose, fructose, and sucrose. The percentage contributions of the sugars changed during ripening. Sugars are a basic parameter in evaluating fruit market quality attributes. Genetic and environmental factors may affect the qualitative and quantitative composition of the sugar fraction by altering the activity of the enzymes involved in synthesis and breakdown processes (Lingle & Dunlap, 1987). In the early growth stages the glucose and fructose content was high and the sugars were present in similar amounts. As ripening progressed the sucrose content increased and the concentrations of the two monosaccharides decreased. Sucrose may be formed by the above mentioned monosaccharides as well as by the breakdown of the

different carbohydrates present in other organs of the plant. Hubbard et al. (1989) recorded the presence of stachyose in leaves and partial breakdown of this substance, with synthesis of sucrose, in the fruit pedicel. Schaffer et al. (1987) reported the presence of the saccharose galactosides raffinose and stachyose, which may be transported to the fruit, though sucrose is the sugar finally accumulated by the fruit. These changes in the sugar composition were in agreement with the findings published by other workers for different melon cultivars (Lester & Dunlap, 1985; McCollum et al., 1988; Wang et al., 1996). Fig. 1 presents two chromatographic profiles, one for immature melon samples and one for ripe melon samples from cultivar Piel de Sapo.

During ripening of the cv. Piel de Sapo melons changes of glucose and fructose were similar. The concentration of glucose was 16.7 g/kg and the concentration of fructose 16.4 g/kg at the initial time of sampling. These levels increased in the early stages of development until the 26th day from fruit set. From that date the levels began to fall until the 35th day after fruit set, after which they held steady until the final sampling date. At this point the values were 15.6 g/kg for glucose and 14.4 g/kg for fructose. In contrast, accumulation of sucrose continued throughout fruit development, increasing from 1.0 g/kg at the beginning of sampling to a peak of 95.4 g/kg on the final sampling date. Concentrations were minimal until 18 days from fruit set. After that there was a steeper increase between the 26th and 35th day from fruit set. Duncan's multiple range test revealed significant differences for glucose and fructose only between 26 and 35 days after fruit set. For sucrose, after the initial period, all the increases were significant.

The initial glucose and fructose contents in cv. Rochet melons were 18.6 and 18.2 g/kg, respectively. The values increased slightly until day 18 and then levelled off. There was a marked decline from the 26th day after fruit set, to values of 13.0 and 10.5 g/kg in the ripe fruit. In contrast, sucrose levels rose from 1.1 g/kg to a final value of 96.6 g/kg. The rise started 18 days after fruit set





Means in the same column followed by different letters are significantly different according to Duncan's test  $(p < 0.05)$ .<br><sup>a</sup> Data are the means of four replications.



Fig. 1. Chromatographic profile for sugars in Piel de Sapo muskmelon pulp: (a) immature; (b) ripe.  $1 =$  Sucrose,  $2 =$  Glucose,  $3 =$  Fructose.

at a constant rate of increase, whereas very low concentrations were recorded in the early stages of development. Duncan's multiple range test revealed significant differences for the monosaccharides over the periods between 10 and 18 days and 26 and 35 days from fruit set. Values held steady over the rest of the period, but sucrose exhibited a statistically significant, progressive increase in synthesis from 18 days from fruit set.

Comparing our results with those reported by Bianco and Pratt (1977) for cv. Honey Dew and cv. Powdery Mildew Resistant no. 45 (PMR-45) melons, the percentage glucose and fructose values for those cultivars were lower throughout the ripening period. The final sucrose levels in cv. PMR-45 and in cv. Honey Dew in particular were very similar to our findings. The trends for the three sugars were similar for all four melon cultivars.

The glucose to fructose ratio  $(G/F)$  remained practically constant over time and was quite similar in the two cultivars, especially until 26 days from fruit set. After that date, the ratio values were somewhat higher in cv. Rochet. The sucrose to reducing sugars ratio  $(S/G + F)$  held steady until 26 days from fruit set at values of less than 1. After that date the ratio increased rapidly to a value close to 4 in cv. Rochet and a value close to 3 in cv. Piel de Sapo. This sharp increase was related to the levelling off of weight and the accumulation of sucrose.

The behavior of the sugars was consistent with the findings reported by Lingle and Dunlap (1987) and by Schaffer et al. (1987), who attributed it to alterations in the activity of the different enzymes during development. In the early stages of fruit development activity of acid invertase was high and that of sucrose phosphate synthase (SPS) low, but as the fruit developed, the level of activity of the former decreased as that of SPS rose.

McCollum et al. (1988) suggested that sucrose is the source of the hexoses needed by melon metabolism during development and that the high activity of invertase prevents accumulation early in development. Physiologically, invertase plays a role in maintaining the osmotic pressure of cells by hydrolyzing sucrose into glucose and fructose. The osmotic pressure must be high so that water can be accumulated during growth of the fruit. Invertase activity declines during the final stages of fruit development, making possible the accumulation of sucrose.

In different studies on the development of cv. Prince melons (Hashinaga, Koga, & Ishida, 1984), cv Perlita melons (Lester & Dunlap, 1985), cv. Galia and Noy Yizre melons (McCollum et al., 1988) trends in glucose, fructose, and sucrose analogous to our results were reported. Glucose and fructose concentrations in these melon cultivars were similar in magnitude to those in our samples. This was not the case for sucrose, which in all cases was approximately half the level recorded in cv. Rochet and cv. Piel de Sapo melons on the final sampling date. This may be ascribed to melon cultivar, because the melons in the present study are long-lasting hybrids with high sugar levels.

The results published by Wang et al. (1996) for cv. Makdimon melons are comparable to ours. However, the fructose concentrations recorded for the Makdimon cultivar were slightly higher than the levels recorded in our study and followed a slightly increasing trend. The behavior of sucrose during development was very similar, with a sharp increase from day 41 to day 44 after anthesis. Although sucrose concentrations in the ripe fruit were high in the cultivars studied by McCollum et al. (1988), they were clearly lower than the sucrose levels in the cultivars of our study.

The results of two-way ANOVA showed that there were no significant differences between the two melon cultivars in weight, sugars and pectins content, while there were significant differences for time and for the interaction of cultivar and time for all the compounds (Table 6). Lack of statistical significance for differences found for some parameters between both cultivars could be due to the same agronomic and climatic conditions.

In the stages studied expression of results must be considered. If results for all the mentioned compounds

Table 6 Two way ANOVA cultivar  $(C)$  and time  $(T)$ : F statistic values

Variable	C	T	CT
Weight	0.9 <sup>ns</sup>	$131***$	2.2 <sup>ns</sup>
Moisture	$5.7^*$	348***	$10.9***$
<b>Brix</b>	$6.9*$	1739***	$16.9***$
PH	$6.6*$	254***	29.4***
Total acidity	0.5 <sup>ns</sup>	$142***$	$15.5***$
Proteins	$10.3**$	1913***	$90.0***$
<b>NDF</b>	279***	$5.7**$	$5.6**$
Pectins	3.8 <sup>ns</sup>	$13.2***$	$11.5***$
Ash	$25.0***$	157.4***	$18.6***$
Glucose	0.1 <sup>ns</sup>	$43.2***$	$5.2*$
Fructose	0.9 <sup>ns</sup>	$44.3***$	$5.2*$
Sucrose	0.1 <sup>ns</sup>	1889***	$15.7***$
Total sugars	0.1 <sup>ns</sup>	$364***$	$6.9**$

were expressed in grams per piece of fruit (instead of grams per kg fresh weight) the ascendent slope should be more pronounced in both cultivars of melon and significant differences should be found among all the stages of maturity analyzed for each cultivar, because this expression is an indication of the ability of the cultivars to accumulate, synthesize or degradate compounds and, obviously, concentration may be affected by fruit growth, especially during the periods of pronounced development (over the 26 first days after fruit set).

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 $\begin{array}{c} \mbox{ns} \\ \mbox{$p<0.05$} \\ \mbox{$p<0.01$} \\ \mbox{***} \end{array}$ 

 $p < 0.001$ .