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Search for suitable maturation parameters to define the harvest maturity of plums (*Prunus domestica* L.): A case study of candied plums

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ABSTRACT

Plums (*Prunus domestica* L.) of a greengage variety, from South–East of Portugal, are used to produce a traditional candied product, "Ameixa d'Elvas", which has a Protected Designation of Origin, recognised by the European Union. To obtain a good texture quality in candied plums, it is necessary to define accurate maturation parameters. Parameters such as the total soluble solids (TSS), titratable acidity (TA), TSS/TA, and pH are not always suitable for this purpose. In order to find a more reliable maturation parameter, plums were collected during the commercial harvesting period, in two orchards, Vila Viçosa and Cano in different years (2003 and 2005). Total polysaccharides (PS) and uronic acids (UA) were quantified in the alcohol-insoluble residues (AIR) of pulp. In all harvests, the content of polysaccharides and uronic acids present in the AIR increased as the maturity of the fruits progressed. To the dataset that comprised the TSS, TA, TSS/TA, pH, PS, and UA measured in these plums, a linear discriminant classifier was applied to obtain a reliable parameter to predict fruit quality upon candying. The models built showed errors of lack of fitness of 0.005% for the content of UA in the AIR and 0.8% for PS, which contrasted with the errors of 17%, 21%, 17%, and 11%, for the TSS, TA, ratio TSS/TA, and pH, respectively. Considering that the variability associated with the content of PS was higher than that observed in UA estimation, and the easy and fast determination of UA, it is proposed that the UA content in AIR be used as a reliable harvesting maturity parameter, complementary to TSS and/or TA, to obtain a high quality candied product. An easy and quick laboratory methodology is proposed for the determination of the UA in plums.

1. Introduction

A large number of physiological, biochemical, and structural changes occur during the ripening of fruits, resulting in modifications identified by measurement of specific physico-chemical parameters. In plums, several maturation parameters have been defined, based on characteristics related to colour of the skin and flesh, fruit firmness, occurrence of specific volatile compounds, soluble solids content, and titratable acidity (Abdi, Holford, McGlasson, & Mizrahi, 1997; Bhutani & Joshi, 1995; Prasanna, Prabha, & Tharanathan, 2007). Skin and flesh colour may be useful indicators of ripening but many plum cultivars develop their pigmentation early in growth; therefore, this character has little value for determining harvest date (Abdi et al., 1997; Bhutani & Joshi, 1995). Fruit firmness also has some limitations, since the decrease of firmness is associated with cell enlargement during fruit growth. This characteristic is largely influenced by fruit size, as large fruits show lower firmness than small ones. The production of characteristic volatile compounds is correlated with fruit ripening in many fruits, although in plums no pattern of production was found, due to high variability between cultivars (Abdi et al., 1997). Soluble solids content, titratable acidity and their ratio have been suggested as the most reliable maturation parameters for plums, since fruit sweetness gradually increases and acidity decreases during ripening (Bhutani & Joshi, 1995; Prasanna et al., 2007). However, these parameters also have limitations for following maturation, since soluble solids content and titratable acidity have been shown to vary with fruit position on the tree and with environmental conditions (Abdi et al., 1997). For each cultivar, specific maturity parameters need to be defined and, in addition, these parameters need to be adapted depending on the final purpose of the fruit.

Plums (*Prunus domestica* L.) of a special type of greengage variety, "Rainha Cláudia Verde", from Alto Alentejo (South–East of Portugal) can be utilised to obtain a traditional candied plum, "Ameixa d'Elvas", which has a Protected Designation of Origin (PDO) granted by the European Union. To process this variety, the maturity stage at harvest is one of the most important factors for a high texture quality in the final product. For this reason, plums used to produce "Ameixa d'Elvas" are harvested at a defined maturation point evaluated by their total soluble solids (16 °Brix) and titratable acidity (1.0 meq malic acid/100 g fruit flesh weight). However,

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it has been found that plums from an orchard (Cano, CA), although fulfilling these criteria, produced a candied product of low or even no commercial use, due to appreciable loss of pulp firmness and skin disruption. However, fruit from another orchard (Vila Viçosa, VV) produced a candied product with good textural properties (Nunes et al., in press), when harvested at the same maturation stage as that of CA plums, as determined by total soluble solids and titratable acidity.

The major textural changes resulting in the softening of fruits are due to enzyme-mediated alterations in the composition and structure of cell wall polysaccharides, such as pectic polysaccharides and cellulose, which leads to their partial solubilisation (Waldron, Smith, Parr, Ng, & Parker, 1997). Cell walls are complex in composition and structure, thus, it is unlikely that any particular enzyme alone is able to significantly modify their properties. A combined action of a number of enzymes acting synergistically is a more plausible cause for the occurrence of the changes needed to make any significant texture changes of fruits (Ali, Chin, & Lazan, 2004). The preparation of alcohol-insoluble residues (AIR) is a relatively easy and quick method for obtaining plum cell wall polymers (Nunes, Saraiva, & Coimbra, in press). This method efficiently inactivates enzymes while avoiding significant degradation of cell wall polysaccharides (Coimbra, Delgadillo, Waldron, & Selvendran, 1996).

In order to determine if plum cell wall polysaccharides could be used to define more reliable maturity parameters and harvesting fruits for candying, the AIR of plums were prepared from fruits harvested at different dates in two different years and in two different orchards (CA and VV). A linear discriminant classifier was used to relate the maturity characteristics of the fruits to the texture characteristics obtained upon candying. The characteristics assayed to assess maturity were total soluble solids, titratable acidity, total soluble solids/titratable acidity, pH, total polysaccharides and uronic acids content in the AIR.

2. Materials and methods

2.1. Plant material and sample preparation

"Rainha Cláudia Verde" is the name given in the PDO to the plums (*P. domestica* L.) of greengage variety. These plums were collected from two orchards, Vila Viçosa (VV) and Cano (CA), within the PDO region. VV and CA plums were collected in 2003 at five harvest dates: VV plums on 10th, 15th, 18th, 22nd, and 25th of July, and CA plums on 15th, 22nd, 25th, and 30th of July and 1st of August. In 2005, the VV plums were harvested at three harvest dates on 13th, 19th, and 26th of July, and CA plums at two stages ripening on 19th and 26th of July. The plums were supplied by Fruteco-Fruticultura Integrada, Lda. (Borba, Portugal) and were brought to the laboratory immediately after harvesting.

2.2. Total soluble solids, pH, and titratable acidity

The plums' juice was obtained by squeezing the plum flesh, followed by filtration through a glass fibre filter (Whatman GF/C). Total soluble solids, pH, and titratable acidity were determined on the filtrate. Total soluble solids (°Brix) were determined by measuring the refractive index of the juice with a hand held refractometer (ATC-1E, Atago, Tokyo, Japan). Titratable acidity was measured by titration with 0.1 M NaOH to an endpoint of pH 8.1, using an automatic titrator (TT2022, Crison SA., Alella, Spain) using 6 g of juice diluted with 50 ml of distilled water. Titratable acidity was calculated and expressed as meq of malic acid/100 g of deseeded fruit.

2.3. Preparation of cell wall material

Plums were deseeded and peeled and the flesh was dispersed in ethanol, using a proportion of 4 ml to 1 g of solids. The suspension was boiled for 10 min. The mixture was cooled and filtered through a glass fibre filter (Whatman GF/C). The residue was dispersed again in 85% ethanol, boiled for 10 min and filtered. The residue was then washed with diethyl ether and allowed to dry at room temperature. The dried material was considered to be the alcohol-insoluble residue (AIR).

2.4. Carbohydrate analysis

Monosaccharides were released from cell wall polysaccharides by a pre-hydrolysis in 0.2 ml of 11 M H_2SO_4 for 3 h at room temperature followed by 2.5 h hydrolysis in 1 M H_2SO_4 at 100 °C (Selvendran, March, & Ring, 1979). Neutral sugars were analysed after conversion to their alditol acetates by GC, using 2-deoxyglucose as internal standard (Coimbra et al., 1996), and GC analysis as described by Nunes, Rocha, Saraiva, and Coimbra (2006). Cellulosic glucose was calculated as the difference between the content found with and without 11 M H_2SO_4 pre-hydrolysis.

Uronic acids (UA) were quantified by a modification (Coimbra et al., 1996) of the 3-phenylphenol colorimetric method (Blumenkrantz & Asboe-Hansen, 1973). Samples were prepared by hydrolysis in 0.2 ml of 11 M H_2SO_4 for 3 h at room temperature followed by 1 h in 1 M H_2SO_4 at 100 °C. A calibration curve based on D-galacturonic acid as standard was used to calculate UA concentration.

2.5. Statistical analysis

Quantitative analyses are presented as mean values and the reproducibility of the results is expressed as standard deviation. Statistical analysis of the experimental results was carried out based on Student's *t*-test. Differences were considered significant at the level of $p < 0.05$.

2.6. Linear discriminant classifier

A linear discriminant classifier (Vandeginste et al., 1998) was applied to assess the classification ratios of total soluble solids, titratable acidity, total soluble solids/titratable acidity, pH, total polysaccharides, and uronic acids. This classifier was based on least squares regression by estimating the slope and the intercept, defining a line boundary that can be used to classify samples. The values of the above parameters (summarised in Table 1) were used to build the classifier. A dummy vector was filled with 0 (zeros) and 1 (ones) for the cases of poor and good final product, respectively. A Monte-Carlo model validation, in tandem with the linear discriminant classifier, was used to compute the classification ratios of each parameter of interest; two thirds of the samples were used for calibration purpose and one third was used for validation. Each Monte-Carlo model was run 1000 times. From these 1000 models for each parameter the averaged linear classifier parameters (slope and intercept) and model's lack of fitness (LOF) were calculated. In addition, and for each parameter, a random permutation test was performed.

3. Results and discussion

3.1. Characterisation of plum samples

The dataset used in this study was produced from fruit collected from two orchards (VV and CA), in two years (2003 and 2005),

Table 1
Total soluble solids (TSS), titratable acidity (TA), TSS/TA, and pH of the fruits, and total polysaccharides (PS) and uronic acids (UA) of AIR from plums from Vila Viçosa (VV) and Cano (CA)

Harvesting day	TSS (°Brix) (n = 3)	TA (meq of malic acid/100 g) (n = 3)	TSS/TA (°Brix/meq of malic acid) (n = 3)	pH (n = 2)	PS (mg/g AIR) (n = 4)	UA (mg/g AIR) (n = 4)
VV 2003						
10 July 03	14.8 ± 0.1[a]	1.12 ± 0.02[a]	13.3 ± 0.1[a]	3.21	534 ± 21[a]	239 ± 18[a]
15 July 03	15.2 ± 0.2[a]	1.02 ± 0.01[b]	14.8 ± 0.2[b]	3.19	598 ± 32[b]	197 ± 14[a]
18 July 03	16.6 ± 0.1[b]	1.00 ± 0.01[b]	16.6 ± 0.2[c]	3.26	781 ± 25[c]	386 ± 14[b]
22 July 03	18.8 ± 0.2[c]	0.94 ± 0.01[c]	20.0 ± 0.3[c]	3.29	825 ± 19[c]	425 ± 17[c]
25 July 03	21.2 ± 0.1[d]	0.90 ± 0.01[d]	23.7 ± 0.1[d]	3.30	906 ± 14[d]	453 ± 12[c]
VV 2005						
13 July 05	14.5 ± 0.1[a]	1.14 ± 0.03[a]	12.7 ± 0.2[e]	3.19	493 ± 12[a]	190 ± 5[a]
19 July 05	16.1 ± 0.1[e]	1.03 ± 0.01[b]	15.7 ± 0.3[f]	3.22	623 ± 25[b]	260 ± 21[a]
26 July 05	19.6 ± 0.1[f]	0.90 ± 0.01[d]	21.7 ± 0.1[g]	3.32	756 ± 38[c]	359 ± 35[b]
CA 2003						
15 July 03	12.8 ± 0.1[g]	1.16 ± 0.01[a]	11.0 ± 0.2[h]	3.22	644 ± 28[b]	293 ± 78[a]
22 July 03	15.6 ± 0.1[a]	1.10 ± 0.01[a]	14.2 ± 0.0[b]	3.28	747 ± 29[c]	416 ± 26[b,c]
25 July 03	19.7 ± 0.1[f]	1.02 ± 0.01[b]	19.3 ± 0.2[c]	3.34	823 ± 14[c]	432 ± 14[c]
30 July 03	21.5 ± 0.2[d]	0.92 ± 0.01[c,d]	23.3 ± 0.1[d]	3.38	834 ± 11[c]	472 ± 82[c]
1 August 03	21.9 ± 0.1[d]	0.88 ± 0.01[d]	24.9 ± 0.3[i]	3.49	917 ± 24[d]	486 ± 21[c]
CA 2005						
19 July 05	13.7 ± 0.1[h]	1.18 ± 0.01[a]	11.6 ± 0.1[h]	3.16	690 ± 18[e]	255 ± 20[a]
26 July 05	15.7 ± 0.2[a]	1.04 ± 0.01[b]	15.1 ± 0.2[b]	3.27	784 ± 27[c]	357 ± 25[b]

Mean ± standard deviation. Within columns, means with different superscript letters are significantly different ($p < 0.05$).

harvested at different maturities (Table 1). The content of total soluble solids (TSS) varied from 12.8 °Brix in CA harvested on 15th July 2003 to 21.9 °Brix in CA harvested on 1st August 2003; this parameter increased as the fruit from all locations and years became more mature. Titratable acidity (TA) varied from 0.88 meq of malic acid in CA harvested on 1st August 2003 to 1.18 in CA harvested on 19th July 2005, and decreased as the fruit matured. TSS/TA ranged from 11.0 °Brix/meq of malic acid in CA harvested on 15th July 2003 to 24.9 in CA harvested on 1st August 2003, and this parameter increased with fruit maturity in all four ripening sets. The pH of the fruits' juice ranged from 3.16 for CA plums harvested on 19th July 2005 to 3.49 for CA plums harvested on 1st August 2003 and increased as the fruit ripened.

The plums used to produce candied "Ameixa d'Elvas" are commonly harvested when the TSS is approximately at 16 °Brix and the TA is higher than 1.0 meq of malic acid. According to these criteria, the plums from VV orchard collected until 15th July 2003 and 19th July 2005, and the plums from CA orchard collected until 22nd July 2003 and 26th July 2005 should produce good quality candied fruits. Fig. 1a shows the TSS of the plums from the two orchards. For VV, all samples with a TSS higher than 16.6 were unsuitable for processing; hence, this criterion of maturity was applicable to this orchard. However, this criterion seems not to be applicable to the plums from CA orchard. In fact, the CA plums collected on 22nd July 2003 and 26th July 2005, although having values of TSS suitable to classify them as adequate to be candied, produced poor final products with low pulp consistency and appreciable skin disruption (Nunes et al., in press). TA is also not a reliable predictor of processing quality, as the plums collected from CA on 22nd and 25th July 2003 and 26th July 2005, although having values of TA suitable to classify them as adequate to be candied, produced poor texture quality products. TSS/TA was also unsuitable as a criterion to evaluate the plums from CA (Fig. 2a). In contrast to TSS and TA and their ratio, the measurement of juice pH allows a separation of fruit that give good products from those that do not (Fig. 2b). However, the pH range separation of good from poor quality fruit is too small to obtain an unequivocal definition of the harvesting day for candying, based on this parameter. As these parameters seem not to be reliable enough to evaluate the maturity stage of plums as well as to determine precisely the moment to harvest fruits for processing, other parameters, based on cell wall polysaccharide composition of the fruits, were assessed.

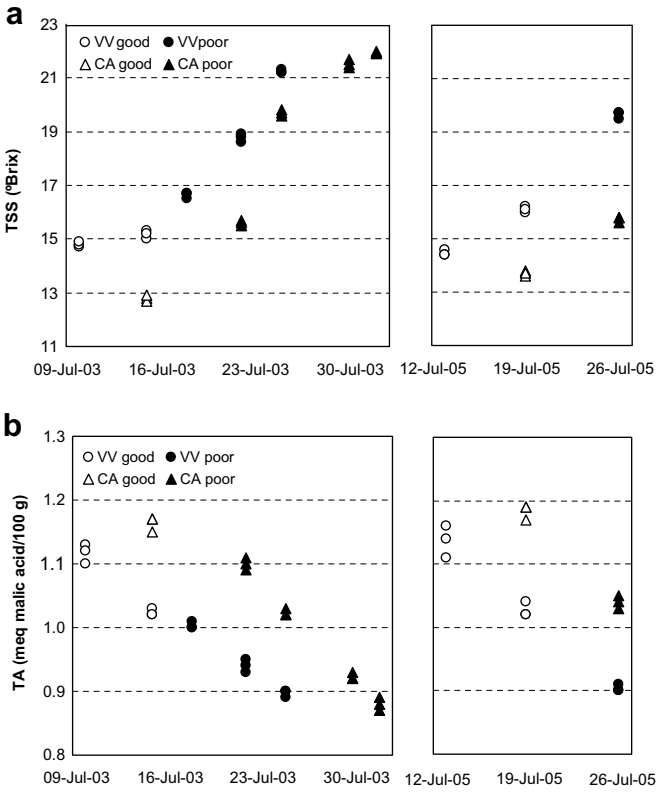

Fig. 1. (a) Total soluble solids (TSS) and (b) titratable acidity (TA) of plums from Vila Viçosa (VV) and Cano (CA) orchards in 2003 and 2005.

3.2. Total polysaccharides and uronic acids content in AIR

The total polysaccharides (PS) present in the AIR varied from 493 mg/g AIR in VV plums harvested on 13th July 2005 to

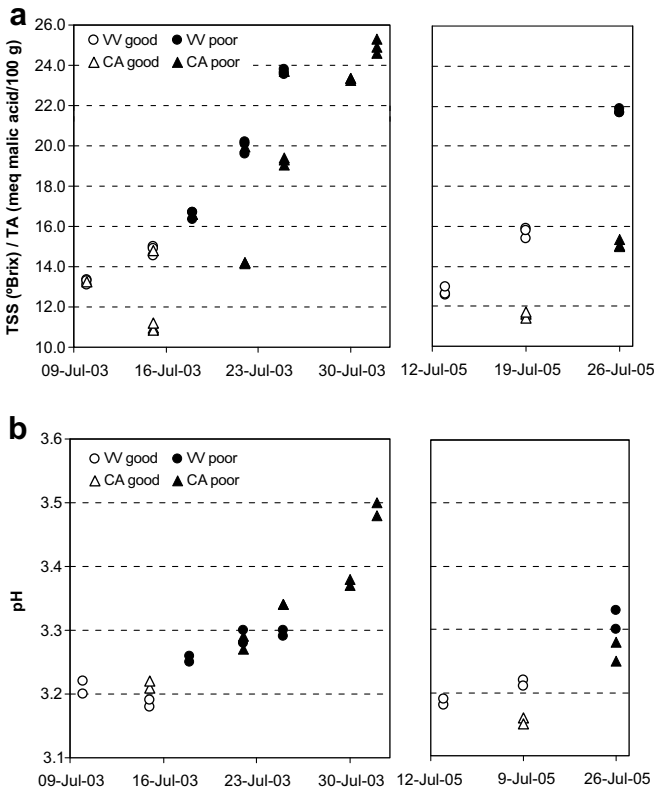

Fig. 2. (a) Ratio of total soluble solids and titratable acidity (TSS/TA) and (b) pH of plums from Vila Viçosa (VV) and Cano (CA) orchards in 2003 and 2005.

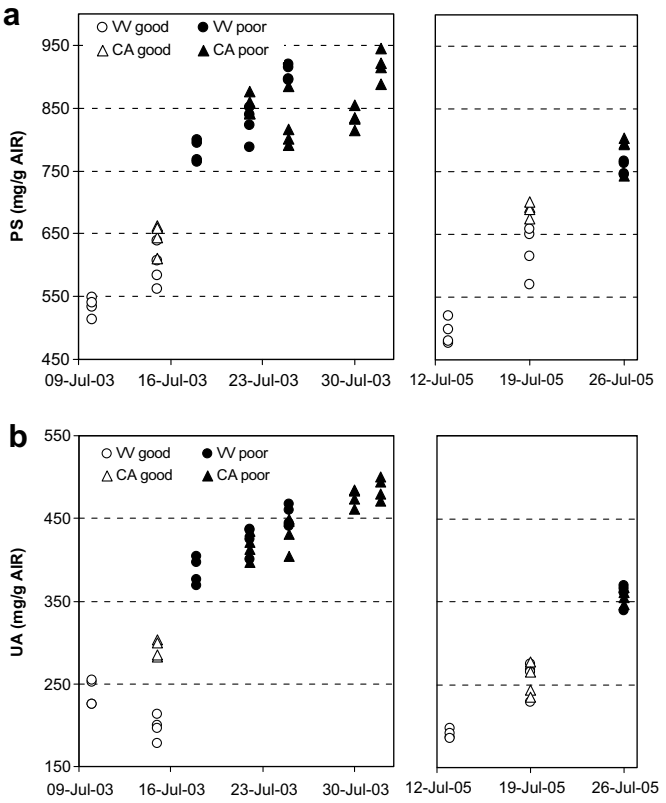

Fig. 3. (a) Total polysaccharides (PS) and (b) uronic acids (UA) content in AIR of plums from Vila Viçosa (VV) and Cano (CA) orchards in 2003 and 2005.

917 mg/g AIR in CA plums harvested on 1st August 2003 (Table 1). This parameter increased as the maturity of the fruit progressed in all four ripening sets studied. The uronic acids (UA) present in the AIR, which represent the pectic polysaccharides of the fruit, varied from 190 mg/g AIR in VV harvested on 13th July 2005 to 486 mg/g AIR in CA harvested on 1st August 2003. As observed for the PS, the content in UA increased as the maturity of the fruit proceeded in all four ripening sets studied.

Fig. 3 shows the content of PS (Fig. 3a) and UA (Fig. 3b) in the AIR of plums from the two orchards. A visible separation is obtained between the samples that give good or poor texture quality product for both parameters. Cell wall polysaccharide composition seems to provide the information about the stage of ripening of the fruit suitable to predict its texture behaviour upon candying.

3.3. Linear discriminant classifier

In order to assess the classification ratios of TSS (three replicates of 15 fruit samplings), TA (three replicates of 15 fruit samplings), TSS/TA (three replicates of 15 fruit samplings), pH (two replicates of 15 fruit samplings), PS (four replicates of 15 fruit samplings), and UA (four replicates of 15 fruit samplings) for the determination of a reliable classifier for the prediction of the ideal ripening stage of plums for candying, a linear discriminant classifier was applied. The results obtained are shown in Table 2.

Using the slope and the intercept for each parameter, it is possible to predict the texture of the fruits upon candying. A value near one means that the fruit is suitable for candying according to this parameter and, on the contrary, a value near zero means that the fruit is not suitable. When the TSS is used as a maturity parameter to select the fruits for candying, an error (LOF) of 17% is obtained. Also, an error of the same order of magnitude is obtained for TA (21%) and TSS/TA (17%), whereas the error given by the pH of the fruit's juice is 11%. However, the errors obtained were relatively lower when the PS and the UA content in AIR of plums were used as maturity parameters to select the fruits for candying (0.8% for PS and 0.005% for UA content). Considering the high standard error in the slope for PS, the content of UA in the AIR seems the parameter of choice. In order to assess if the models proposed were not due to chance, permutation tests were performed. This is based on the principle that a random permutation of the samples grouping should give 50% of correct answers and 50% of incorrect ones due to the arbitrary classification of the samples into 0 or 1. The permutation test performed confirmed that the content of UA in the AIR is a reliable parameter to predict the final quality of the candied plums, since the lack of fitness obtained when this test was performed was near 50%.

Table 2
Linear discriminant classifier results as a function of the total soluble solids (TSS), titratable acidity (TA), TSS/TA, and pH of the fruit, and total polysaccharides (PS) and uronic acids (UA) of AIR

Parameter	Number of samples	Slope	Intercept	LOF (%)	Permutation (LOF, %)
TSS (°Brix)	45	−0.127 ± 0.008	2.58 ± 0.16	17	~59
TA (meq malic acid)	45	3.55 ± 0.20	−3.23 ± 0.20	21	~59
TSS/TA	45	−0.08 ± 0.01	1.78 ± 0.20	17	~59
pH	30	−4.47 ± 0.86	15.0 ± 2.8	11	~57
PS (mg/g AIR)	60	−0.0033 ± 0.0034	2.9 ± 0.1	0.8	~49
UA (mg/g AIR)	60	−0.0045 ± 0.0001	1.95 ± 0.06	0.005	~49

Mean ± standard deviation.

4. Conclusion

This work shows that the estimation of the total amount of uronic acids in the AIR of the plums is a reliable maturation parameter able to define the harvesting point of "Rainha Cláudia Verde" plums for candying. This methodology, which is based on a colorimetric method, can be performed in an easy and quick way within a laboratory and provides a more accurate assessment of harvest date for candying than is provided by total solids or titratable acidity. Total solids and titratable acidity are reliable parameters to follow the maturation of the plum with little extra time or effort. However, for the definition of the harvesting point for candying, complementary data from UA is required. The preparation of the AIR by boiling, in a test tube, of 0.5 g of plum flesh in 2 ml of ethanol, filtration, wash with diethyl ether, and drying, can be performed in less than 30 min. From it, UA can be released after 1 h in 1 M H_2SO_4 at 100 °C, and colorimetrically quantified in the following 30 min. The time required for the analysis of a single sample is not very different from that required if 10–12 samples are used simultaneously. This gives a maximum time of laboratorial analysis of 2 h, which is a feasible time for the maturity control of the fruits of the orchard.

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