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DEIVED UILIAN DE CARVALHO

**DESEMPENHO HORTICULTURAL, COMPOSTOS  
ANTIOXIDANTES E CERAS EM PÓS-COLHEITA DE  
LARANJAS-DOCES DE MATURAÇÃO TARDIA NO  
NOROESTE DO PARANÁ**

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Tese apresentada ao Programa de Pós-graduação em Agronomia - Curso de Doutorado da Universidade Estadual de Londrina, área de concentração em Fitotecnia.

Orientador: Pesq. Dr. Rui Pereira Leite Júnior.  
Co-Orientadora: Prof<sup>a</sup>. Dr<sup>a</sup>. Carmen Silvia Vieira  
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Londrina, 14 de março de 2022.

*Dedico este trabalho aos meus pais Maria Casturina Machado de Carvalho e Obadias Teixeira de Carvalho, por toda dedicação, educação, perseverança e fé em Deus.*

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## RESUMO

Atualmente, a região noroeste do Paraná é reconhecida como o maior polo citrícola do Estado, possuindo extenso cultivo de laranjas-doces (*Citrus xsinensis*) para o processamento de sucos. No entanto, verifica-se a predominância de poucos genótipos de laranjas-doces nos pomares da região, tornando-os vulneráveis às adversidades bióticas e abióticas. Dessa maneira, neste estudo avaliou-se o desempenho horticultural, incluindo desenvolvimento vegetativo, produção e qualidade físico-química dos frutos, incidência de cancro cítrico e de Huanglongbing (HLB), índice de desempenho para o mercado de fruta fresca e indústria processadora de suco, assim como a estimativa de espaçamento entre plantas e linhas, produtividade projetada e índice tecnológico de 19 seleções de laranjas-doces de maturação tardia enxertadas sobre limão Cravo (*Citrus xlimonia*) no noroeste do Paraná de 2012 à 2021 (Artigo A). Também foi avaliada a caracterização físico-química de frutos e tecidos (flavedo, albedo, membrana da parede, vesículas de suco e eixo central) e uso de ceras em pós-colheita para as laranjas-doces Valencia Late e Natal IAC (Artigo B). Para o Artigo A, o delineamento experimental adotado foi blocos ao acaso, com 19 tratamentos (laranjas-doces) e três blocos de 5 plantas por parcela. Para o Artigo B, os frutos de laranjas-doces foram caracterizados em 2019 e tratados ou não com cera de carnaúba/colofônia sob armazenamento refrigerado por 0, 15, 30, 45 e 60 dias em 2020. O índice de cor, perda de peso, parâmetros físico-químicos e sensorial dos frutos foram monitorados na colheita e após cada período de armazenamento. O delineamento estatístico tanto para a caracterização dos frutos e tecidos, quanto para o ensaio pós-colheita foi inteiramente casualizado e complementado em esquema fatorial para o ensaio pós-colheita (2 cultivares × 5 períodos de armazenamento). Os resultados do Artigo A indicam que as seleções de Valência foram mais vigorosas, destacando-se a Olinda, Frost e #121 que registraram altura de planta e volume de copa superior à 4,20 m e 43 m<sup>3</sup>, respectivamente. Plantas de Natal África do Sul e Whit's Late Valencia foram as mais produtivas, com produção acumulada superior a 650 kg por planta. Com exceção da Olinda Valencia, todas as seleções produziram frutos de excelente qualidade físico-química, apresentando similaridades para o mercado de frutas frescas, enquanto a Natal África do Sul e Charmute de Brotas foram mais adequadas para a indústria de processamento de suco. Frost Valencia e Valencia Late Fla. tiveram a maior incidência de frutos com cancro cítrico (> 20%). Plantas de Valencia Mutação apresentaram a maior incidência acumulada de HLB após nove anos de cultivo (93%), enquanto as de Natal IAC e Folha Murcha IAC tiveram a menor incidência da doença (13%). Com base nos resultados do Artigo B, os diferentes tecidos dos frutos de Valencia Late e Natal IAC apresentaram alta capacidade antioxidante, que podem ser utilizados como fonte antioxidante de baixo custo, com destaque ao flavedo e albedo. O tratamento com cera de carnaúba/colofônia integrado ao armazenamento refrigerado foi eficiente para diminuir a perda de peso e preservar o avanço da cor, qualidade físico-química e sensorial dos frutos por até 60 dias.

**Palavras-chave:** *citrus ×sinensis* (L.) Osbeck; maturação tardia; desenvolvimento vegetativo; qualidade de fruto; doenças; pós-colheita de citros; cera de carnaúba/resina de colofônia.

CARVALHO, Deived Uilian de. **Horticultural performance, antioxidant compounds and waxes in postharvest of late-season sweet oranges in northwest Paraná.** 2022. 125 p. Thesis (Doctorate in Agronomy) – Universidade Estadual de Londrina, Londrina, 2022.

## ABSTRACT

Currently, the northwest region of Paraná is the largest citrus-growing area in the state, with extensive cultivation of sweet oranges (*Citrus x sinensis*), mainly for processing. However, there is a predominance of few sweet orange genotypes in the orchards of the region, which is a vulnerability factor for biotic and abiotic stresses. Thus, this study evaluated the horticultural performance, including vegetative growth, fruit yield and physicochemical quality, incidence of citrus canker and huanglongbing (HLB), performance index for fresh fruit market and processing, as well as the spacing, yield and SST yield estimation of 19 selections of late-season sweet oranges grafted onto Rangpur lime (*Citrus x limonia*) in northwestern Paraná from 2012 to 2021 (Article A). The physicochemical characterization of fruits and tissues (flavedo, albedo, segment wall, juice vesicles and central core) and the use of waxes/resin in postharvest of Valencia Late and Natal IAC sweet oranges (Article B) were also evaluated. For Article A, the experimental design was randomized blocks, with 19 treatments (sweet oranges) and three blocks of 5 trees per plot. For Article B, sweet orange fruits were characterized in 2019 and treated or not with carnauba wax/rosin under cold storage for 0, 15, 30, 45 and 60 days in 2020. Color index, weight loss, physicochemical and sensory parameters of the fruits were monitored at harvest and after each storage period. The statistical design, for both the characterization and postharvest assay was completely randomized and complemented in factorial arrangement for the postharvest assay (2 cultivars × 5 storage periods). The results of Article A indicate that the Valencia selections were the most vigorous, particularly Olinda, Frost and #121, which had tree height and canopy volume greater than 4.20 m and 43 m<sup>3</sup>, respectively. Trees of Natal África do Sul and Whit's Late Valencia were the most productive, with cumulative yield higher than 650 kg per tree. Except for Olinda Valencia, all selections produced fruits of excellent physicochemical quality, showing similarities for the fresh fruit market, while Natal África do Sul and Charmute de Brotas were more suitable for processing. Frost Valencia and Valencia Late Fla. had the highest incidence of citrus canker on fruits (> 20%). Valencia Mutação trees had the highest cumulative incidence of HLB after nine years of planting (93%), while Natal IAC and Folha Murcha IAC had the lowest incidence (13%). Based on the results from Article B, the different tissues of the Valencia Late and Natal IAC fruits showed high antioxidant capacity, which can be used as a low-cost source of antioxidants, particularly the flavedo and albedo tissues. The treatment with carnauba wax/rosin integrated with cold storage was efficient for reducing weight loss and preserving the color development, physicochemical and sensory quality of the fruits for up to 60 days.

**Key words:** *Citrus x sinensis* (L.) Osbeck; late-season maturing; tree growth; fruit quality; diseases; citrus postharvest; carnauba wax/rosin resin.

## LISTA DE TABELAS

<b>Table 3.3.1</b> - Origin of the 19 selections of sweet orange ( <i>C. xsinensis</i> ) assessed in this study. ....	47
<b>Table 3.4.1</b> - Tree size of late-season sweet orange selections grafted onto Rangpur lime rootstock and determined in the 2019 season. Guairaçá, state of Paraná, Brazil. ....	55
<b>Table 3.4.2</b> - Yield performance of late-season sweet orange selections grafted onto Rangpur lime rootstock from 2016 to 2021. Guairaçá, state of Paraná, Brazil. ....	57
<b>Table 3.4.3</b> - Three-season average fruit quality of late-season sweet oranges produced in Guairaçá, state of Paraná, Brazil, from 2019 to 2021. ....	60
<b>Table 3.4.4</b> - Three-season average juice quality of late-season sweet oranges produced in Guairaçá, state of Paraná, Brazil, from 2019 to 2021. ....	61
<b>Table 3.4.5</b> - Estimates of minimum row and tree spacing, maximum tree density, fruit yield, and TSS yield for late-season sweet orange selections grafted onto Rangpur lime rootstock, based on field performance in Guairaçá, state of Paraná, Brazil. ....	63
<b>Table 3.4.6</b> - Fresh fruit and industrial processing indices of late-season sweet orange selections grafted onto Rangpur lime rootstock under humid subtropical conditions in Guairaçá, state of Paraná, Brazil, from 2016 to 2021 cropping season average. ....	65
<b>Table 3.4.7</b> - Incidence of harvested fruit with citrus canker on late-season sweet orange selections grafted onto Rangpur lime rootstock harvested in 2020 and 2021 in Guairaçá, state of Paraná, Brazil. ....	67
<b>Table 4.4.1</b> - Physicochemical quality of Valencia Late and Natal IAC sweet orange fruits evaluated in the 2019 harvest. ....	88
<b>Table 4.4.2</b> - Total soluble solids (TSS), titratable acidity (TA), and ratio (TSS.TA-1) of Valencia Late and Natal IAC sweet orange fruits subjected to carnauba wax/rosin resin coating treatment and different cold storage period. ....	93

## LISTA DE FIGURAS

- Figure 3.3.1** - Monthly rainfall and maximum (Tmax), medium (Tmed) and minimum (Tmin) average temperatures from 2016 to 2021 in Guairaçá, state of Paraná, Brazil. ....46
- Figure 3.4.1** - Cumulative incidence of huanglongbing (HLB)-affected trees in 19 late-season sweet orange selections grafted onto Rangpur lime rootstock after nine years of planting in Guairaçá, state of Paraná, Brazil.....68
- Figure 3.4.2** - Principal component analysis (PCA) for tree size, yield, fruit quality, estimation of plant density and yield, incidence of citrus canker on fruits, and incidence of huanglongbing (HLB) in trees of 19 late-season sweet orange selections grafted onto Rangpur lime rootstock .....70
- Figure 4.4.1** - Different fruit tissues extracted from Valencia Late and Natal IAC, including flavedo, albedo, central core, juice sacs and segment wall. ....85
- Figure 4.4.2** - Total phenolic content (mg GAE.100 g of dry tissue<sup>-1</sup>), total flavonoid content (mg hesperidin per 100 g of dry tissue), and percentage of DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity of different fruit tissues (flavedo, albedo, central core, juice sacs and segment wall) of Valencia Late and Natal IAC sweet orange fruits.....90
- Figure 4.4.3** - Weight loss (%) of Valencia Late and Natal IAC sweet orange fruits treated with carnauba wax/rosin resin and maintained under different cold storage periods (15, 30, 45 and 60 days) .....91
- Figure 4.4.4** - Citrus color indices (CCI) of Valencia Late and Natal IAC sweet orange fruits subjected to carnauba wax/rosin resin coating treatment after 0 (harvest), 15, 30, 45 and 60 days of cold storage .....92
- Figure 4.4.5** - Juice content of Valencia Late and Natal IAC sweet orange fruits subjected to carnauba wax/rosin resin coating treatment after 0 (harvest), 15, 30, 45, and 60 days of cold storage .....94
- Figure 4.4.6** - Sensory evaluation for color, texture, firmness, aroma, flavor,

juiciness, and preference of Valencia Late and Natal IAC sweet orange fruits treated/non-treated with carnauba wax/rosin resin coating at harvest (A) and after 15 (B), 30 (C), 45 (D), and 60 days (E) of cold storage. ....96

## LISTA DE ABREVIATURAS E SIGLAS

ADAPAR	Agência de Defesa Agropecuária do Paraná
AOAC	Association of Official Analytical Chemists
BAG	Banco Ativo de Germoplasma
CCSM	Centro de Citricultura “Sylvio Moreira”
EMATER	Empresa Paranaense de Assistência Técnica e Extensão Rural
FAO	Food and Agriculture Organization of the United Nations
FCOJ	Frozen Concentrated Orange Juice
HLB	Huanglongbing
IAC	Instituto Agronômico de Campinas
IAPAR	Instituto Agronômico do Paraná
IDR	Instituto de Desenvolvimento Rural do Paraná – IAPAR/Emater
IBGE	Instituto Brasileiro de Geografia e Estatística
LDC	Louis Dreyfus Commodities
MAPA	Ministério da Agricultura, Pecuária e Abastecimento
NFC	Not From Concentrate
SEAB	Secretaria de Estado da Agricultura e do Abastecimento do Paraná
USDA	United States Department of Agriculture

## SUMÁRIO

<b>1</b>	<b>INTRODUÇÃO</b> .....	14
<b>2</b>	<b>REVISÃO DE LITERATURA</b> .....	17
2.1	PANORAMA DA CITRICULTURA NO MUNDO .....	17
2.2	PRODUÇÃO DE LARANJAS-DOCES NO BRASIL .....	19
2.3	PRODUÇÃO DE LARANJAS-DOCES NO PARANÁ .....	20
2.4	CULTIVARES DE LARANJAS-DOCES COM FOCO EM TARDIAS.....	22
2.4.1	Laranja-Doce Valência .....	24
2.4.2	Laranja-Doce Natal.....	26
2.4.3	Laranja-Doce Folha Murcha .....	26
2.4.4	Laranja-Doce Charmute de Brotas .....	28
2.5	DESENVOLVIMENTO VEGETATIVO E PRODUÇÃO DE CITROS .....	28
2.6	CRESCIMENTO E DESENVOLVIMENTO DE CITROS .....	30
2.7	MATURAÇÃO DE CITROS .....	32
2.8	QUALIDADE FÍSICO-QUÍMICA DE CITROS .....	33
2.9	QUALIDADE SENSORIAL DE CITROS .....	37
2.10	PÓS-COLHEITA DE CITROS .....	38
<b>3</b>	<b>ARTIGO A</b> .....	42
3.1	ABSTRACT .....	42
3.2	INTRODUCTION.....	42
3.3	MATERIALS AND METHODS.....	45
3.3.1	Field Location .....	45
3.3.2	Plant Material.....	46
3.3.3	Orchard Management.....	47
3.3.4	Vegetative Growth Measurements.....	48
3.3.5	Fruit Yield and Production Efficiency .....	48
3.3.6	Fruit Quality Evaluations.....	49
3.3.7	Estimates for Planting Density and Yield .....	50
3.3.8	Sweet Orange Performance Index for Fresh Fruit Market and Juice Processing.....	51
3.3.9	Incidence of Fruit with Citrus Canker.....	52

3.3.10	Incidence of Trees with HLB.....	52
3.3.11	Data Analyses .....	53
3.4	RESULTS.....	53
3.4.1	Vegetative Growth Measurements.....	53
3.4.2	Fruit Yield and Production Efficiency .....	56
3.4.3	Fruit Quality Evaluations.....	58
3.4.4	Estimates for Planting Density and Yield.....	62
3.4.5	Sweet Orange Performance Index for Fresh Fruit Market and Juice Processing.....	64
3.4.6	Incidence of Citrus Canker and Huanglongbing (HLB) .....	65
3.4.7	Multivariate Analysis.....	68
3.5	DISCUSSION .....	71
3.6	CONCLUSIONS .....	78
<b>4</b>	<b>ARTIGO B</b> .....	<b>80</b>
4.1	ABSTRACT .....	80
4.2	INTRODUCTION.....	81
4.3	MATERIALS AND METHODS.....	82
4.3.1	Fruit Harvest and Treatments .....	82
4.3.2	Fruit Analyses.....	83
4.3.3	Postharvest Assay .....	86
4.3.4	Statistical Analysis.....	87
4.4	RESULTS .....	87
4.4.1	Pre-storage Fruit Evaluation .....	87
4.4.2	Postharvest Fruit Evaluation.....	91
4.4.2.1	Weight loss .....	91
4.4.2.2	Fruit color index.....	92
4.4.2.3	Juice quality.....	92
4.4.2.4	Sensory analysis.....	94
4.5	DISCUSSION .....	97
4.6	CONCLUSIONS .....	103
	<b>REFERÊNCIAS</b> .....	<b>105</b>

## 1 INTRODUÇÃO

A laranja-doce [*Citrus xsinensis* (L.) Osbeck] é uma das frutas mais consumidas no mundo, seja 'in natura', como também na forma de sucos ou derivados. Dentre os países produtores, o Brasil se destaca na primeira posição, sendo responsável por aproximadamente 25% da produção mundial, com 16,9 milhões de toneladas produzidas em 2020, seguido da Índia e China com 9,7 e 7,6 milhões de toneladas, respectivamente (FAO, 2020; USDA-FAS Foreign Agricultural Service, 2022). No Brasil, o estado de São Paulo lidera a produção de laranjas-doces com 78% do total produzido, seguido de Minas Gerais com 6.0%, Bahia com 3.6% e Paraná com 3.5% (IBGE, 2020). No estado do Paraná, a produção de laranjas-doces concentra-se nas regiões norte e noroeste (AULER et al., 2014). Investimentos em tecnologias e técnicas adequadas de produção e manejo têm garantido um aumento significativo na produção de laranjas no Paraná. Atualmente, a região noroeste do estado detém o maior parque citrícola, com mais de 11.000 hectares destinados ao cultivo de laranjas-doces para atender principalmente à indústria produtora de suco concentrado e congelado (FCOJ – frozen concentrated orange juice) e o mercado de frutas frescas do estado. As principais cultivares de laranjas-doces cultivadas na região noroeste do Paraná são IAPAR 73 (maturação precoce), Pera (meia estação), Folha Murcha e Valencia (maturação tardia) predominantemente enxertadas sobre o limão Cravo [*Citrus xlimonia* (L.) Osbeck] (AULER et al., 2014).

O Brasil apresenta avançada tecnologia no processamento industrial de laranjas-doces visando a produção de suco, o principal produto resultante da citricultura brasileira. Aproximadamente 70% da produção nacional de laranjas-doces são destinadas ao processamento, destacando-se o suco concentrado e congelado de laranja (FCOJ) e o não concentrado (NFC – not from concentrate) (SPREEN et al., 2020; CARVALHO et al., 2021a). Em torno de 95% dos sucos produzidos são exportados para os mercados europeu, norte-americano e, mais recentemente, o asiático (NEVES et al., 2019; SPREEN et al., 2020). Mesmo atendendo à demanda nacional, o país destina um volume insignificante de laranjas-doces para o mercado externo de frutas de mesa. Associado à qualidade inadequada dos frutos e às questões fitossanitárias que restringem a ampliação da exportação de citros de mesa, as laranjas-doces brasileiras podem atender o mercado externo desde que se invista em cultivares (principalmente do grupo Valencia) e técnicas adequadas de manejo e

conservação pós-colheita (CARVALHO et al., 2020a).

Os pomares de laranjas-doces no Brasil são formados predominantemente pelas cultivares de maturação precoce Hamlin, de meia-estação Pera, e tardias Valência e Natal (CARVALHO et al., 2019a). Observa-se a preferência pelos produtores por cultivares de maturação tardia, pois apresentam alta produção e elevado teor de sólidos solúveis no suco (NEVES et al., 2019). No entanto, este estreito número de cultivares utilizadas nos pomares torna a citricultura brasileira mais vulnerável aos problemas bióticos e abióticos. Assim, há necessidade de realizar a diversificação dos pomares com novas alternativas de laranjas-doces. Portanto, a diversificação dos pomares com cultivares copa com diferentes épocas de maturação e porta-enxertos alternativos é importante, permitindo que o produtor comercialize sua produção por um período mais amplo do ano. Isto garante maiores lucros e ampliação do período de extração de sucos pelas indústrias de processamento (NEVES et al., 2019). Esta diversificação pode reduzir também problemas fitossanitários, favorecendo o manejo de pragas e doenças, assim como a redução no impacto das condições adversas de clima e solo (EMMANOUILIDOU; KYRIACOU, 2017; CARVALHO et al., 2019b; NEVES et al., 2019; CARVALHO et al., 2021a).

Mesmo sob intensa pressão fitossanitária ocorrida no histórico da citricultura brasileira, destacando-se o vírus da tristeza (CTV) na década de 1930, o surto de cancro cítrico nos anos 1970, a clorose variegada dos citros (CVC) a partir de 1987 e o huanglongbing (HLB, ex. *greening* dos citros) a partir de 2004 (BOVÉ, 2006), o Brasil tem adaptado diversas práticas culturais e um eficiente sistema de manejo de plantas (CARVALHO et al., 2019a; SILVEIRA et al., 2020; SPREEN et al., 2020). Dessa maneira, o Instituto de Desenvolvimento Rural do Paraná – IAPAR/EMATER (IDR - Paraná), órgão responsável pela pesquisa e recomendação de cultivares para o estado, tem introduzido diversos materiais no seu Banco Ativo de Germoplasma de Citros (BAG - Citros) para dar suporte ao estabelecimento e crescimento da atividade citrícola paranaense. Baseado nesses estudos, o IDR - Paraná tem indicado para cultivo diversas cultivares de laranjas-doces destinadas ao mercado de frutas frescas, processamento de suco ou com dupla aptidão, entre elas Navelina, IAPAR 73, Salustiana e IPR Cadenera de maturação precoce, Pera, Shamouti e IPR Jaffa de meia-estação e as tardias Folha Murcha, Valência e Natal (LEITE JÚNIOR, 1992; TAZIMA; LEITE JÚNIOR, 2000; 2002; PARANÁ, 2021).

A qualidade pós-colheita e a vida de prateleira de frutos são aspectos cada vez

mais importantes na produção de alimentos, especialmente quando a produção é restrita a um período específico do ano (LADANAYA, 2008). Técnicas como armazenamento refrigerado, tratamento térmico, irradiação com raios ultravioleta (UV) e gama, controle de umidade e temperatura, movimento do ar, balanço de gases atmosféricos (atmosfera modificada) e revestimentos com ceras e resinas podem ser empregadas na conservação pós-colheita de frutas (LADANAYA, 2008; GUTIÉRREZ et al., 2019). A aplicação em frutas de materiais de revestimento à base natural, como ceras e resinas, é reconhecida como uma abordagem ecologicamente correta e segura, podendo ser adotada por países produtores de citros onde os recursos naturais são disponíveis (ANSORENA; PONCE, 2019). Esta técnica tem se tornado popular entre as práticas pós-colheita de citros, pois têm a capacidade de retardar a senescência natural dos frutos e reduzir a perda de água através da permeabilidade diferencial do CO<sub>2</sub>, O<sub>2</sub> e vapor d'água, além de aumentar as propriedades antimicrobianas da fruta revestida (LADANIYA, 2008; ANSORENA; PONCE, 2019; MIRANDA et al., 2021). Esses materiais podem ser extraídos de diferentes fontes naturais e renováveis como carnaúba [*Copernica prunifera* (Mill.) HE Moore], cana-de-açúcar [*Saccharum officinarum* (L.)], soja [*Glycine max* (L.) Merrill], candelilla (*Euphorbia antisyphilitica* Zucc.), madeira (ex. *Pinus* spp.) e cera de abelhas (HAGENMAIER, 2000; JONGLERTJUNYA et al., 2014; BARMAN et al., 2019; MOTAMEDDI et al., 2018; DE FREITAS et al., 2019; BASWAL et al., 2020; NASRIN et al., 2020; FEI et al., 2021).

Diante do exposto, neste estudo avaliou-se o desempenho agrônômico, incluindo crescimento vegetativo, produção, qualidade físico-química de frutos, estimativa de densidade de plantas e produtividade, e incidência de cancro cítrico e HLB em 19 seleções de laranjas-doces de maturação tardia na região noroeste do estado do Paraná, Brasil. Também foi objeto do estudo a avaliação de ceras naturais de recobrimento e determinação de propriedade antioxidantes de laranjas-doces para ampliar o período de vida útil dos frutos e reduzir as perdas na cadeia produtiva de citros de mesa.

## 2 REVISÃO DE LITERATURA

### 2.1 PANORAMA DA CITRICULTURA NO MUNDO

Os citros são amplamente cultivados em diferentes regiões do mundo, seja nas áreas tropicais, subtropicais e do mediterrâneo. Os frutos apresentam sabor característico, refrescante e agradável ao paladar humano, com elevado valor nutricional, sendo fonte de ácido ascórbico que é a principal forma biologicamente ativa da vitamina C (DAVEY et al., 2000; SCHERER et al. 2012). Além do ácido ascórbico, os frutos contêm vários compostos fitoquímicos que desempenham papel neutracêuticos, como carotenoides, limonoides, flavanonoides, complexo de vitamina B e nutrientes relacionados (LADANIYA, 2008).

Os citros são basicamente destinados para o mercado de frutas frescas e ao processamento. Os produtos cítricos processados incluem sucos, tanto não concentrados quanto concentrados, e derivados. Os produtos processados também geram subprodutos comercializáveis, incluindo polpa e casca que geralmente são transformadas em ração animal, melação e óleos essenciais e compostos como o d-limoneno (CARVALHO et al., 2019a; SPREEN et al., 2020).

Os principais grupos de citros comercializáveis no mundo são as laranjas-doces, tangerinas e limões, incluindo as limas-ácidas. Em 2020, os principais países produtores de citros foram, em ordem decrescente da produção, China, Brasil, Índia, México, Espanha e Estados Unidos (FAO, 2020; USDA-FAS Foreign Agricultural Service, 2021). No entanto, as diferentes regiões produtoras de citros se destacam na produção de grupos específicos para atender às diversas demandas do mercado interno e externo. A China se destaca na produção de tangerinas, com um volume total de 23,1 milhões de toneladas da fruta em 2020, além de 7,6 milhões de toneladas de laranjas doces e 2,7 milhões de toneladas de limões e limas-ácidas (FAO, 2020). Na China, os citros são produzidos principalmente em pequenas propriedades e comercializados predominantemente no mercado interno (SPREEN et al., 2020). Por outro lado, o Brasil é líder na produção de laranjas-doces, tendo atingido o volume total de 16,7 milhões de toneladas em 2020, somados a 1,6 milhão de toneladas de limões e limas-ácidas e 1,0 milhão de toneladas de tangerinas (FAO, 2020; IBGE, 2020). A citricultura brasileira se concentra em médias e grandes propriedades, sendo aproximadamente 70% da produção destinada à indústria de processamento de

sucos de laranja (CARVALHO et al., 2020a; SPREEN et al., 2020). Cabe salientar que 98% do suco produzido é exportado para Europa Ocidental, América do Norte e Ásia (NEVES et al., 2010; 2019; CARVALHO et al., 2019a; SPREEN et al., 2020). A comercialização dos citros produzidos no Brasil gera receita anual de bilhões de dólares e garante milhares de empregos nos diversos setores da cadeia produtiva dos citros, como no manejo da cultura, colheita, pós-colheita, processamento industrial, transporte, logística e comercialização (NEVES et al., 2019).

Índia e México também têm obtido espaço na citricultura mundial. Em 2020, a Índia produziu cerca de 9,7 milhões de toneladas de laranjas-doces e 3,6 milhões de toneladas de limões e limas-ácidas, enquanto o México produziu 4,7 milhões de toneladas de laranjas-doces e 2,8 milhões de toneladas de limões e limas-ácidas (FAO, 2020). A Espanha se caracteriza pela produção de frutos frescos para consumo '*in natura*', visto que o clima mediterrâneo favorece a produção de frutos de alta qualidade com coloração acentuada e homogênea, característica de cada espécie (NEVES et al., 2010). Em 2020, a Espanha registrou volumes de 3,3 milhões de toneladas de laranjas-doces, 2,0 milhões de toneladas de tangerinas e aproximadamente 1,0 milhão de toneladas de limões e limas-ácidas (FAO, 2020). Parte da produção espanhola é comercializada no próprio mercado interno e outra parcela significativa é exportada para diversos países da União Europeia (SPREEN et al., 2020).

Nesse cenário, os Estados Unidos têm apresentado significativa redução em sua produção de laranja-doce nos últimos anos, visto que o país já foi o segundo maior produtor mundial (FAO, 2020; USDA-FAS Foreign Agricultural Service, 2021). Isto está associado diretamente à alta ocorrência nos pomares de citros do Huanglongbing (HLB, ex. *greening* dos citros), presente também em diversas outras áreas produtoras de citros. A citricultura estadunidense é composta por pequenas, médias e grandes propriedades (SPREEN et al., 2020). Observa-se uma desistência da produção de citros por parte dos pequenos e médios produtores, devida a redução expressiva da produtividade e da qualidade dos frutos, associado ao alto custo de produção requerido pelo manejo dos citros após a introdução da bactéria ('*Candidatus Liberibacter asiaticus*', CLas) do HLB em 2005 (GOTTWALD et al., 2007). A incidência de HLB aumentou rapidamente de 0,2% em 2005–2006 no país, quando os primeiros relatos da doença foram registrados (GOTTWALD et al., 2007), para os expressivos 100% em 2020 (GRAHAM et al., 2020). O HLB tem causado enormes perdas

econômicas aos Estados Unidos, com redução de mais de 70% na produção de laranjas-doces entre 2004 e 2020 (USDA-NASS National Agricultural Statistic Service, 2021; KUNWAR et al., 2021).

## 2.2 PRODUÇÃO DE LARANJAS-DOCES NO BRASIL

O Brasil é líder na produção de laranjas-doces, assim como na produção e exportação de suco dessa fruta, como o suco concentrado e congelado de laranja (FCOJ) e o não concentrado (NFC), os principais produtos resultantes da cadeia agroindustrial da citricultura brasileira (FAO, 2020; USDA-FAS Foreign Agricultural Service, 2021). De acordo com a Organização das Nações Unidas para Agricultura e Alimentação (FAO, 2020), no período de 2010 a 2020, o Brasil teve participação de 24% na produção mundial de laranjas-doces, se destacando com produção média anual de aproximadamente 17,5 milhões de toneladas.

A produção de laranjas-doces se concentra no sudeste, nordeste e sul do Brasil (IBGE, 2020). O estado de São Paulo é o maior produtor nacional, sendo responsável por aproximadamente 78% da safra de 2020, com 13,0 milhões de toneladas de frutos colhidos em 362.764 hectares, seguido por Minas Gerais com volume total de 1,0 milhão de toneladas produzidos em 38.284 hectares, Bahia com 595,404 toneladas produzidos em 49,332 hectares e Paraná com 585,721 toneladas de frutos produzidos em 20.046 hectares (IBGE, 2020).

O Brasil detém mais de 50% da produção mundial de suco concentrado e congelado de laranja (FCOJ), exportando 98% de sua produção total para os mercados da União Europeia, Estados Unidos, e mais recentemente o asiático como Japão e China (NEVES et al., 2010; NEVES et al., 2019). Tudo isto se deve ao investimento em pesquisas e tecnologias no segmento citrícola brasileiro, desde o manejo dos pomares até o processamento industrial dos frutos. As principais indústrias extratoras de suco de laranjas no estado de São Paulo são Citrosuco S/A Agroindústria, Sucocítrico Cutrale Ltda e a LDC - Louis Dreyfus Commodities Sucos S.A. (NEVES et al., 2019). Algumas dessas indústrias também possuem unidades de processamento de suco de laranja no estado da Flórida, Estados Unidos (SPREEN et al., 2020). Apesar de o Brasil ser líder na produção mundial de laranjas-doces, o país apresenta baixa produção de laranja-doce de mesa para exportação, principalmente devido à restrição fitossanitária imposta pelos países importadores

associada à necessidade de investimentos em técnicas mais eficazes de conservação pós-colheita, as quais têm limitado a expansão da cultura de laranja de mesa no país.

Mesmo sob grande pressão fitossanitária, como o vírus da tristeza dos citros (CTV) nas décadas de 1930 e 1940, um surto de cancro cítrico na década de 1970, a ocorrência e disseminação da clorose variegada dos citros (CVC) nas décadas de 1980 e 1990, e o huanglongbing (HLB) a partir de 2004, a citricultura brasileira tem se adaptado aos novos cenários, adotando eficientes sistemas de controle de pragas e doenças e manejo das plantas cítricas, assim como a expansão da produção para novas áreas (SPREEN et al., 2020; NEVES et al., 2019). Isto possibilitou que a citricultura brasileira se mantivesse na liderança da produção mundial de laranjas-doces. As unidades processadoras de suco de laranja no estado de São Paulo desenvolveram um sistema altamente eficiente de recebimento e processamento de laranjas-doces e logística no transporte a granel de suco. Parcerias estratégicas têm sido formadas também entre indústrias processadoras de suco de laranja e distribuidores para distribuição de suco não concentrado (NFC), uma vez que a demanda por este produto vem aumentando no Brasil e no mundo (SPREEN et al., 2020; NEVES et al., 2019). Com isso, as pequenas unidades processadoras iniciaram suas operações com marcas locais em 2010, aumentando significativamente o consumo de suco de laranja não processado (SPREEN et al., 2020).

### 2.3 PRODUÇÃO DE LARANJAS-DOCES NO PARANÁ

O estado do Paraná encontra-se em quarto lugar no ranking nacional de produção de laranjas-doces, responsável por 3,5% da safra em 2020 (IBGE, 2020). A produção de laranjas-doces no estado se concentra nas regiões norte e noroeste (AULER et al., 2014). Em relação à produtividade, o Paraná alcançou 29,2 ton.ha<sup>-1</sup> na safra de 2020, similar ao rendimento médio nacional e próximo ao obtido pelo estado de São Paulo, 35,7 ton.ha<sup>-1</sup>, o maior produtor nacional (IBGE, 2020).

Apesar da atual participação no cenário brasileiro, a produção de citros no Paraná esteve restrita à região do Vale do Ribeira até o final da década de 1980, devido à ocorrência de cancro cítrico (*Xanthomonas citri* subsp. *citri*, Xcc) nas regiões norte, noroeste e oeste do Estado. O primeiro relato desta doença no Paraná ocorreu em 1957, no município de Lupionópolis, a partir de então, as regiões que apresentavam incidência do cancro cítrico foram impedidas de cultivar citros

(AMARAL, 1957), o que prevaleceu por mais de 30 anos, mesmo apresentando condições edafoclimáticas adequadas para a citricultura. Mediante isto, foram adotadas medidas severas para o controle da doença baseadas na erradicação de plantas nas áreas infestadas, e em critérios rígidos para liberação de áreas para cultivo (IAPAR, 1992; LEITE JÚNIOR, 2007).

Para a viabilização técnica e política dessas medidas, foram conduzidos estudos de pesquisa envolvendo práticas de prevenção e o controle do cancro cítrico, os quais proporcionaram a retomada na produção de citros em todo o estado no final de 1980, através do desenvolvimento do programa de manejo integrado (LEITE JÚNIOR; MOHAN, 1990). Este programa preconizou o plantio de mudas saudáveis, plantio de cultivares mais resistentes, utilização de quebra-vento, controle do minador-dos-citros (*Phyllocnistis citrella* Stainton) e a aplicação preventiva e regular de bactericidas cúpricos. Além destas diretrizes, o programa também incluiu medidas regulatórias como a quarentena de material propagativo a ser introduzido no estado, inspeções em viveiros e pomares e regulamentação na implantação de pomares (LEITE JÚNIOR, 1992). Com isso, foi observada uma crescente expansão da área cultivada por esta cultura no estado, demonstrando um potencial para o aumento da produção, o que tem levado à necessidade de mais pesquisas voltadas para o desenvolvimento da citricultura e aperfeiçoamento do manejo dos pomares, visando maior produtividade e rentabilidade ao citricultor paranaense.

A região noroeste do estado do Paraná é a mais recente na implantação da citricultura, tendo iniciado na década de 1990, a partir da instalação da primeira indústria de suco concentrado de laranja pela Cocamar Cooperativa Agroindustrial Ltda. no município de Paranavaí em 1994. Apesar de recente, a citricultura tem proporcionado um desenvolvimento tecnológico e econômico efetivo nesta região, transformando os municípios que a compõem, como o caso de Paranavaí que se tornou no maior polo produtor de laranjas-doces do estado (LEITE JÚNIOR, 2007; IBGE, 2020).

A região Noroeste compreende o maior parque citrícola do Paraná, com mais de 11.000 hectares destinados ao cultivo de laranjas-doces e uma grande indústria extratora de suco, a LDC - Louis Dreyfus Commodities Sucos S.A., responsável pelo processamento de laranjas. O suco extraído é quase que totalmente exportado na forma de suco concentrado e congelado (FCOJ), sendo o restante destinado ao mercado interno para suprir o abastecimento da indústria de sucos e refrigerantes.

Em conjunto com uma segunda indústria extratora de suco de laranja, a Cooperativa Integrada, localizada no município de Uraí no norte paranaense, produzem cerca de 50.000 toneladas de suco concentrado e congelado de laranja (FCOJ) que são exportados principalmente para Europa e Estados Unidos (LEITE JÚNIOR, 2007; IBGE, 2020).

A região noroeste é representada pelo núcleo regional de Paranaíba que engloba diversos municípios produtores de laranjas-doces, como Guairaçá e Alto Paraná. De acordo com os últimos dados da cultura no estado, 44% da área cultivada com laranjas-doces encontram-se nessa região, apresentando participação de aproximadamente 53% na produção paranaense de laranjas-doces. A produtividade é superior à média do estado de São Paulo, com 44 ton.ha<sup>-1</sup>, demonstrando o alto desempenho dessa cultura na região e a sua importância na economia estadual (IBGE, 2020).

#### 2.4 CULTIVARES DE LARANJAS-DOCES COM FOCO EM TARDIAS

As laranjas-doces são divididas comercialmente em quatro subgrupos conforme suas características, sendo elas: comum (Hamlin, Iapar 73, Pera, Valência, Natal, Rubi, Westin e Folha Murcha); de umbigo (Bahia, Baianinha, Washington Navel, Navelina, Navelate, Lane Late e Mars Navel); sanguíneas (Moro, Sanguinelli, Sanguinello, Tarocco e Sanguínea); e de baixa acidez (Lima, Piralima, Lima Tardia e Serra d'água) (ALBRIGO et al., 2019). As laranjas comuns são destinadas tanto para consumo '*in natura*' como para o processamento industrial para produção de sucos e óleos essenciais, diferentemente das de umbigo que são preferencialmente comercializadas no mercado de frutas frescas devido à presença de limonina manolactona no albedo e endocarpo dos frutos (SIQUEIRA; SALOMÃO, 2017), conferindo sabor amargo ao suco após a sua oxidação (ALBRIGO et al., 2019). A maioria das cultivares de laranjas-doces teve sua origem em mutações somáticas (MOURÃO FILHO et al., 2008).

As laranjas-doces de cada subgrupo podem ainda ser agrupadas de acordo com sua época de maturação, compreendendo maturação precoce, meia-estação ou tardia (SIQUEIRA; SALOMÃO, 2017). As precoces atingem maturação entre 6 e 9 meses após a antese, como é o caso das laranjas Hamlin, Westin e Iapar 73. As laranjas de meia-estação, principalmente os clones de Pera, estão aptas para serem

colhidas entre 9 e 12 meses após a antese, enquanto as cultivares tardias atingem ponto de colheita entre 12 e 18 meses após a antese, como as laranjas Valência, Natal e Folha Murcha (POZZAN; TRIBONI, 2005; ALBRIGO et al., 2019). O período de maturação depende das condições de desenvolvimento dos frutos, que estão relacionados a fatores climáticos, e dos critérios estabelecido para definir a maturação.

As cultivares comercialmente disponíveis de laranjas-doces também podem ser classificadas com base na ausência (*'seedless'*) ou presença de sementes nos frutos, variando de sem sementes (0–8 sementes), moderadamente com sementes (9–15 sementes) a muitas sementes (>15 sementes) (ALBRIGO et al., 2019). Embora algum cultivares com sementes sejam destinadas para o consumo doméstico de frutos de mesa, as cultivares comerciais mais importantes no Brasil como Valência, Pera e as laranjas de umbigo são comercialmente classificadas como sem sementes.

O estabelecimento de uma fonte confiável de material de plantio é essencial para o sucesso da citricultura moderna, pois a disseminação de mudas doentes ou geneticamente inferiores pode ter efeitos prejudiciais (ALBRIGO et al., 2019). Os principais países produtores de citros detêm regulamentações rigorosas para produção de material de propagação e mudas de citros, alguns já exigindo o uso de ambientes protegidos de vetores (FOCHESATO et al., 2007; CARVALHO et al., 2019a). Assim, o emprego de material de propagação aos viveiristas e das mudas de citros por eles produzidas para os produtores deve ser registrado e regulamentado pelos órgãos responsáveis de cada região e país. No Paraná, os viveiros devem ser credenciados junto ao Ministério da Agricultura, Pecuária e Abastecimento (MAPA) e à Agência de Defesa Agropecuária do Paraná (ADAPAR).

As cultivares de citros permitidas para cultivo no estado do Paraná devem, ainda, apresentar tolerância ou resistência à bactéria *Xcc*, praga quarentenária A2, agente causal do cancro cítrico (PARANÁ, 2021). De acordo com as resoluções do Paraná (PARANÁ, 2021), as laranjas-doces Pera e Folha Murcha foram permitidas para o cultivo no estado somente a partir de 1985, com base na resolução 038/85 da Secretaria de Estado da Agricultura e do Abastecimento (SEAB). Em 1987, com a resolução 066/87, também da SEAB, foram liberadas para cultivo as laranjas doces Valência e Navelina e, em 1992, foi inserida a IAPAR 73 com a resolução 198/94 da SEAB. Já em 2004, houve a inserção de mais quatro cultivares de laranjas-doces, a Salustiana, Shamouti, IPR Cadenera e IPR Jaffa. Mais recentemente, em 2015, a

laranja-doce Natal foi também liberada para o cultivo no Estado através da portaria 176/2015 (PARANÁ, 2021).

Apesar da diversidade de materiais disponíveis de laranjas-doces para cultivo no Paraná, os pomares são constituídos predominantemente por quatro variedades, sendo elas IAPAR 73, Pera, Folha Murcha e Valência (STENZEL et al., 2005; AULER et al., 2014). No entanto, o predomínio de poucas cultivares de laranjas-doces nos pomares pode aumentar o risco de problemas bióticos e abióticos devido à homogeneidade genética no campo. Assim, há uma constante necessidade de avaliar cultivares potenciais de laranjas-doces para o estado. Dentre as seleções de laranja-doce estudadas no presente estudo, encontram-se alguns de cultivo já tradicional, como Valência, Natal, Folha Murcha e Charmute de Brotas.

#### 2.4.1 Laranja-Doce Valência

A Valência tem se tornado a cultivar de laranja-doce de maior importância no mundo, sendo amplamente cultivada nas principais regiões produtoras de citros (ALBRIGO et al., 2019). As plantas são vigorosas e relativamente tolerantes ao cancro cítrico (LEITE JÚNIOR, 1992), de porte alto e copa arredondada (BASTOS et al., 2014). As plantas de Valência têm boa produtividade variando de moderada (40–50 t.ha<sup>-1</sup>) a alta (> 60 t.ha<sup>-1</sup>) em algumas regiões de cultivo (ALBRIGO et al., 2019).

Os frutos de Valência geralmente amadurecem de fevereiro a outubro no hemisfério norte e de agosto a abril no hemisfério sul, e permanecem na planta após maduros sem grandes perdas na qualidade. No entanto, é interessante ressaltar que as altas temperaturas da primavera e a umidade adequada do solo podem estimular a síntese de clorofila nos frutos maduros de Valência, revertendo a coloração laranja para verde, um processo denominado '*re-greening*' ou reverdecimento (ALBRIGO, et al., 2019). Neste caso, os cromoplastos reverterem para cloroplastos (THOMSON et al., 1967). Embora possua amplo espectro de adaptação climática, a laranja Valência apresenta exigência térmica bastante alta para a maturação dos frutos (LEITE JÚNIOR, 1992). Por exemplo, os frutos somente atingem qualidades comerciais adequadas nas regiões mais quentes do estado do Paraná (LEITE JÚNIOR, 1992). Nessas condições, a cultivar tem boa produtividade, produzindo frutos com qualidade e tamanho para a indústria de sucos e para o mercado interno e externo de frutos de mesa (LEITE JÚNIOR, 1992; BASTOS et al., 2014).

Os frutos de Valência são de tamanho médio, esféricos a oblongos, de coloração amarelo-alaranjado e casca lisa, apresentando poucas sementes e suco abundante, ligeiramente ácido (LADANIYA, 2008; BASTOS et al., 2014). A qualidade do suco é excelente, principalmente devido ao desenvolvimento da coloração amarela intensa e aos altos níveis de sólidos solúveis totais (SST), qualidades estas altamente desejadas pela indústria de processamento de sucos, que a utiliza em misturas com sucos de qualidade inferior (ALBRIGO et al., 2019).

Várias seleções de Valencia são apreciadas no mercado de frutas frescas e utilizados na indústria de processamento de sucos, incluindo Olinda, Frost, Campbell, Midnight, Delta, Cutter, Berry, Rhode Red, Folha Murcha, Whit's Late, Chafeei Late, Late Fla., entre outros (HODGSON et al., 1967; OLIVEIRA et al., 2008; CASTLE; BALDWIN, 2011; BARRY et al., 2020). Esses genótipos diferem principalmente no formato do fruto, qualidade e espessura da casca, bem como no período de maturação. No entanto, todas as frutas são muito semelhantes morfologicamente e todas amadurecem no final da estação (ALBRIGO et al., 2019).

A seleção original da cultivar Valência, também conhecida como Valencia Late, provavelmente ocorreu em Portugal (UCR, 2021). A cultivar foi levada das ilhas dos Açores, Portugal, para a Inglaterra e renomeada de Excelsior em 1865 (BARRY et al., 2020; UCR, 2021). Posteriormente, foi levada para Nova York, Estados Unidos, e depois para a Califórnia em 1876, onde passou a ser chamada de Rivers Late (BARRY et al., 2020; UCR, 2021). Em 1887, o nome da seleção foi alterado para Valencia Late, conforme sugerido por um visitante espanhol que a considerou semelhante a uma cultivar de maturação tardia produzida na região de Valência, Espanha (BARRY et al., 2020).

A seleção Frost Valencia teve origem em 1915, obtida por H. B. Frost no Citrus Research Center em Riverside, CA, Estados Unidos, e liberada para cultivo em 1952. As plantas são vigorosas e produtivas, sendo o clone nucelar mais antigo de Valência (HODGSON et al., 1967; UCR, 2021). A seleção da Olinda Valencia também ocorreu na Califórnia, Riverside, encontrado e selecionado em 1939 por H. J. Webber e L. D. Batchelor em um pomar localizado em Olinda, CA, Estados Unidos. Esta seleção foi liberada para cultivo em 1957 (HODGSON et al., 1967).

A Cutter Valencia foi selecionada em um pomar comercial de Valência pertencente ao produtor J. C. Cutter em Riverside, CA, em 1935 por H. S. Fawcett do Citrus Research Center, de Riverside, CA. A Cutter Valencia foi liberada para cultivo

apenas em 1957. Este clone é bastante vigoroso, porém mais lento para iniciar a produção de seus frutos do que as outras seleções de Valência (HODGSON et al., 1967).

A seleção Campbell Valencia foi selecionada em 1942, em pomar de laranja Early Campbell nas proximidades de Santa Ana, CA, Estados Unidos, estabelecido em 1871 (UCR, 2021). Durante as últimas décadas, a Campbell Valencia tem sido muito plantada em pomares da Califórnia (UCR, 2021). Mais recentemente, a linhagem nucelar da Campbell Valencia alcançou grande popularidade entre os citricultores (UCR, 2021; USDA, 2021).

#### 2.4.2 Laranja-Doce Natal

A laranja Natal é uma cultivar brasileira de origem desconhecida e se assemelha à Valência em muitas de suas características (ALBRIGO et al., 2019). É uma cultivar de maturação tardia amplamente cultivada no Brasil, principalmente para processamento industrial para produção de sucos (ALBRIGO et al., 2019). A planta é vigorosa e produtiva, com porte alto e copa compacta (BASTOS et al., 2014), produzindo frutos de tamanho médio, formato globoso, com poucas sementes, polpa de coloração amarelo-claro, casca finamente granulada e cor amarela-alaranjada (LADANIYA, 2008). Os frutos amadurecem em época próxima do Natal no Brasil. Cabe salientar que o período de colheita é mais amplo do que o da Valência (BARRY et al., 2020). No entanto, esta cultivar é mais susceptível à podridão floral dos citros (PFC), uma doença causada pelos fungos *Colletotrichum acutatum* e *C. gloeosporioides* (ALBRIGO et al., 2019).

#### 2.4.3 Laranja-Doce Folha Murcha

A cultivar Folha Murcha também tem ganhado destaque na citricultura brasileira devido, principalmente, à sua produtividade e à maturação tardia, época de alta demanda por laranjas (STENZEL et al., 2005; AZEVEDO et al., 2015). Essa cultivar teve origem provavelmente em mutação espontânea da Valência, sendo selecionada em Araruama, RJ, Brasil. Foi introduzida pelo Banco Ativo de Germoplasma de Citros do Centro APTA Citros Sylvio Moreira do Instituto Agrônomo de Campinas na década de 1940 (TEÓFILO SOBRINHO et al., 1994).

Esta cultivar é caracterizada por apresentar folhas permanentemente enroladas ou retorcidas, característica esta que lhe garantiu o nome de Folha Murcha (STUCHI; DONADIO, 2000). Também é denominada como Valência Folha Murcha, existindo variantes de outras cultivares com esta característica de folhas, como a Natal Folha Murcha (NOVELLI et al., 2006) e a Seleta Folha Murcha (PAIVA et al., 1993). As plantas apresentam porte médio e copa arredondada, com elevada resistência ao cancro cítrico (LEITE JÚNIOR, 1992; BASTOS et al., 2014).

As plantas de Folha Murcha apresentam um desenvolvimento vegetativo menos vigoroso quando comparado com outras cultivares comerciais de laranja-doce, como Valência e Natal, que chegam a apresentar volumes de copas superiores a 20 m<sup>3</sup> (STUCHI; DONADIO, 2000). Com período de colheita de frutos de novembro até março (CARVALHO et al., 2019a). Azevedo et al. (2015) observaram que o espaçamento mais adensado proporcionou uma maior produtividade de pomar com Folha Murcha enxertada em limão Cravo nas condições edafoclimáticas de Cordeirópolis, SP, Brasil. Plantas menos vigorosas são mais indicadas para o adensamento de plantio, pois proporcionam um melhor aproveitamento da fertilização, maior eficiência nos tratamentos fitossanitários, e maior facilidade na colheita e manejos, tornando-se mais eficientes (STUCHI et al., 2003).

A produtividade desta cultivar é dependente do material utilizado, pois existem vários clones de Folha Murcha, sendo alguns de baixa produtividade (PACE et al., 1981). Stenzel et al. (2005), avaliando o desempenho agrônomo da Folha Murcha enxertada em sete porta-enxertos, observaram produções superiores a 100 kg em plantas adultas sobre tangerina Cleópatra (*C. reshni* Hort. ex Tanaka) e limão Rugoso da África (*C. jambhiri* Lush.) nas condições edafoclimáticas de Paranavaí, PR, Brasil.

Os frutos dessa cultivar são de excelente qualidade, pois apresentam poucas sementes (PACE et al., 1981), possuem coloração alaranjada e casca lisa, com alto conteúdo de suco e baixa acidez, podendo ser direcionados tanto para a indústria quanto para o mercado de citros de mesa (BASTOS et al., 2014), qualidades estas que se assemelham a Valência e Natal (STUCHI; DONADIO, 2000). Os frutos de plantas cultivadas na região de Londrina, PR, Brasil, apresentam em média 191 g, com rendimento de suco de aproximadamente 51%, teores de sólidos solúveis totais (SST) de 10 °Brix, acidez titulável (AT) de 0,81% e índice de maturação de 12,4 (SST/AT) (LEITE JÚNIOR, 1992).

#### 2.4.4 Laranja-Doce Charmute de Brotas

A Charmute de Brotas vem sendo cultivada na região de Brotas, SP, Brasil, há alguns anos. Esta cultivar tem origem desconhecida, mas teria sido selecionada no município de Engenheiro Coelho, SP, Brasil (NASCIMENTO et al., 2005). Ela se caracteriza por altas produções de frutos de elevada qualidade, baixo número de sementes (~3), alto teor de sólidos solúveis totais (SST) com maturação no período de novembro a janeiro, sendo que a colheita pode se estender de outubro a abril. Uma característica importante é que os frutos permanecem nas plantas por um longo período sem perder as qualidades organolépticas (NASCIMENTO et al., 2005). Tem boa aceitação tanto para o consumo *'in natura'* quanto para a indústria de processamento de produção de sucos (NASCIMENTO et al., 2005).

Um estudo realizado em Brotas, SP, Brasil, em 2004 mostrou que os frutos de Charmute de Brotas, Valência e Folha Murcha não diferiram entre si quanto ao peso de fruto e rendimento de suco (NASCIMENTO et al., 2005). No entanto, os frutos da laranja Charmute de Brotas apresentaram melhor qualidade em novembro que os frutos de Valencia e Folha Murcha, indicando uma maturação mais precoces dos frutos dessa cultivar em relação as outras duas cultivares analisadas. A laranja Charmute de Brotas apresentou também maior teor SST por caixa de 40,8 kg de frutos no período de novembro a janeiro, tornando-os interessante para a produção de suco de laranja concentrado congelado (FCOJ) e não concentrado (NFC). Nascimento et al. (2005) relatam que a Charmute de Brotas se assemelha aos clones de Valência Delta e Midnight, amplamente cultivadas na Austrália e África do Sul, enquanto Hodgson (1967) menciona sua similaridade a cultivar israelense Shamouti.

#### 2.5 DESENVOLVIMENTO VEGETATIVO E PRODUÇÃO DE CITROS

O ciclo de vida de uma planta de citros pode ser dividido em quatro distintas fases, sendo elas propagação, formação da planta, produção de frutos e senescência, em que a duração de cada fase é dependente de diversos fatores como cultivar copa, porta-enxerto, clima, solo, manejo do pomar e incidência de pragas e doenças.

A fase de propagação ocorre entre a sementeira do porta-enxerto, enxertia copa/porta-enxerto e plantio das mudas enxertadas no campo. A duração do período de produção da muda é variável, pois depende do sistema de produção utilizado,

porta-enxerto e manejos como adubação, irrigação e controle de insetos pragas e doenças (SIQUEIRA; SALOMÃO, 2017). Ao todo, esse processo pode levar até 12 meses e se torna um gargalo quando a demanda por mudas é grande (VASHISTH et al., 2020). Por outro lado, os citricultores precisam avaliar suas necessidades com antecedência e podem ter que esperar pelo menos um ano para iniciar novos plantios.

Após o plantio das mudas no campo, a fase de formação da planta é iniciada, pois ela apresenta crescimento ativo nesta fase, formando toda sua arquitetura vegetativa, incluindo raízes, tronco, ramos e folhas, para sustentar a produção futura. As mudas enxertadas com gemas de plantas adultas iniciam suas produções a partir do segundo e terceiro ano após o plantio. No entanto, o volume de copa é pequeno, levando a baixas produções nos primeiros anos, mas aumenta gradativamente à medida que a planta se estabelece no campo (CASTLE et al., 2010). De um modo geral, as plantas jovens produzem frutos de maior tamanho, com casca mais grossa, rugosa e com pouco rendimento de suco, diferente do tipo característico de cada espécie e cultivar (AGUSTÍ et al., 1996; PRIMO-MILLO; AGUSTÍ, 2020). O raleio de frutos nos primeiros anos de formação da planta pode ser empregado para evitar o consumo excessivo de água, nutrientes e fotoassimilados pelo dreno (frutos) em detrimento do desenvolvimento da planta. Geralmente, a duração média dessa fase é de quatro a cinco anos (SIQUEIRA; SALOMÃO, 2017).

A fase de produção das plantas é normalmente iniciada entre dois e cinco anos após o plantio (CASTLE et al., 2010). As plantas atingem produção máxima aos oito e 10 anos após o plantio, mantendo-se estável por vários anos. O crescimento vegetativo da planta cítrica é pequeno nesse período, ocorrendo apenas manutenção de folhas, ramos e raízes. Os pomares de citros, quando bem manejados, possuem potencial para produzir comercialmente por mais de 30 anos. Após a fase de produção, as plantas entram no estágio de senescência com redução gradual da produção de frutos (SIQUEIRA; SALOMÃO, 2017). Atualmente, a vida útil dos pomares cítricos nas principais regiões produtoras tem sido reduzida devido à alta incidência de doenças e pragas, com destaque para o HLB. Esta doença tem causado grandes mudanças na citricultura mundial (VASHISTH et al., 2020). Dessa maneira, planejar pomares com vida útil mais curta passa a ser uma estratégia a ser considerada visando a economicidade (STUCHI, 2005). O adensamento de plantio pode ser uma alternativa simples na convivência com o HLB, pois a alta densidade de plantas permite um retorno financeiro mais rápido do investimento inicial e pode

compensar a redução da população decorrente da eliminação de plantas doentes, e a não necessidade de replantio. Ainda, esta estratégia evita a presença de plantas mais jovens entre árvores já estabelecidas, fator favorável ao aumento da incidência da doença (AZEVEDO et al., 2015), além de aumentar a produção de frutos por área.

## 2.6 CRESCIMENTO E DESENVOLVIMENTO DE CITROS

De acordo com Bain (1958) e outros autores (CAO et al., 2019), o crescimento e desenvolvimento do fruto cítrico é definido em três distintos estádios. O estádio I é iniciado na antese com abertura de  $\geq 50\%$  dos botões florais. Esta primeira etapa do desenvolvimento do fruto é concluída quando ocorre a polinização e a fertilização do óvulo (TADEO et al., 2020). Este período tem duração de aproximadamente um a dois meses. Nesta fase, os pequenos frutos estão vulneráveis a danos superficiais causados por ventos e insetos, devido à ausência da cutícula que é uma camada que protege contra ações externas (LADANIYA, 2008). Este problema é relativamente maior em regiões tropicais e subtropicais onde a pluviosidade é intensificada no período pós-floração, o que facilita infecções superficiais e ataque de pragas (LADANIYA, 2008). Este período é reconhecido, ainda, pelo crescimento lento do fruto, porém com uma alta taxa de divisão celular, na qual o flavedo sofre grande aumento de espessura e há diferenciação e crescimento das vesículas de suco (TADEO et al., 2020).

O estádio II é marcado pelo rápido crescimento do fruto devido à expansão celular e acúmulo de água nas vesículas de suco. Aumento significativo do tamanho e peso dos frutos são observados nesse estádio, assim como a formação de solutos orgânicos (LADANIYA, 2008; TADEO et al., 2020). Estes solutos são inicialmente ricos em ácidos, mas pobres em açúcares, especialmente para o grupo das laranjas-doces. No entanto, esta relação é invertida com a maturação dos frutos, quando o conteúdo de açúcares aumenta e o de ácidos diminui (ALFEREZ et al., 2021). A época de maturação dos frutos para as diversas cultivares é definida neste estádio, em que os frutos permanecem por um maior período (SIQUEIRA; SALOMÃO, 2017). A duração dessa fase varia de acordo com a variedade copa, porta-enxerto, clima, práticas culturais e outros fatores, podendo ser de 2 a 10 meses (SIQUEIRA; SALOMÃO, 2017). Para as cultivares precoces, essa fase tem duração de até 2

meses, enquanto para as tardias, a duração varia de 5 a 10 meses (AGUSTÍ et al., 1996). Em regiões subtropicais, onde as temperaturas durante o inverno são mais baixas, a duração do estágio II é maior que nas regiões tropicais, sendo esta a razão pela qual os frutos de uma mesma variedade cultivada em diferentes regiões são colhidos em épocas distintas (SIQUEIRA; SALOMÃO, 2017). Tadeo et al. (2008) complementam que na transição do estágio I para o II, o desenvolvimento dos frutos alterna do metabolismo básico de "utilização" para o de "armazenamento".

A maturação dos frutos se completa no estágio III, quando o crescimento dos frutos cessa e picos de reações bioquímicas são iniciados (CAO et al., 2019). Esse estágio tem duração de 2 a 3 meses, dependendo da cultivar (ALBRIGO et al., 2019). Durante essa fase, os frutos sofrem processo de maturação não-climatérica, caracterizado pela ausência do pico da taxa de respiração e liberação do etileno nos tecidos (ERICKSON, 1968). Nesse estágio, ocorre mudança na coloração do flavedo devido à degradação enzimática da clorofila e biossíntese de carotenoides (SARTORI et al., 2002; LADANIYA, 2008). Esse estágio é caracterizado, ainda, pelo aumento significativo dos teores de SST e compostos nitrogenados, principalmente aminoácidos, e redução substancial de ácidos orgânicos, em destaque o cítrico (ERICKSON, 1968; AGUSTÍ et al., 1995). Esta combinação resulta nos atributos organolépticos característicos dos frutos cítricos (ALFEREZ et al., 2021). Portanto, os frutos cítricos atingem suas propriedades físicas, como tamanho, forma, cor e textura, e químicas, como sabor e aroma, nos estágios II e III. Nesses estágios, os frutos também adquirem o valor nutricional final típico de cada espécie, incluindo quantidade de nutrientes minerais, antioxidantes e vitaminas (TADEO et al., 2020).

A duração completa do processo de crescimento e desenvolvimento dos frutos depende das condições edafoclimáticas, assim como das espécies e variedades. Com isso, de um modo geral, o período entre a floração e a maturação completa dos frutos de citros varia de 6 a 18 meses, dependendo dos fatores mencionados anteriormente (LADANIYA, 2008; SIQUEIRA; SALOMÃO, 2017; TADEO et al., 2020). Para as cultivares precoces, como as laranjas Hamlin e algumas de umbigo, a colheita normalmente inicia-se de 6 a 7 meses após a floração; enquanto para as cultivares tardias, como a Valência, a colheita é apenas realizada além de 12 meses após o florescimento. Interessantemente, as laranjas de maturação tardia, assim como a laranja Pera e os pomelos, apresentam duas safras ao mesmo tempo. Ambos os frutos, imaturos e botões florais da nova safra como os frutos maduros da safra

anterior, podem ser observados na mesma planta (LADANIYA, 2008).

## 2.7 MATURAÇÃO DE CITROS

O desenvolvimento e a maturação dos frutos de citros são resultados de uma interação de processos bioquímicos e fisiológicos, governados por fatores internos, como hormônios, e externos, como clima e solo. A transição do crescimento do fruto para a maturação envolve mudanças no metabolismo de açúcares, amolecimento e coloração de diferentes tecidos do fruto (ALFEREZ et al., 2021).

Os citros são classificados como frutos não-climatéricos, ou seja, não apresentam aumento significativo do etileno e da respiração nos tecidos após a colheita (ALBRIGO et al., 2019). Assim, os frutos necessitam completar a maturação ainda nas plantas, já que não amadurecem e não mostram qualquer aumento respiratório e mudanças significativas no sabor e na composição bioquímica após a colheita (LADANIYA, 2008; ALFEREZ et al., 2021).

As laranjas-doces, tangerinas e pomelos são consideradas maduras quando o rendimento de suco e a relação entre sólidos solúveis totais (SST) e acidez tenham atingido um limite mínimo de palatabilidade (LADANIYA, 2008). Em geral, os SST em suco de laranjas são constituídos de aproximadamente 80% de açúcares, 10% de ácidos orgânicos e 10% de compostos nitrogenados (LADANIYA, 2008; LADO et al., 2014; CARVALHO et al., 2020b). Os açúcares são compostos principalmente por sacarose, o principal componente, glicose e frutose, e a sua proporção é geralmente de 2:1:1, respectivamente (LEE; COATES, 2000). A sacarose, principal fotoassimilado encontrado no floema dos citros, é fonte de carbono para o metabolismo primário celular (TADEO et al., 2020).

Os ácidos orgânicos são o segundo maior grupo de compostos naturais presentes nas laranjas-doces (SCHERER et al., 2012; CARVALHO et al., 2020b). Sua natureza e concentração são importantes nos frutos destinados tanto para o mercado de frutas frescas como para as indústrias de sucos e refrigerantes. Os ácidos orgânicos têm grande influência nas propriedades organolépticas e na estabilidade dos sucos, sendo um importante índice para determinar a autenticidade dos sucos e bebidas derivadas de sucos naturais (KELEBEK et al., 2009; SCHERER et al., 2012). No entanto, seu perfil e concentração em citros estão relacionados ao conteúdo de açúcares e são dependentes das cultivares, solo, clima e condições de estresse

(JONES, 1998; LADO et al., 2014). Em geral, os ácidos cítrico, ascórbico e málico são os ácidos orgânicos mais abundantes encontrados em laranjas-doces (LADO et al., 2014; CARVALHO et al., 2020b). De acordo com Scherer et al. (2012), os ácidos cítrico e málico são comumente usados como acidulantes para realçar as bebidas, enquanto o ácido ascórbico é utilizado como agente antioxidante.

De acordo com Bartholomew e Sinclair (1943), os SST apresentam forte correlação com a acidez total. Aumento no conteúdo de açúcares e redução progressiva da acidez é observado na fase de maturação dos frutos. Independentemente da finalidade do fruto, seja para consumo '*in natura*' ou para processamento industrial, a maturação dos frutos é determinada principalmente com base na relação açúcares/acidez ou *ratio*, conhecido também como índice de maturação.

A leitura do conteúdo de SST nos frutos é usual durante a colheita, principalmente nas áreas rurais mais remotas, onde o produtor não detém de outros meios para medir o índice de maturação dos frutos. O refratômetro portátil pode fornecer um perfil quase que exato da maturidade do fruto no pomar, expresso em °Brix (LADANIYA, 2008). A mudança na coloração dos frutos de um verde mais profundo para um amarelo-alaranjado em quase todos os frutos da planta é também um critério simples para determinar a maturação dos frutos. No entanto, este parâmetro é subjetivo podendo levar muitas vezes à uma conclusão errônea sobre a maturação dos frutos, pois cada avaliador possui certa singularidade neste tipo de avaliação, aliado ao fato que os frutos de uma mesma cultivar apresenta desenvolvimento de cor variável dependendo da região de cultivo. De acordo com Goldweber et al. (1957), o tamanho do fruto também não pode ser um critério confiável na avaliação da maturidade de citros, visto que o fruto pode permanecer pequeno, dependendo da cultivar copa e porta-enxerto, produção, disponibilidade hídrica, incidência de doenças e do estado nutricional da planta.

## 2.8 QUALIDADE FÍSICO-QUÍMICA DE CITROS

A qualidade externa e interna dos frutos de citros é modulada por diferentes fatores, incluindo clima, solo, cultivar, porta-enxerto, volume de produção, ocorrência de pragas e doenças e práticas culturais, dentre outros (ALBRIGO et al., 2019).

A cor interna e externa dos citros é uma característica indispensável para a

qualidade comercial e aceitação pelo consumidor. Dois tipos principais de pigmentos derivados de isoprenoides, os terpenoides ou terpenos, são responsáveis pela coloração dos citros: as clorofilas que conferem a coloração verde ao flavedo dos frutos imaturos e os carotenoides que atribuem o amarelo e tons de laranja e vermelho no flavedo e na polpa dos frutos maduros (LADANIYA, 2008; TADEO et al., 2020). No gênero *Citrus*, as laranjas do grupo sanguíneas são exceções, já que a coloração roxa dos frutos se deve às antocianinas, um grupo de pigmentos derivados dos flavonoides (TADEO et al., 2020). No início do desenvolvimento e crescimento do fruto, as células do flavedo apresentam altos níveis de clorofila, permitindo que o fruto produza alguns metabólitos fotossintéticos; no entanto, com o avanço da maturação natural dos frutos, a clorofila se degrada e os cloroplastos são convertidos em cromoplastos contendo pigmentos de coloração (THOMSON et al., 1967; ALBRIGO et al., 2019). De uma maneira geral, a coloração dos frutos é substancialmente influenciada por fatores exógenos, incluindo temperatura, umidade, luminosidade, solo e porta-enxerto, e fatores endógenos como as giberelinas (GAs), ácido abscísico (ABA), compostos nitrogenados e carboidratos (ALFEREZ et al., 2021).

Em regiões de clima tropical, onde as temperaturas médias permanecem altas durante todo o ano, os frutos de laranjas-doces podem apresentar qualidade interna adequada para consumo, mas permanecer com uma coloração externa esverdeada devido ao elevado nível de clorofila e baixa síntese de carotenoides, pois o conteúdo desses compostos é reduzido com temperaturas acima de 35 °C (MEREDITH; YOUNG, 1969; YOUNG; JAHN, 1972; ALBRIGO et al., 2019). Em contrapartida, os frutos de alguns cultivares de maturação tardia, como as laranjas-doces Valência e Natal, produzidas em regiões com invernos mais frios, com temperatura média abaixo de 15 °C durante sua maturação, apresentam coloração amarela uniforme devido à conversão dos cloroplastos em cromoplastos (THOMSON et al., 1967), porém com qualidade interna inadequada para o consumo devido à elevada acidez e baixos teores de açúcares (SIQUEIRA; SALOMÃO, 2017; ALBRIGO et al., 2019). Portanto, o cultivo de laranjas-doces em regiões de clima quente durante o dia e moderado à noite pode favorecer a maturação dos frutos e promover uma melhor qualidade externa e interna dos frutos, como na região do mediterrâneo.

O volume da produção tem um impacto significativo no tamanho do fruto, especialmente para tangerinas e seus híbridos que apresentam alternância de produção. A quantidade e o tamanho do fruto estão relacionados de forma distinta,

pois, quanto maior a quantidade de frutos por planta, maior a competição por fotoassimilados, resultando em frutos de menor calibre e de baixa qualidade, geralmente sem valor comercial no mercado de frutas frescas (GUARDIOLA et al., 1982). A técnica do raleio e o uso de reguladores de crescimento são práticas comuns na citricultura moderna para regular o volume de produção, principalmente em tangerinas. Outros manejos culturais, como irrigação, nutrição e poda também influenciam o tamanho do fruto, mas, no caso do excesso de irrigação, pode diluir o conteúdo de sólidos solúveis e acidez total (ALBRIGO et al., 2019).

A variação no formato do fruto geralmente não é uma grande preocupação para os produtores de frutas frescas. Entretanto, este parâmetro pode variar de acordo com as condições climáticas. Albrigo et al. (2019) relataram que o formato do fruto não é influenciado pelo abortamento de sementes, como observado em outras espécies frutíferas, exceto para os frutos produzidos em plantas afetadas pelo HLB que apresentam deformações no formato.

O rendimento de suco dos citros é geralmente expresso com base no peso. Os frutos de mesa são valorizados por seu conteúdo de suco, além de outros parâmetros qualitativos que são limitantes para a comercialização (CEAGESP, 2011). Entretanto, o teor de suco diminui com o avanço da maturação e extensão do período do fruto na planta (LADANIYA, 2008). Vários são os fatores reguladores do conteúdo de suco nos frutos, com destaque para os climáticos. Períodos de estresse hídrico podem comprometer a qualidade dos frutos durante seu desenvolvimento e maturação, promovendo a perda de água e formação de granulação nos frutos.

Assim como o rendimento de suco, o teor de SST em frutos de citros depende da cultivar copa, porta-enxerto, tratos culturais, solo, clima e outros. O efeito do clima sobre esta variável é muito importante, sendo responsável pelas variações no índice de maturação e relação açúcares/acidez em diferentes anos e locais. De uma maneira geral, os frutos produzidos em regiões com temperaturas mais elevadas durante o período de maturação apresentam maiores teores de açúcares do que frutos provenientes de regiões de clima mais frio (SIQUEIRA; SALOMÃO, 2017). Os SST no suco podem ser aferidos a partir do índice de refração e os refratômetros portáteis ou de bancada são calibrados para fornecer °Brix ou valores percentuais de SST diretamente (LADANIYA, 2008).

A porcentagem de acidez total do suco de citros é outro importante parâmetro qualitativo. A acidez total é resultado da presença de diferentes ácidos orgânicos, com

predomínio do ácido cítrico que representa aproximadamente 80 a 85% da acidez total do suco (LADANIYA, 2008; CARVALHO et al. 2020b). O conteúdo de ácido orgânico se acumula durante o início do desenvolvimento do fruto, mais precisamente durante o estágio II (HUSSAIN et al., 2017; LADO et al., 2018). A acidez atinge valores máximos quando o volume do fruto está em torno de 50% do seu valor final e, então, diminui gradativamente à medida que o fruto amadurece (LADO et al., 2018; TADEO et al., 2020). A redução na concentração de ácidos durante a maturação dos frutos é devida principalmente à sua diluição favorecida pelo aumento do volume de suco (SIQUEIRA; SALOMÃO, 2017). Para a maioria das cultivares de citros, o maior aumento no teor de sólidos solúveis ocorre durante a fase de declínio da acidez e maturação do fruto (TADEO et al., 2020).

A acidez total em suco de citros pode ser determinada aplicando 0,1 N NaOH (TING; ROUSEFF, 1986), estabelecida como ácido cítrico anidro e expressa como porcentagem em peso (AOAC, 2019; LADANIYA, 2008). A fenolftaleína é geralmente utilizada como indicador visual do ponto final de titulação ou ponto de 'viragem', na qual a solução se torna rósea. O ponto final de titulação também pode ser determinado com pHmêtro (LADANIYA, 2008). Laranjas e tangerinas devem apresentar entre 0,5 e 1,0% de ácido cítrico quando maduras (PEREIRA et al., 2006), pois valores divergentes a esta faixa podem ser responsáveis por sabor insípido ou muito ácido dos frutos, tornando-os impróprios à comercialização em alguns casos.

A doçura ou acidez relativa dos citros é definida pela relação entre açúcares e ácidos, designado como *ratio* ou índice de maturação. Como a maioria dos sólidos solúveis em laranjas, tangerinas, toranjas, pomelos e seus híbridos são constituídos por açúcares, a proporção entre sólidos solúveis e ácidos é utilizada por conveniência (LADANIYA, 2008). Esta proporção fornece uma medida relativa da maturidade dos frutos e este padrão varia conforme a preferência dos consumidores nas diferentes regiões. Portanto, o *ratio* adequado depende do destino da produção, da cultivar, da época do ano e da preferência dos consumidores, pois cada local de destino apresenta suas tradições e peculiaridades. Valores iguais a 9,5 de °Brix/acidez são considerados como mínimo aceitável para laranjas-doces e tangerinas de mesa no Brasil (CEAGESP, 2011). Já frutos com proporções superiores a 19 normalmente não são apreciadas pelos consumidores, pois perdem o aroma e o sabor, tornando-se 'plano', muito doce e com pouco ácido (LADANIYA, 2008). Esta mudança é resultado principalmente da hidrólise dos ésteres que contribuem significativamente no sabor e

aroma dos sucos cítricos (PLOTTO et al., 2008; WANG et al., 2020).

Segundo Jones e Cree (1965), as laranjas-doces apresentam qualidade ideal para consumo '*in natura*' quando a relação açúcares/acidez atinge entre 8,8 e 15,4. Teores mais elevados de açúcares, desde que adequadamente equilibrados com os teores de ácidos, geralmente resultam em frutos com sabor mais agradável (SIQUEIRA; SALOMÃO, 2017). No Brasil e na Flórida (EUA), o processamento industrial de suco de laranjas é iniciado quando o *ratio* atinge 12 e 13 (KIMBALL, 1991; MARCHI, 1993; POZZAN; TRIBONI, 2005).

O rendimento industrial é um importante parâmetro qualitativo utilizado pelas indústrias de processamento de laranjas-doces para produção de sucos. O rendimento industrial é calculado com base no índice tecnológico ou quantidade de SST no suco obtido dos frutos de uma caixa-padrão industrial de citros que é de 40,8 kg (DI GIORGI et al., 1990). O índice tecnológico pode ser utilizado como indicador da qualidade do fruto (SINCLAIR, 1984; SOULE; GRIERSON, 1986), além de demonstrar a rentabilidade do pomar.

Por outro lado, a qualidade de laranjas-doces destinada ao mercado '*in natura*' esta principalmente relacionada ao tamanho, relação açúcares/acidez, aparência, ausência de defeitos, forma e cor. Entretanto, a maioria dos países têm seus próprios padrões e legislação para os mercados domésticos de citros de mesa. No Brasil, a qualidade dos citros de mesa é determinada com base nas Normas de Classificação de Citros de Mesa, regulamentada pelo Programa Brasileiro para Modernização da Horticultura. Este programa foi criado em 1997 por decisão das câmaras setoriais de frutas e de hortaliças da Secretaria da Agricultura de Abastecimento do estado de São Paulo. A Companhia de Entrepostos e Armazéns Gerais de São Paulo (CEAGESP), através do Centro de Qualidade em Horticultura, é a sua gestora (CEAGESP, 2011).

## 2.9 QUALIDADE SENSORIAL DE CITROS

Assim como a qualidade físico-química dos frutos, a qualidade sensorial é importante para estabelecer uma descrição completa dos atributos qualitativos dos frutos, visando o seu desempenho para com os potenciais consumidores. No entanto, técnicas abordando os aspectos sensoriais, como a análise sensorial, não têm sido muito empregadas para determinar o desempenho e a aceitação de frutos antes da

comercialização. Dessa maneira, o consumo se baseia apenas nas características físico-químicas, obtendo-se um perfil incompleto do produto (CASTRO et al., 2013; TARANCÓN et al., 2021). Isto gera a necessidade de mais estudos relacionados aos atributos sensoriais para compreender o hábito do consumidor.

Mediante este contexto, a análise sensorial consiste em uma ferramenta importante para a ciência e a indústria de alimentos, pois possibilita a caracterização do produto pela interpretação de suas propriedades qualitativas com base nos sentidos humanos, como visão, olfato, gosto, tato e audição. Isto é fundamental para compreender a relação entre as propriedades do alimento e o comportamento humano (STONE; SIDEL, 2004; NAES et al., 2010). Stolzenbach et al. (2016) complementam que é necessária a compreensão dos parâmetros que influenciam a intenção de compra pelo gosto do consumidor para garantir uma introdução bem-sucedida e competitiva do produto no mercado. Além do gosto do consumidor, esses parâmetros estão também relacionados aos significados, ideias, compreensão, pensamentos e noções dos consumidores. Desta maneira, o provador pode entender e atribuir significado às percepções de acordo com seus conceitos (STONE; SIDEL, 2004; STOLZENBACH et al., 2016). A análise sensorial permite avaliar a aceitabilidade de citros quanto aos parâmetros qualitativos relacionados ao tamanho, textura e coloração interna e externa, aroma, sabor, suculência e preferência (MALGARIM et al., 2007a; MALGARIM et al., 2009; CASTRO et al., 2013; PACHECO et al., 2017; TARANCÓN et al., 2020, 2021; CARVALHO et al., 2020a).

## 2.10 PÓS-COLHEITA DE CITROS

Os frutos, quando em plena maturação, expressam seu maior potencial de qualidade. Cabe salientar que o estágio de maturação na colheita é determinante na manutenção da qualidade pós-colheita (MOURA et al., 2013). O estágio de maturação mais adequado para colheita depende da interação das características fisiológicas intrínsecas a cada espécie e cultivar e da tecnologia de conservação pós-colheita a ser empregada. Após a colheita, a qualidade dos citros tende a decrescer pelo processo natural de senescência (CARVALHO et al., 2020a). A partir disso, o tempo de vida útil do fruto, também conhecido como “vida de prateleira”, é variável dependendo da espécie e do tipo de acondicionamento empregado (KAFKAS et al., 2007; SANTOS et al., 2006). Técnicas como o uso do armazenamento refrigerado

(cadeia de frio), tratamento térmico, irradiação com raios UV e gama, embalagens e ambientes de atmosfera modificada ( $O_2/CO_2$ ), tratamento químico, controle de umidade, recobrimento com ceras naturais ou sintéticas, dentre outros, podem ser empregadas no processo de conservação pós-colheita de citros (LADANIYA, 2008; BADIA-MELIS et al., 2018; BARMAN et al., 2019; GIL; BEAUDRY, 2019).

Estima-se que mais de 30% dos alimentos produzidos no mundo é desperdiçado por ano, devido a falhas na cadeia de suprimentos de alimentos desde o campo até o consumo final (FAO, 2011). As perdas podem variar de 5 a 10% nos países desenvolvidos durante o manuseio de frutas frescas, mas aumentam para 30% ou mais nos países subdesenvolvidos ou em desenvolvimento, onde os alimentos são perdidos principalmente na pós-colheita (LADANIYA, 2008; FAO, 2011). Esta diferença está relacionada às condições limitadas de infraestruturas e refrigeração presente nos países de baixa renda, assim como à falta de conhecimento quanto ao manuseio de produtos perecíveis (GOEDHALS-GERBER; KHUMALO, 2020). Diferentemente, os países desenvolvidos detêm de tecnologias mais eficientes para tratamento pós-colheita de frutos, onde estes entram na cadeia de frio na colheita e permanecem sob baixa temperatura até o consumo final do produto (TARANCÓN et al., 2021). Investimentos em infraestrutura e cadeia de frio são limitados nos países de baixa renda, onde políticas com esforços para educação e conscientização sobre práticas pós-colheitas e padrões sanitários, com gestão de alimentos seguros e não perigosos, necessitam ser aperfeiçoados.

Dessa maneira, é fundamental o desenvolvimento de práticas pós-colheita que visam estabelecer condições que retardem a senescência dos frutos, mantendo a qualidade, prolongando a vida útil, garantindo o máximo lucro econômico e, o mais importante, evitando perdas na cadeia produtiva de alimentos. Durante o armazenamento, a redução das atividades de respiração e transpiração são fundamentais (PALIYATH et al., 2008; MOURA et al., 2013). Malgarim et al. (2007b) complementam, ainda, que as características atribuídas à qualidade do fruto, como aparência, coloração, sabor e textura, podem ser mantidas com o uso de práticas pré e/ou pós-colheita.

Os revestimentos de frutos com ceras e resinas de origem natural são reconhecidos como alternativas sustentáveis para reduzir as perdas pós-colheita e, ao mesmo tempo, prolongar a vida útil de frutos (GUTIÉRREZ et al., 2019). Alguns revestimentos naturais extraídos de plantas como carnaúba [*Copernicia prunifera*

(Mill.) HE Moore] (MOTAMEDI et al., 2018; DE FREITAS et al., 2019), cana-de-açúcar [*Saccharum officinarum* (L.)] (JONGLERTJUNYA et al., 2014), soja [*Glycine max* (L.) Merrill] (FEI et al., 2021), coco [*Cocos nucifera* (L.)] (NASRIN et al., 2020), candelilla [*Euphorbia antisyphilitica* Zucc.] (HAGENMAIER, 2000), pinheiro [*Pinus* spp.] (BARMAN et al., 2019), fécula de mandioca [*Manihot esculenta* Crantz] (LIMA et al., 2012), assim como a cera de abelha melífera [*Apis mellifera* (L.)] (BASWAL et al., 2020; NASRIN et al., 2020), podem ser aplicadas em frutos com o objetivo de intensificar o brilho e a aparência, reduzir a perda de peso, a respiração e a transpiração normal dos frutos, sem provocar condições de anaerobiose (MANNHEIN; SOFFER, 1996; LIMA et al., 2012). Estes materiais podem ser aderidos no manejo pós-colheita de frutos nos diversos países produtores e exportadores de frutas frescas, principalmente os de baixa renda, onde a matéria-prima é disponível. A cera de carnaúba e a resina de madeira (resina vegetal ou colofônia) são permitidas como ingredientes não sintéticos em revestimentos de citros orgânicos de mesa (LADANIYA, 2008).

A cera de carnaúba é uma cera vegetal que apresenta diversas possibilidades de aplicações, sendo a matéria-prima de inúmeros produtos industriais (DE FREITAS et al., 2019). Ela é extraída das folhas da palmeira da carnaúba, de origem brasileira, que se encontra em condições de exploração econômica no ambiente seco da caatinga nordestina (DE CARVALHO; GOMES, 2008). Esta cera vegetal é amplamente utilizada em composições comerciais de revestimento de frutas para aumentar a durabilidade e o brilho (BARMAN et al., 2019; DE FREITAS et al., 2019). Malgarim et al. (2007a) complementam que a utilização do recobrimento à base de cera de carnaúba, sem diluição, intensifica o brilho do flavedo da laranja Navelina, reduzindo a perda de massa e a ocorrência de podridões, possibilitando o armazenamento refrigerado por até 90 dias, sem comprometer a qualidade sensorial das frutas. Won e Min (2018) observaram que os revestimentos com cera de carnaúba, incorporados com extrato de sementes de toranja e óleo de orégano, foram capazes de reduzir a taxa de incidência de *Penicillium italicum* Wehmer nas superfícies de tangerinas (*C. unshiu* Marc.) quando comparados ao controle. O revestimento do tangor Ortanique [*C. xsinensis* (L.) Osbeck × *C. reticulata* Blanco] com cera de carnaúba em condições de armazenamento comercial ( $22 \pm 2$  °C,  $60 \pm 5\%$  UR) também foi eficaz na preservação da qualidade sensorial e visual dos frutos por meio da redução da perda de peso e desidratação (MACHADO et al., 2012).

A resina natural é uma substância orgânica resultante do metabolismo secundário de várias espécies vegetais do gênero *Pinus* (GAILLARD et al., 2011). A colofônia é obtida da resina bruta de madeira de pinheiro após destilação da fração volátil (óleo de *Pinus* spp. e terebintina), utilizada como matéria-prima em diversas aplicações (GAILLARD et al., 2011). A colofônia e seus derivados têm importante valor na área de agroquímicos, por apresentar capacidade de inibição de doenças fúngicas, como o mofo cinzento causada por *Botrytis cinerea* (De Bary) Whetzel, uma das principais doenças de pós-colheita de frutas frescas (TAO et al., 2020). A adição de diferentes ceras e/ou resinas também é observada nas formulações comerciais dos produtos para recobrimento de frutos, podendo potencializar/otimizar suas propriedades. Portanto, o desenvolvimento de técnicas de manuseio pós-colheita é extremamente importante para manter e assegurar os parâmetros de qualidade dos frutos para que tenham boa aceitação pelo consumidor (LIMA et al., 2011).

### 3 ARTIGO A

## PERFORMANCE OF LATE-SEASON SWEET ORANGE SELECTIONS UNDER HUANGLONGBING AND CITRUS CANKER ENDEMIC CONDITION IN THE BRAZILIAN HUMID SUBTROPICAL REGION

### 3.1 ABSTRACT

The Brazilian citrus orchards are comprised by few genotypes, which increases the risk of pest and disease outbreaks. The diversification of sweet oranges (*Citrus xsinensis*) in orchards also generates off-season revenue and extend the fruit processing period. This study aimed to evaluate several horticultural traits of 19 late-season sweet orange selections under citrus canker and huanglongbing (HLB) endemic condition in northwestern Paraná state, Brazil, in a long-term field experiment. Tree size, yield, fruit quality for fresh fruit and industrial markets, estimates of tree density and yield, and citrus canker and HLB incidences were assessed. The experimental design was a randomized block with three replicates and five trees per unit. The orchard was drip-irrigated and arranged at tree spacing of 6.5 m × 4.5 m. All scions were graft-compatible with Rangpur lime (*C. xlimonia*). Valencia selections had the tallest trees and largest canopies, particularly Olinda, Frost and #121 with heights and volumes greater than 4.20 m and 43 m<sup>3</sup>, respectively. Natal África do Sul and Whit's Late Valencia trees were the most productive with cumulative yields above 640 kg per tree. Most of the selections produced fruits of excellent physicochemical quality attending the fresh fruit and industrial market requirements. All selections showed similar horticultural characteristics for the fresh market, while Natal África do Sul and Charmute de Brotas were more suitable for juice processing. Frost Valencia and Valencia Late Fla. had the highest incidence of citrus canker on fruits, above 20%, whereas IPR Folha Murcha, Natal IAC, Charmute de Brotas and some Valencia selections (Chafeei Late, Campbell 479, Campbell 294, Olinda, Mutação and Whit's Late) exhibited low incidence. At nine years, Valencia Mutação trees had high HLB incidence (93%). In contrast, Natal IAC and Folha Murcha IAC showed the lowest HLB incidence (13%). Our results revealed that Natal IAC, Folha Murcha IAC, IPR Folha Murcha, Natal Murcha, Campbell 479 Valencia and Valencia Late Fla. had the best horticultural performance in addition to low HLB incidence. Together, these late-season sweet oranges are the most advantageous selections for citrus orchard diversification under citrus canker and HLB endemic conditions in humid subtropical regions.

Keywords: *Citrus xsinensis* (L.) Osbeck; late-season cultivar; fruit quality and yield; tree size; disease incidence; orchard diversification.

### 3.2 INTRODUCTION

Brazil is the world's largest producer of sweet oranges [*Citrus xsinensis* (L.) Osbeck] accounting for around one-quarter of the global production and three-quarters of the orange juice exports (FAO, 2020; SPREEN et al., 2020; USDA-FAS Foreign

Agricultural Service, 2021). In 2020, the Brazilian production of sweet oranges was 17M tons, followed by India with 9.9M tons, China with 7.5M tons and United States with 4.8M tons (FAO, 2020). The main citrus-growing area in Brazil is in the state of São Paulo, where sweet oranges are mainly produced for juice processing (~70%), followed by Minas Gerais, Bahia, and Paraná (IBGE, 2020; SPREEN et al., 2020; USDA-FAS Foreign Agricultural Service, 2021).

In the last decades, the adoption of advanced cultural practices in the citrus orchards was critical for Brazil to maintain leadership in the global sweet orange production (CARVALHO et al., 2019a; BASSANEZI et al., 2020; FAO, 2020). These practices include irrigation, fertilization, high tree density, pest and disease control, and planting of citrus cultivars that are more productive and adapted to a wide range of environmental conditions (BOTEON; NEVES, 2005; CARVALHO et al., 2019a; SILVEIRA et al., 2020). The presence and progression of citrus diseases in orchards are frequent under the humid subtropical climate. Nevertheless, even under intense disease pressure, such as citrus tristeza virus (CTV), citrus canker, citrus variegated chlorosis (CVC), citrus black spot, leprosis and huanglongbing (HLB, a.k.a. citrus greening), the Brazilian citrus industry has adapted to a challenging scenario relying on an efficient management system (SPREEN et al., 2020; BASSANEZI et al., 2020).

Citrus canker and HLB are particularly important for the citrus industry in the state of Paraná. Historically, most of the area of Paraná was prohibited to grow citrus until the late 1980s due to the occurrence of citrus canker (*Xanthomonas citri* subsp. *citri*, *Xcc*) and the lack of efficient control measures by then (LEITE JUNIOR et al., 1987; BEHLAU, 2021). An integrated disease management program was developed to prevent and control the citrus canker in new areas across the state (LEITE JUNIOR; MOHAN, 1990). This program involves some cultural and preventive measures such as planting resistant or less susceptible citrus cultivars, establishment of windbreaks, periodic sprays of copper-based bactericides, and control of the citrus leafminer (*Phyllocnistis citrella* Stainton) (LEITE JUNIOR et al., 1987; LEITE JUNIOR; MOHAN, 1990; BEHLAU et al., 2021). Citrus cultivars have a broad range of susceptibility to citrus canker, varying from resistant to highly susceptible (AMARAL et al., 2010; VARGAS et al., 2013; CARVALHO et al., 2015). Based on previous investigations, only genotypes that show certain resistance to citrus canker are authorized for planting in Paraná, which includes the early-season sweet oranges IAPAR 73, Navelina, Salustiana, and Cadenera; the mid-season Pera, Shamouti and Jaffa; and the late-

season Valencia, Folha Murcha and Natal (IAPAR, 1992; AULER et al., 2014). Therefore, planting canker-resistant genotypes is the most efficient and inexpensive measure to control this disease (BEHLAU et al., 2021).

More recently, HLB has become the most serious threat to the citrus industry worldwide (BASSANEZI et al., 2020). The '*Candidatus Liberibacter asiaticus*' (CLAs), a phloem-limited bacterium associated with the HLB pathosystem, was first identified in Paraná in 2007 (NUNES et al., 2007; SAUER et al., 2015) and rapidly spread across the main orange-growing areas of the state (GOTTWALD, 2010; PAULA et al., 2019). Integrated management approaches for mitigating HLB have been established in Brazil such as planting of healthy nursery trees; monitoring of the citrus-growing and surroundings areas; area-wide control of the CLAs vector, the Asian citrus psyllid (ACP) (*Diaphorina citri* Kuwayama); removal of symptomatic trees; and release of ACP's parasitoids, as the *Tamarixia radiata* Waterston (NUNES et al., 2010; BASSANEZI et al., 2020).

The Brazilian orange belt is composed of a few genotypes, with Hamlin (early-season), Pera (mid-season), Valencia and Natal (late-season) being the predominant cultivars, as they are highly demanded for juice processing (CARVALHO et al., 2019a; NEVES et al., 2019). The late-season Valencia and Natal accounts for at least 55% of the planted sweet orange trees in the Brazilian orchards, as these cultivars are very productive and bear fruit of remarkable juice quality (NEVES et al., 2019). However, the limited number of cultivars in citrus orchards may favor outbreaks of pests and diseases due to the narrow genetic pool (EMMANOUILIDOU; KYRIACOU, 2017; CARVALHO et al., 2019a; CARVALHO et al., 2019b; CARVALHO et al., 2021a). Therefore, the diversification of scion and rootstock citrus genotypes is of paramount importance to improve citrus protection. Moreover, this strategy may allow growers to obtain higher profits when the fruit supply is low, enhance the options of sweet oranges for the fresh market, besides enabling the industry to extend the juice processing period.

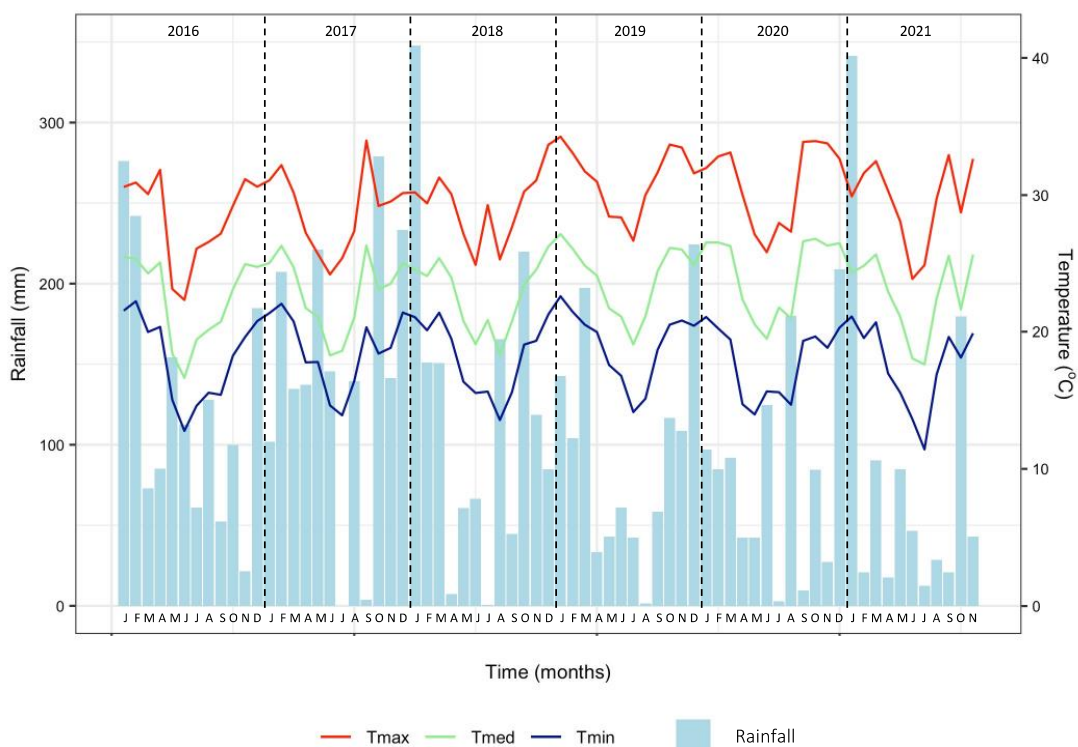
Several citrus scion and rootstock accessions have been introduced and selected in different breeding programs in Brazil. The Instituto de Desenvolvimento Rural do Paraná (IDR-Paraná) maintains a program that constantly evaluates potential citrus selections to be included in the state of Paraná, aiming at orchard diversification and production of high-quality fruits for the fresh market and processing (CARVALHO et al., 2020a; PAULA et al., 2022). The juice processing values sweet

orange cultivars that are more productive and show notable juice quality particularly total soluble solids concentration (TSS), color index and juice content (RAMOS et al., 2021), whereas the fresh market requires fruit with attractive coloration, large size, rich content of TSS, and no or few number of seeds (CAPUTO et al., 2012; CARVALHO et al., 2019a; RAMOS et al., 2021). Within this context, this study reports the evaluation of several horticultural traits of 19 late-season sweet orange selections based on a long-term field experiment established in northwestern Paraná, Brazil. The results led to a better understanding of the suitability of the selections for fresh fruit market and/or juice processing, which will contribute to a more strategic and oriented establishment of new orchards.

### 3.3 MATERIALS AND METHODS

#### 3.3.1 Field Location

The experimental orchard was conducted from 2012 to 2021 in the Experimental Station of Cocamar Cooperativa Agroindustrial in the municipality of Guairaçá, state of Paraná, southern Brazil (22° 56' 04" S, 52° 41' 08" W, 518 m of altitude). The climate of the region is humid subtropical (Cfa) according to the Köppen-Geiger climate classification, with annual maximum and minimum temperatures of 28.4 and 17.8 °C, respectively, annual average rainfall of 1,527 mm (Figure 3.3.1) and relative humidity of 69% (IDR-Paraná, 2021). The soil is a Typic Haplustox with 90% sand, 1% clay, base saturation of 20%, and pH of 3.9 in the 0–40 cm layer (LARACH, 1984).



**Figure 3.3.1** Monthly rainfall and maximum (Tmax), medium (Tmed) and minimum (Tmin) average temperatures from 2016 to 2021 in Guairaçá, state of Paraná, Brazil (Source: IDR-PARANÁ, 2021).

### 3.3.2 Plant Material

The nursery trees of 19 late-season selections of Valencia, Charmute de Brotas, Natal and Folha Murcha sweet orange cultivars were provided by the Citrus Active Germoplasm Bank of the Instituto Agrônomo de Campinas – IAC/Centro de Citricultura “Sylvio Moreira” in Cordeirópolis, state of São Paulo, Brazil, and the Instituto de Desenvolvimento Rural do Paraná – IAPAR/EMATER (IDR-Paraná) in Londrina, state of Paraná (Table 3.3.1). All trees were grafted onto the Rangpur lime [*C. xlimonia* (L.) Osbeck] rootstock, which is graft-compatible with almost all scion cultivars and adapted to a wide range of soil and climate conditions. The experimental design was a randomized block with 19 treatments, three replicates and five trees per plot. The experimental orchard was planted in December 2012 at tree spacing of 6.5 m × 4.5 m corresponding to 341 trees.ha<sup>-1</sup>. The trial also had sides planted with two-row windbreaks of *Eucalyptus grandis* (Hill) ex Maiden.

**Table 3.3.1** Origin of the 19 selections of sweet orange (*C. xsinensis*) assessed in this study.

Sweet orange selection	Origin of selection	Material source	Name in the source institution
Whit's Late Valencia	USA	CCSM <sup>1</sup>	Valencia Whit's Late IAC 1373
Cutter Valencia	USA	CCSM	Valencia Cutter IAC 1726
Berry Valencia	Australia	CCSM	Berry Valencia IAC 1335
Frost Valencia	USA	CCSM	Valencia Frost IAC 1727
Valencia Mutação	Brazil	CCSM	Valencia IAC 1754
Valencia IAC	Brazil	CCSM	Valencia IAC
Olinda Valencia	USA	CCSM	Valencia Olinda IAC 478
Chafeei Late Valencia	Australia	CCSM	Valencia Chafeei Late IAC 1357
Campbell 479 Valencia	USA	CCSM	Valencia Campbell IAC 1724
Campbell 294 Valencia	USA	CCSM	Valencia Campbell EECB – 294
Valencia Late 161	USA	CCSM	Valencia Late IAC 161
Valencia Late Fla.	USA	CCSM	Valencia Late IAC 1361
Valencia #121	Cuba	CCSM	Valencia IAC 1430
Charmute de Brotas	Brazil	CCSM	Charmute de Brotas IAC 2007
Natal África do Sul	South Africa	CCSM	Natal África do Sul IAC 481
Natal IAC	Brazil	CCSM	Natal IAC
Natal Murcha	Brazil	CCSM	Natal Folha Murcha IAC 491
Folha Murcha IAC	Brazil	CCSM	Folha Murcha IAC 474
IPR Folha Murcha	Brazil	IDR <sup>2</sup>	IPR 172 Folha Murcha

<sup>1</sup>CCSM – Centro de Citricultura “Sylvio Moreira” Cordeirópolis, SP, Brazil; <sup>2</sup>IDR-Paraná – Instituto de Desenvolvimento Rural do Paraná - IAPAR/EMATER, Londrina, PR, Brazil.

### 3.3.3 Orchard Management

Citrus tree management was based on the recommendations for the state of Paraná, Brazil (IAPAR, 1992; NUNES et al., 2010). Fertilizations were performed based on soil analysis. Weeds were managed with periodic mowing using an ecological rotary mower and herbicides sprays, between and within rows, respectively. Trees were irrigated with a localized drip irrigation system. The amount of water supplied to the trees was determined according to the crop evapotranspiration ( $ET_c$ ), which is determined by the crop coefficient procedure whereby the effect of the various weather conditions is incorporated into the reference crop evapotranspiration ( $ET_o$ ), and the crop characteristics into the single crop coefficient ( $K_c$ ) (ALLEN et al., 1998):

$$ET_c = ET_o \times K_c,$$

where  $ET_c$  = crop evapotranspiration,  $ET_o$  = reference crop evapotranspiration, and  $K_c$  = single crop coefficient.

Disease and insect pest management included monthly preventive sprays to control citrus canker and leafminer (BEHLAU et al., 2010; 2021), and two-monthly insecticide sprays to control the ACP (*Diaphorina citri* Kuwayama) from fruit set to fruit maturation (NUNES et al., 2010). Top and hedge pruning were not performed in order to evaluate the natural tree growth.

### 3.3.4 Vegetative Growth Measurements

The vegetative growth of the trees was assessed in early-spring of 2019, when the trees were seven-year-old. Tree canopy volume (CV) was determined based on tree height (TH) and canopy diameter (CD) measured with a graduate pole according to Mendel (1956):

$$CV = \frac{2}{3} \times \pi \times CR^2 \times TH,$$

where  $CR$  = canopy radius (m) and  $TH$  = tree height (m).  $CV$  is expressed in  $m^3$ .

Trunk circumference was measured 10 cm above and 10 cm below the graft union using a cloth tape measure and converted to diameter. The trunk index was calculated based on the relationship between the trunk diameter above and below the graft union.

### 3.3.5 Fruit Yield and Production Efficiency

Fruit yield was evaluated annually from 2016 to 2021 in November of each year, about 15 months after the main bloom from the tree innermost trees. Cumulative yield was determined after the annual harvests. Yield efficiency was determined based on the relationship between the fruit yield average (kg per tree) and canopy volume ( $m^3$  per tree) assessed in 2019. The result was expressed in kilograms per cubic meter ( $kg \cdot m^{-3}$ ). The alternate bearing index was determined according to Pearce and

Doberšek-Urbanc (1967):

$$ABI = \frac{1}{n-1} \times \left\{ \frac{|a_2 - a_1|}{a_2 + a_1} + \frac{|a_3 - a_2|}{a_3 + a_2} + \dots + \frac{|a_n - a_{n-1}|}{a_n + a_{n-1}} \right\},$$

where *ABI* = alternate bearing index, *n* = number of years, and *a*<sub>1</sub>, *a*<sub>2</sub>, ..., *a*<sub>(*n*-1)</sub>, *a*<sub>(*n*)</sub> = yields of the corresponding years.

### 3.3.6 Fruit Quality Evaluations

Fruit quality attributes were determined based on 10-fruit samples collected from the three innermost trees of each block. Fruit was randomly collected at 1–2 m tree height in October–November of each year from 2019 to 2021, before the annual harvests, with the averages for the evaluation period being presented. Fruit length and diameter were measured with a digital Vernier caliper (Mitutoyo, ABS, Kawasaki, Konagawa, Japan), weighed and classified according to the fresh citrus standards (CEAGESP, 2011). The fruit shape index was calculated based on the relationship between fruit length and diameter.

Fruit and juice colors were measured using a portable chroma meter (Minolta CR-400, Konica Minolta, Tokyo, Osaka, Japan) and the *CIELab* color system (McGUIRE, 1992). The device was calibrated before color assessments with a white tile. Fruit color was measured by readings taken at four equidistant points in the equatorial circumference of the fruit. Juice color was determined for each sample by readings taken through a 10 mL cuvette filled with juice as previously described by Li et al. (2021). The citrus color index (CCI) was calculated based on previous report (JIMENEZ-CUESTA et al., 1981):

$$CCI = \frac{1000 \times a^*}{L^* \times b^*},$$

where *CCI* = citrus color index, *a*<sup>\*</sup> = red-green color value, *b*<sup>\*</sup> = yellow-blue color value, *L*<sup>\*</sup> = lightness.

The CCI is a comprehensive indicator for color impression with positive values for red, negative values for blue-green, and zero for an intermediate mixture of red, yellow, blue, and green (ZHOU et al., 2010). After assessing fruit color, juice aliquots

were sampled using a Croydon extractor (Croydon, Duque de Caxias, RJ, Brazil). Juice content (JC) was determined according to the following equation and expressed as percentage:

$$JC = \frac{JW}{FW} \times 100,$$

where  $JW$  = juice weight (g) and  $FW$  = fruit weight (g).

Total soluble solids (TSS) concentration was measured with a digital refractometer (Atago Co., Ltd, PAL-3, Tokyo, Kantō, Japan) in 0.3 mL of undiluted juice. The results were expressed in °Brix units. Titratable acidity (TA) was determined in 25 mL juice and 0.1 N NaOH in a TitroLine easy titrator (Schott Instruments GmbH, Mainz, Rhineland-Palatinate, Germany), and expressed in grams of citric acid per 100 mL of juice ( $\text{g}\cdot 100\text{mL}^{-1}$ ) (AOAC, 2019). The maturity index (TSS.TA<sup>-1</sup> ratio) was calculated to determine the fruit maturity. The technological index, which indicates the amount of TSS content per standard citrus box (total capacity of 40.8 kg), was calculated according to the equation proposed by Di Giorgi et al. (1990):

$$TI = \frac{TSS \times JC \times 40.8}{10,000},$$

where  $TI$  = technological index ( $\text{t TSS}\cdot\text{ha}^{-1}$ ),  $TSS$  = total soluble solids (°Brix), and  $JC$  = juice content (%).

### 3.3.7 Estimates for Planting Density and Yield

Based on the tree size measures in 2019, the corresponding number of trees per hectare was estimated for all sweet orange selections included in this study, assuming a free spacing of 2.5 m between-rows (canopy diameter + 2.5 m) and 25% tree overlap in-rows (canopy diameter  $\times$  0.75) (DE NEGRI; BLASCO, 1991). Fruit yield was estimated according to the theoretical number of trees per hectare and the average fruit yield per tree determined for the 2017–2021 cropping seasons, when all trees were bearing. The soluble solids yield was determined according to the estimated yield and expressed in tons of TSS per hectare ( $\text{t TSS}\cdot\text{ha}^{-1}$ ):

$$TSS\ Yield = \frac{TSS \times JC \times YE}{10000},$$

where *TSS Yield* = soluble solids yield (t TSS.ha<sup>-1</sup>), *TSS* = total soluble solids (°Brix), *JC* = juice content (%), and *YE* = yield estimation. Adapted from Di Giorgi et al. (1990).

### 3.3.8 Sweet Orange Performance Index for Fresh Fruit Market and Juice Processing

To calculate the performance indices for each evaluated sweet orange selection, the data were normalized using the equations proposed by Ramos et al. (2021):

$$N1 = \left| \frac{max - min}{2} \right|$$

$$N2 = \left| \frac{N1 \times 100}{max} \right|$$

$$N3 = \left| \frac{N2 \times V}{N1} \right|,$$

where *max* = maximum value of each variable, *min* = minimum value of each variable, *V* = value of the variable.

Results were used to calculate the sweet orange performance indices for the fresh fruit market (FFI – fresh fruit index) and for the juice processing (JPI – juice processing index) according to Ramos et al. (2021):

$$FFI\ or\ JPI = \left| \frac{(A \times a) + (B \times b) + \dots + (N \times n)}{(max - min)} \right|,$$

where *A, B, ..., N* = correspond to the indices of each variable; *a, b, ..., n* = relative importance attributed to each variable (%); *max* = maximum value of each variable; and *min* = minimum value of each variable (CAPUTO et al., 2012).

The scores of each variable were assigned according to their relative importance for the fresh fruit market as it follows: 30% for cumulative yield, 20% for

TSS, 20% for fruit color, 10% for number of seeds, and 20% for fruit weight; and for juice processing: 30% for cumulative yield, 30% for TSS, 30% for juice content, and 10% for juice color (RAMOS et al., 2021).

### 3.3.9 Incidence of Fruit with Citrus Canker

Citrus canker incidence on fruits was evaluated at the annual harvests of 2020 and 2021 by visually assessing canker lesions on a 100-fruit sample per plot. Fruit of each selection were collected from the three middle trees of each plot. The incidence of fruit with citrus canker at harvest was expressed in percentage (%).

### 3.3.10 Incidence of Trees with HLB

Molecular analyses using the conventional polymerase chain reaction (PCR) technic were performed to determine the infection rate of the citrus trees by the huanglongbing (HLB) putative causal agent, '*Candidatus Liberibacter asiaticus*' (CLas). Leaves, asymptomatic and HLB-symptomatic for leaves that showed typical symptoms, were sampled from the sweet orange trees in the late spring of 2020 (growth season) and in the late fall (appearance of HLB-symptom) and late spring of 2021. Total genomic DNA was extracted (MURRAY; THOMPSON, 1980) from 10-leaf sample per tree individually (five trees per plot) and subjected to PCR amplification with primers sets A2/J5 (HOCQUELLET et al., 1999) and Oi1/Oi2c (JAGOEIX et al. 1994). PCR reaction was performed in 20  $\mu\text{L}$  of reaction mixture containing 0.1  $\mu\text{L}$  of each primer (0.5 mM), 0.8  $\mu\text{L}$  of dNTP (5 mM), 0.8  $\mu\text{L}$  of  $\text{MgCl}_2$  (50 mM), 2.0  $\mu\text{L}$  of buffer (10 $\times$ ), 1.0  $\mu\text{L}$  of Taq DNA Polymerase Recombinant (5 U  $\mu\text{L}^{-1}$ ) (Invitrogen, Carlsbad, CA, USA), 14.2  $\mu\text{L}$  of ultrapure water and 1  $\mu\text{L}$  of total genomic DNA. PCR reactions were performed in a thermal cycler (Veriti<sup>TM</sup> 96-Well, Applied Biosystems<sup>®</sup>, Waltham, MA, USA). For the A2/J5 primers, the thermal cycler was setup to 35 cycles at 92 °C for 20 s, 62 °C for 20 s, and 72 °C for 45 s (HOCQUELLET et al., 1999); while for the Oi1/Oi2c primers, the device was configurated to 35 cycles at 92 °C for 40 s, and 72 °C for 90 s (JAGOEIX et al., 1994). After amplification, one aliquot of each PCR reaction mixture was electrophoresed on 1.0% (w/v) agarose gel and visualized

with SYBR™ Gold Nucleic Acid Gel Stain (ThermoFisher Scientific, Carlsbad, CA, USA). The gel was visualized through a photo documenter (L-PIX EX, Loccus do Brasil Ltd., Cotia, SP, Brazil) under UV light.

### 3.3.11 Data Analyses

The data were evaluated according to the experimental design, tested for normal distribution and homogeneity at  $p \leq 0.05$ , and then submitted to analysis of variance (ANOVA). The citrus canker and HLB incidences data were transformed to  $\arcsin \sqrt{(x/100)}$  before ANOVA. Means were grouped using the Scott-Knott's test at  $p \leq 0.05$ . Incidence of fruit with citrus canker was analyzed in a randomized block design with a factorial arrangement (19 sweet orange selections  $\times$  two years). Significant variables were taken together and submitted to the multivariate analyses using a mean value for each selection and variable, and a principal component analysis (PCA) was plotted. All data were processed in *R* v. 4.0.2 (The R Foundation for Statistical Computing, Vienna, Austria) using the packages *ExpDes*, *ggplot2*, and *Facto MineR* for graphics and visualization of the statistical data.

## 3.4 RESULTS

### 3.4.1 Vegetative Growth Measurements

Significant differences ( $p \leq 0.05$ ) among the 19 late-season sweet orange selections were observed for the vegetative measurements, including tree height, canopy diameter, canopy volume, and rootstock-scion trunk diameters (Table 3.4.1). Olinda, Frost, Chafeei Late, #121, Campbell 479, Whit's Late, Berry and Late Fla. Valencia selections had the tallest trees while Natal IAC, Valencia Late 161 and IPR Folha Murcha trees were the shortest ones. Furthermore, trees of Olinda Valencia, Frost Valencia, Valencia #121 and Valencia Mutaç o exhibited the largest canopy diameters, which differed from Campbell 479 Valencia, Natal IAC, Natal Murcha, Folha Murcha IAC and IPR Folha Murcha trees. Olinda Valencia showed the greatest canopy volume, with an average of 49 m<sup>3</sup> per tree, and contrasted significantly with Natal IAC, Natal Murcha, Folha Murcha IAC and IPR Folha Murcha trees, which developed a

canopy volume smaller than 30 m<sup>3</sup>.

The rootstock trunk diameters of the Valencia selections Olinda, Frost, Berry, and Chafeei Late grafted onto Rangpur lime were significantly larger than those observed for Natal Murcha, Folha Murcha IAC and IPR Folha Murcha (Table 3.4.1). Frost and Chafeei Late Valencia trees scored the largest scion trunk diameters among the evaluated sweet oranges (~17 cm), while IPR Folha Murcha had the smallest diameter (12 cm). Similarly, the scion-rootstock trunk diameter ratios, expressed as trunk diameter indices, also varied among the evaluated selections. The highest trunk diameter index was observed for IPR Folha Murcha (0.97), which differed significantly from those of some Valencia selections including Campbell 294, Mutação, Olinda, Berry and Cutter.

**Table 3.4.1** Tree size of late-season sweet orange selections grafted onto Rangpur lime rootstock and determined in the 2019 season. Guairaçá, state of Paraná, Brazil.

Sweet orange selection	Tree height (m)	Canopy diameter (m)	Canopy volume (m <sup>3</sup> )	Rootstock trunk diameter <sup>1</sup> (cm)	Scion trunk diameter <sup>1</sup> (cm)	Trunk diameter index <sup>2</sup>
Whit's Late Valencia	4.18 a <sup>3</sup>	4.12 b	37.2 c	17.8 b	15.6 b	0.88 c
Cutter Valencia	4.03 b	3.96 c	33.3 d	17.2 b	14.5 c	0.86 d
Berry Valencia	4.17 a	4.00 c	35.1 d	18.6 a	15.4 b	0.83 d
Frost Valencia	4.40 a	4.39 a	44.3 b	19.1 a	17.0 a	0.89 c
Valencia Mutação	4.08 b	4.38 a	41.0 c	15.4 c	13.2 d	0.85 d
Valencia IAC	3.89 b	3.87 c	30.7 d	16.4 c	14.5 c	0.89 c
Olinda Valencia	4.50 a	4.56 a	49.3 a	19.1 a	15.8 b	0.83 d
Chafeei Late Valencia	4.30 a	4.22 b	40.1 c	18.4 a	16.7 a	0.91 c
Campbell 479 Valencia	4.24 a	3.72 d	30.7 d	16.7 c	14.5 c	0.87 c
Campbell 294 Valencia	4.03 b	3.89 c	31.9 d	17.1 b	13.5 d	0.79 d
Valencia Late 161	3.79 c	3.89 c	30.0 d	16.5 c	15.4 b	0.93 b
Valencia Late Fla.	4.13 a	3.83 c	31.7 d	17.4 b	15.5 b	0.89 c
Valencia #121	4.27 a	4.39 a	43.1 b	18.1 b	15.7 b	0.87 c
Charmute de Brotas	3.94 b	4.10 b	34.6 d	16.2 c	14.3 c	0.88 c
Natal África do Sul	3.98 b	4.08 b	34.7 d	16.2 c	14.5 c	0.89 c
Natal IAC	3.56 c	3.74 d	26.2 e	15.9 c	13.9 c	0.87 c
Natal Murcha	3.91 b	3.65 d	27.3 e	14.9 d	13.6 d	0.91 c
Folha Murcha IAC	3.95 b	3.56 d	26.2 e	14.1 d	13.7 d	0.97 a
IPR Folha Murcha	3.81 c	3.68 d	27.1 e	13.2 d	12.2 e	0.92 b
CV (%)	3.88	3.66	8.28	5.09	4.95	3.04
<i>F</i> value	6.34***	11.53***	15.95***	10.59***	8.54***	7.02***

<sup>1</sup>Trunk diameters were based on trunk circumference measurements, 10 cm above and 10 cm below the graft union. <sup>2</sup>Expressed as the ratio between scion and rootstock trunk diameters. <sup>3</sup>Means followed by the same letter in the column belong to the same group according to the Scott-Knott's test. Significance level: \*\*\*,  $p \leq 0.001$ .

### 3.4.2 Fruit Yield and Production Efficiency

The highest fruit yields were observed for Whit's Late Valencia and Natal África do Sul over the evaluated period (Table 3.4.2). The annual yields for these cultivars were above 100 kg per tree in most of the assessed years (Table 3.4.2). In 2016, when trees were near four years old, all trees exhibited lower yields or even did not bear any fruit, as was the case of Olinda Valencia. However, a significant increase on fruit production was observed from 2018 onwards for most of the selections, as the case of Whit's Late Valencia and Natal África do Sul. Similarly, these selections had the highest cumulative yield after six consecutive years of fruit production, with 683 kg per tree for Whit's Late Valencia and 646 kg per tree for Natal África do Sul (Table 3.4.2). Most of the evaluated selection grouped for the highest yield efficiency, except the Valencia selections Berry, Frost, Mutaç o, Olinda, Chafeei Late, Late 161 and #121 that exhibited poor yield efficiency. The lowest alternate bearing indices were found for the Valencia selections Cutter, IAC, Campbell 479, Late Fla and for Charmute de Brotas and Folha Murcha IAC, differing significantly from Olinda Valencia.

**Table 3.4.2** Yield performance of late-season sweet orange selections grafted onto Rangpur lime rootstock from 2016 to 2021. Guairaçá, state of Paraná, Brazil.

Sweet orange selection	Yield (kg per tree)						Cumulative yield CY (kg)	Alternate bearing index ABI	Yield efficiency YE (kg.m <sup>-3</sup> ) <sup>1</sup>
	2016	2017	2018	2019	2020	2021			
Whit's Late Valencia	47.1 b <sup>2</sup>	120.3 a	125.3 a	128.7 a	158.0 b	103.8 c	683.1 a	0.20 b	3.48 a
Cutter Valencia	64.1 a	89.8 b	118.4 a	123.7 a	115.3 c	98.0 c	609.3 b	0.10 c	3.30 a
Berry Valencia	49.5 b	58.2 c	73.4 b	67.6 b	145.5 b	86.5 d	480.3 c	0.22 b	2.45 b
Frost Valencia	40.4 b	59.6 c	106.8 a	84.5 b	104.8 c	102.5 c	498.7 c	0.20 b	2.08 b
Valencia Mutaç�o	31.0 b	115.1 a	116.5 a	118.1 a	143.3 b	74.5 e	598.4 b	0.23 b	2.78 b
Valencia IAC	43.0 b	93.5 b	104.5 a	101.3 b	132.7 c	120.0 b	594.9 b	0.15 c	3.70 a
Olinda Valencia	--- <sup>3</sup>	46.4 c	96.0 a	74.3 b	180.8 a	72.5 e	475.9 c	0.34 a	1.94 b
Chafeei Late Valencia	46.9 b	80.0 b	100.6 a	72.2 b	125.5 c	99.0 c	524.2 c	0.20 b	2.38 b
Campbell 479 Valencia	57.2 a	90.6 b	89.6 a	97.2 b	147.5 b	117.8 b	599.9 b	0.14 c	3.55 a
Campbell 294 Valencia	54.4 a	89.9 b	91.5 a	94.8 b	143.0 b	57.0 e	530.4 c	0.22 b	2.98 a
Valencia Late 161	28.3 b	52.9 c	66.2 b	80.2 b	101.0 c	64.0 e	392.6 c	0.22 b	2.42 b
Valencia Late Fla.	54.0 a	84.1 b	55.1 b	111.8 a	100.5 c	101.5 c	507.0 c	0.17 c	2.86 a
Valencia #121	45.7 b	41.5 c	83.1 b	80.8 b	152.0 b	144.0 a	547.2 c	0.20 b	2.33 b
Charmute de Brotas	71.3 a	74.1 c	106.0 a	104.5 a	146.0 b	87.1 d	588.7 b	0.14 c	3.00 a
Natal �frica do Sul	59.7 a	114.0 a	81.2 b	141.2 a	161.0 b	89.0 d	646.1 a	0.22 b	3.38 a
Natal IAC	58.5 a	83.4 b	75.1 b	79.2 b	109.8 c	65.8 e	471.8 c	0.14 c	3.15 a
Natal Murcha	34.5 b	78.7 b	66.3 b	101.8 a	124.3 c	98.3 c	503.8 c	0.19 b	3.46 a
Folha Murcha IAC	54.1 a	71.9 c	61.7 b	94.9 b	112.2 c	72.2 e	466.9 c	0.15 c	3.15 a
IPR Folha Murcha	45.0 b	66.8 c	78.4 b	81.0 b	126.5 c	79.8 d	477.5 c	0.21 b	3.23 a
CV (%)	19.90	16.72	21.52	24.03	12.66	13.20	7.80	24.17	11.35
F value	4.03***	8.51***	3.40***	2.38*	5.67***	9.88***	9.18***	3.77***	7.45***

<sup>1</sup>Yield efficiency was based on the average yield from 2017 through 2021 and the canopy volume assessed in 2019. <sup>2</sup>Means followed by the same letter in the column belong to the same group according to the Scott-Knott's test. Significance level: \*,  $p \leq 0.05$ ; \*\*\*,  $p \leq 0.001$ . <sup>3</sup>No data for the respective year.

### 3.4.3 Fruit Quality Evaluations

Based on the average of three consecutive cropping seasons (2019 to 2021), significant differences were observed among the sweet orange selections for all fruit quality attributes assessed (Tables 3.4.3 and 3.4.4). The largest fruit length was recorded for fruits of Folha Murcha IAC and some Valencia selections, including Olinda, #121, Frost, Chafeei Late, Whit's Late, and Late Fla. (Table 3.4.3). Similarly, Valencia #121 had the largest fruit diameter. Moreover, fruit produced by Folha Murcha IAC was oblong in shape, differing from all other selection, which produced round-shaped fruit. Fruit weight ranged from 197 to 251 g, depending on the selection. The Valencia selections #121, Whit's Late, Late 161, and Frost, and the Charmute de Brotas fruit were significant heavier than fruit of all other sweet oranges (Table 3.4.3). The number of seeds per fruit were low, one to five seeds per fruit, for all sweet orange selections. Olinda Valencia, Valencia Late 161 and Charmute de Brotas produced fruit with a much lower number of seeds than Campbell 479 Valencia fruits. In regard to the fruit color, the fruits of all Valencia selections showed higher color index, within – 0.04 and 1.34 CCI, than Natal África do Sul, Natal Murcha and Folha Murcha IAC (Table 3.4.3).

The color of the fresh-squeezed juice among the studied selections ranged from –4.97 up to –1.70 CCI (Table 3.4.4). Cutter Valencia, Natal África do Sul, Folha Murcha IAC, Natal Murcha and Charmute de Brotas presented high standards for this juice attribute. Juice contents were higher for most of the Valencia selections ( $\geq 39\%$ ), except for Olinda that ranked below all assessed sweet orange, with 27% (Table 3.4.4). The total soluble solid (TSS) content in the juices ranged from 9.1 to 11.1 among the selections. The lowest TSS was for the juice of Olinda Valencia while Frost Valencia, Valencia IAC, Campbell 479 Valencia, Valencia Late 161, Valencia Late Fla., Valencia #121, Charmute de Brotas, Natal África do Sul, Natal IAC, Natal Murcha and IPR Folha Murcha had the highest TSS concentration, above 10.4 °Brix (Table 3.4.4). The Valencia selections Whit's Late and Frost had the highest level of juice acidity (titratable acidity – TA), with 1.08 and 1.06 g.100mL<sup>-1</sup> of juice (citric acid), respectively. On the other hand, all other selections showed acidity levels below 1.0 g.100mL<sup>-1</sup>. The maturity index (MI), i.e. TSS.TA<sup>-1</sup> ratio, fluctuated from 9.4 to 16.8, depending on the sweet orange. Juice from Natal Murcha fruits showed the highest maturity index, while the ones from Valencia selections including Whit's Late, Frost

and Berry ranked at the bottom for this qualitative attribute. Most selections produced fruit of proper technological potential ( $TI \geq 1.70$ ) to be processed by the juice industry. However, Olinda Valencia sweet orange exhibited the lowest TI performance ( $\leq 1.10$ ), due basically to its low-quality juice.

**Table 3.4.3** Three-season average fruit quality of late-season sweet oranges produced in Guairaçá, state of Paraná, Brazil, from 2019 to 2021.

Sweet orange selection	Fruit length FL (mm)	Fruit diameter FD (mm)	Fruit shape FS (FL.FD <sup>-1</sup> )	Fruit weight FW (g)	Number of seeds NS	Peel color PC (CCI)
Whit's Late Valencia	78.9 a <sup>1</sup>	78.5 b	1.00 c	240 a	3 b	1.34 a
Cutter Valencia	77.8 b	78.5 b	0.99 c	222 b	3 b	0.10 a
Berry Valencia	76.8 b	76.2 b	1.01 c	211 b	3 b	0.89 a
Frost Valencia	79.7 a	79.3 b	1.00 c	231 a	2 c	0.90 a
Valencia Mutaç�o	77.2 b	77.7 b	0.99 c	214 b	3 b	1.27 a
Valencia IAC	76.6 b	77.5 b	0.99 c	217 b	4 b	0.07 a
Olinda Valencia	80.2 a	77.1 b	1.04 b	212 b	1 d	0.81 a
Chafeei Late Valencia	79.6 a	79.6 b	1.00 c	216 b	4 b	0.15 a
Campbell 479 Valencia	75.5 b	76.6 b	0.99 c	209 b	5 a	0.78 a
Campbell 294 Valencia	75.8 b	78.3 b	0.97 c	216 b	4 b	0.01 a
Valencia Late 161	76.9 b	77.6 b	0.99 c	233 a	1 d	0.71 a
Valencia Late Fla.	78.7 a	77.9 b	1.01 c	211 b	4 b	0.91 a
Valencia #121	80.2 a	81.5 a	0.98 c	251 a	3 b	0.09 a
Charmute de Brotas	76.7 b	76.2 b	1.01 c	226 a	1 d	-0.04 a
Natal �frica do Sul	75.0 b	75.6 b	0.99 c	197 b	4 b	-0.53 b
Natal IAC	76.6 b	76.2 b	1.00 c	205 b	2 c	0.19 a
Natal Murcha	75.9 b	78.1 b	0.97 c	219 b	3 b	-1.76 b
Folha Murcha IAC	81.9 a	75.8 b	1.08 a	214 b	3 b	-1.76 b
IPR Folha Murcha	76.2 b	76.9 b	0.99 c	209 b	2 c	0.48 a
CV (%)	2.10	2.23	1.50	5.13	21.29	14.97
F value	4.11***	2.17*	8.12***	3.96***	9.42***	3.65***

<sup>1</sup>Means followed by the same letter in the column belong to the same group according to the Scott-Knott's test. Significance level: \*,  $p \leq 0.05$ ; \*\*\*,  $p \leq 0.001$ .

**Table 3.4.4** Three-season average juice quality of late-season sweet oranges produced in Guairaçá, state of Paraná, Brazil, from 2019 to 2021.

Sweet orange selection	Juice color JCr (CCI)	Juice content JCr (%)	Total soluble solids TSS (°Brix)	Titrateable acidity TA (g 100.mL <sup>-1</sup> )	Maturity index MI (TSS.TA <sup>-1</sup> )	Technological index TI (kg TSS.box <sup>-1</sup> )
Whit's Late Valencia	-4.97 c <sup>1</sup>	44.9 a	10.1 b	1.08 a	9.4 e	1.86 a
Cutter Valencia	-2.70 a	43.7 a	10.3 b	0.79 c	13.1 c	1.83 a
Berry Valencia	-4.67 c	43.9 a	10.0 b	0.98 b	10.3 e	1.80 a
Frost Valencia	-3.53 b	39.0 c	10.4 a	1.06 a	9.9 e	1.66 b
Valencia Mutaçao	-3.43 b	44.2 a	10.2 b	0.75 d	13.5 c	1.83 a
Valencia IAC	-4.83 c	44.1 a	10.5 a	0.81 c	13.0 c	1.89 a
Olinda Valencia	-4.70 c	26.9 e	9.1 c	0.74 d	12.3 d	1.06 d
Chafeei Late Valencia	-4.23 c	44.6 a	10.1 b	0.93 b	11.0 d	1.84 a
Campbell 479 Valencia	-4.87 c	43.2 a	10.6 a	0.83 c	12.8 c	1.87 a
Campbell 294 Valencia	-3.60 b	41.0 b	9.9 b	0.72 d	13.7 c	1.65 b
Valencia Late 161	-3.70 b	41.6 b	10.7 a	0.79 c	13.6 c	1.81 a
Valencia Late Fla.	-4.40 c	42.9 a	10.9 a	0.98 b	11.2 d	1.91 a
Valencia #121	-4.77 c	42.0 b	10.5 a	0.90 b	11.7 d	1.80 a
Charmute de Brotas	-2.33 a	36.3 d	11.1 a	0.75 d	15.0 b	1.65 b
Natal África do Sul	-1.70 a	38.8 c	10.6 a	0.69 d	15.5 b	1.67 b
Natal IAC	-3.57 b	36.9 d	11.0 a	0.91 b	12.0 d	1.65 b
Natal Murcha	-2.33 a	39.0 c	11.0 a	0.66 d	16.8 a	1.76 a
Folha Murcha IAC	-2.13 a	37.7 d	9.7 b	0.73 d	13.3 c	1.44 c
IPR Folha Murcha	-3.13 b	39.5 c	10.9 a	0.71 d	15.3 b	1.76 a
CV (%)	15.82	2.25	3.78	6.47	6.36	5.22
F value	9.78***	66.70***	5.31***	16.62***	17.94***	14.41***

<sup>1</sup>Means followed by the same letter in the column belong to the same group according to the Scott-Knott's test. Significance level: \*\*\*,  $p \leq 0.001$ .

#### 3.4.4 Estimates for Planting Density and Yield

The largest row and tree spacings were estimated for Olinda Valencia, Valencia #121, Frost Valencia and Valencia Mutaç o, while Valencia Campbell 479, Folha Murcha IAC, Natal Murcha, IPR Folha Murcha and Natal IAC required the smallest spacing, maximizing tree density to a range from 572 up to 619 trees.ha<sup>-1</sup> (Table 3.4.5). In contrast, the selections that demanded the largest tree and row spacings support no more than 415 and 444 trees.ha<sup>-1</sup>, which poses a restriction for their use in high-density plantings. Furthermore, Natal Murcha, Natal  frica do Sul, and the Valencia selections Campbell 479, IAC, Whit's Late and Cutter showed the highest estimation yield per area (t.ha<sup>-1</sup>), a favorable characteristic for high-density orchards. The estimates study also indicated that the sweet oranges with high TSS yield, as the Valencia selections Whit's Late, IAC and Campbell 479, had their excellent performance due to the good yield and juice quality (Tables 3.4.2 and 3.4.5).

**Table 3.4.5** Estimates<sup>1</sup> of minimum row and tree spacing, maximum tree density, fruit yield, and TSS yield for late-season sweet orange selections grafted onto Rangpur lime rootstock, based on field performance in Guairaçá, state of Paraná, Brazil.

Sweet orange selection	Row spacing (m)	Tree spacing (m)	Tree density (trees.ha <sup>-1</sup> )	Fruit yield (t.ha <sup>-1</sup> )	TSS yield (t TSS.ha <sup>-1</sup> )
Whit's Late Valencia	3.09 b	6.62 b	490 c	62.3 a	2.83 a
Cutter Valencia	2.97 c	6.46 c	522 b	56.9 a	2.57 b
Berry Valencia	3.01 c	6.51 c	512 c	43.9 c	1.93 c
Frost Valencia	3.29 a	6.88 a	443 d	40.6 c	1.64 c
Valencia Mutaç�o	3.29 a	6.88 a	444 d	50.3 b	2.25 b
Valencia IAC	2.90 c	6.37 c	545 b	60.4 a	2.80 a
Olinda Valencia	3.42 a	7.06 a	415 d	39.4 c	0.96 d
Chafeei Late Valencia	3.16 b	6.72 b	471 c	44.9 c	2.02 c
Campbell 479 Valencia	2.79 d	6.22 d	578 a	62.8 a	2.89 a
Campbell 294 Valencia	2.91 c	6.39 c	537 b	51.1 b	2.07 c
Valencia Late 161	2.92 c	6.39 c	536 b	39.0 c	1.72 c
Valencia Late Fla.	2.87 c	6.33 c	551 b	49.9 b	2.34 b
Valencia #121	3.29 a	6.89 a	442 d	44.3 c	1.96 c
Charmute de Brotas	3.07 b	6.60 b	494 c	51.1 b	2.05 c
Natal �frica do Sul	3.06 b	6.58 b	497 c	58.3 a	2.39 b
Natal IAC	2.80 d	6.24 d	572 a	47.1 b	1.91 c
Natal Murcha	2.74 d	6.15 d	596 a	55.9 a	2.41 b
Folha Murcha IAC	2.67 d	6.06 d	619 a	51.1 b	1.87 c
IPR Folha Murcha	2.76 d	6.18 d	587 a	50.8 b	2.20 b
CV (%)	3.66	2.25	5.69	9.08	10.24
F value	11.53***	11.53***	11.85***	7.90***	13.38***

<sup>1</sup>Estimates study was based on vegetative, yield, and fruit quality data of the evaluated selections; tree density and row/tree spacing projections were calculated according to De Negri and Blasco (1991) and used to estimate fruit yield and TSS yield. <sup>2</sup>Means followed by the same letter in the column belong to the same group according to the Scott-Knott's test. Significance level: \*\*\*,  $p \leq 0.001$ .

### 3.4.5 Sweet Orange Performance Index for Fresh Fruit Market and Juice Processing

The suitability for the fresh fruit market and for juice processing were evaluated for most sweet orange selections based on fruit quality and yield performance traits (Tables 3.4.2, 3.4.3, 3.4.4 and 3.4.6), except by Olinda Valencia that did not attend the minimal standards of the fresh fruit and industrial markets. All late-season selections did not show any significant differences for the fresh fruit index (FFI), ranging from 1.04, for Frost Valencia, to 2.23, for Valencia IAC. In contrast, significant differences were observed among the sweet oranges for processing index (IPI) (Table 3.4.6). Natal África do Sul and Charmute de Brotas scored the highest IPIs, excelling for industrial processing. The lowest IPIs were observed for Valencia selections, including Whit's Late, Berry, IAC, Chafeei Late, Campbell 479, Late 161, Late Fla. and #121, while the other cultivars showed intermediate indices for this attribute.

**Table 3.4.6** Fresh fruit and industrial processing indices of late-season sweet orange selections grafted onto Rangpur lime rootstock under humid subtropical conditions in Guairaçá, state of Paraná, Brazil, from 2016 to 2021 cropping season average.

Sweet orange selection	Fresh fruit index (FFI) <sup>1</sup>	Industrial processing index (IPI) <sup>2</sup>
Whit's Late Valencia	1.30 a <sup>3</sup>	2.00 c
Cutter Valencia	1.31 a	4.39 b
Berry Valencia	1.28 a	2.06 c
Frost Valencia	1.04 a	3.83 b
Valencia Mutaçãõ	1.61 a	3.30 b
Valencia IAC	2.23 a	2.06 c
Chafeei Late Valencia	1.80 a	2.39 c
Campbell 479 Valencia	1.13 a	2.24 c
Campbell 294 Valencia	1.72 a	3.70 b
Valencia Late161	1.69 a	2.07 c
Valencia Late Fla.	1.38 a	2.40 c
Valencia #121	1.16 a	2.36 c
Charmute de Brotas	1.77 a	14.16 a
Natal África do Sul	1.35 a	16.55 a
Natal IAC	1.40 a	3.26 b
Natal Murcha	1.39 a	4.89 b
Folha Murcha IAC	1.40 a	3.30 b
IPR Folha Murcha	2.22 a	3.73 b
CV (%)	36.92	19.50
F value	1.12ns	16.08***

<sup>1</sup>Fresh fruit index was based on 30% cumulative yield, 20% TSS, 20% fruit color, 10% number of seeds and 20% fruit weight. <sup>2</sup>Industrial processing index was based on 30% cumulative yield, 30% TSS, 30% juice content and 10% juice color. <sup>3</sup>Means followed by the same letter in the column belong to the same group according to the Scott-Knott's test. Significance level: ns, non-significant; \*\*\*,  $p \leq 0.001$ .

### 3.4.6 Incidence of Citrus Canker and Huanglongbing (HLB)

In regard to citrus canker incidence on harvested fruits, a highly significant ( $p \leq 0.001$ ) interaction between the 19 late-season sweet orange selections and the two assessed harvest seasons, 2020 and 2021, was observed (Table 3.4.7). As main effects, the highest incidence of citrus canker on fruits were found for those harvested from Valencia selections Frost and Late Fla. in both years, with more than 20% of affected fruit. These values were significantly higher ( $p \leq 0.001$ ) than those observed on fruits of IPR Folha Murcha, Natal IAC, Charmute de Brotas and Valencia selections

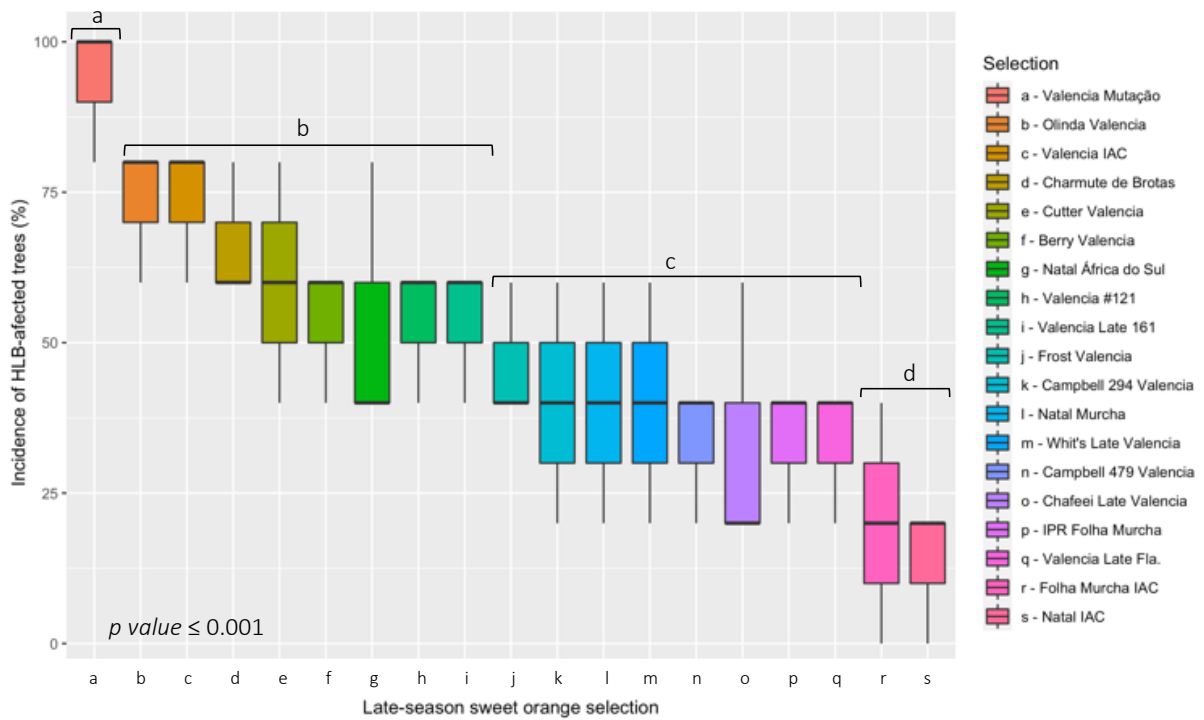
Chafeei Late, Campbell 479, Campbell 294, Olinda, Mutaç o and Whit's Late. Interestingly, Folha Murcha IAC had a substantial development of citrus canker on fruit from 2020 to 2021, showing an increase of 10-fold. This trend was also observed in all other evaluated selections when comparing the 2020 and 2021 harvests (Table 3.4.7).

Significant differences were also observed within the assessed sweet orange selections for the incidence of HLB (Figure 3.4.1). The incidence of trees with PCR-confirmed HLB ranged from 13 to 93%. Nine years old trees of Valencia Mutaç o had the highest cumulative rate of CLas infection, differing significantly from all other selections (Figure 3.4.1). The lowest HLB incidence was observed for trees of Natal IAC and Folha Murcha IAC. All other cultivars showed intermediate levels of HLB incidence (Figure 3.4.1).

**Table 3.4.7** Incidence of harvested fruit with citrus canker on late-season sweet orange selections grafted onto Rangpur lime rootstock harvested in 2020 and 2021 in Guairaçá, state of Paraná, Brazil.

Source of variance	Year <sup>1</sup>	
	2020 harvest	2021 harvest
Sweet orange selection		
Whit's Late Valencia	12.7 Bc <sup>2</sup>	17.7 Ae
Cutter Valencia	9.7 Bc	23.0 Ac
Berry Valencia	8.5 Bd	27.6 Ab
Frost Valencia	21.0 Aa	24.0 Ac
Valencia Mutação	8.0 Ad	10.5 Ag
Valencia IAC	10.0 Bc	27.7 Ab
Olinda Valencia	7.3 Bd	16.0 Ae
Chafeei Late Valencia	3.0 Be	17.7 Ae
Campbell 479 Valencia	6.3 Bd	14.3 Af
Campbell 294 Valencia	9.3 Bc	17.7 Ae
Valencia Late 161	10.3 Bc	21.3 Ad
Valencia Late Fla.	22.0 Ba	33.0 Aa
Valencia #121	17.7 Bb	29.3 Ab
Charmute de Brotas	7.0 Bd	16.5 Ae
Natal África do Sul	7.7 Bd	29.0 Ab
Natal IAC	6.7 Bd	25.7 Ac
Natal Murcha	15.3 Ab	14.5 Af
Folha Murcha IAC	3.3 Be	30.5 Aa
IPR Folha Murcha	4.0 Be	11.7 Ag
CV (%)	13.08	
Cultivar	37.0***	
Year	84.1***	
Cultivar × Year	17.82***	

<sup>1</sup>All data were transformed to  $\arcsin \sqrt{(x/100)}$  before submitted to analysis of variance (ANOVA). <sup>2</sup>Means followed by same letter, capital case in the row and lowercase in the column, belong to the same group according to Scott-Knott's test. Significance level: \*\*\*,  $p \leq 0.001$ .



**Figure 3.4.1** Cumulative incidence of huanglongbing (HLB)-affected trees in 19 late-season sweet orange selections grafted onto Rangpur lime rootstock after nine years of planting in Guairaçá, state of Paraná, Brazil. HLB-affected trees were confirmed by the detection of '*Candidatus Liberibacter asiaticus*' (CLas) infection in a polymerase chain reaction (cPCR) assay. All data were transformed to  $\arcsin \sqrt{(x/100)}$  before submitted to analysis of variance (ANOVA). Means followed by same letter in the in the bar, belong to the same group according to Scott-Knott's test. Significance level: \*\*\*,  $p \leq 0.001$ .

### 3.4.7 Multivariate Analysis

All assessed variables were submitted to the multivariate analysis, and a principal component analysis was carried out (Figure 3.4.2). The first two principal components accounted for 54% of the total variance in the dataset. Principal component 1 (Dim1) explained 37% of the variation while the principal component 2 (Dim2) represented 17%. Segregation among the 19 late-season sweet orange selections was observed when the two first principal components were projected. Four distinct groups (Col. 1, 2, 3, and 4) were formed based on the similarities of the sweet orange selections. The first group (Col. 1) was composed by Valencia selections including Whit's Late, Cutter, Berry, Frost, Mutaçao, IAC, Chafeei Late, Campbell 294,

Campbell 479, Late 161, Late Fla. and #121. These selections were recognized to be more productive and vigorous compared to the other sweet oranges (Figure 3.4.2). The vigorous tendency of Valencia resulted in lower tree density (Figure 3.4.2). Most Valencia trees produced fruits of better external quality including fruit size, weight, and color index. Some qualitative internal attributes were also remarkable within these sweet oranges as TSS, acidity and juice content. Exceptionally, Olinda Valencia formed a single group (Col. 2) far from all other accessions, mainly because this selection scored low for most horticultural traits assessed. Similarly, Folha Murcha IAC formed another single group (Col. 3), characterized to produce fruit of intense juice color but with a greenish peel color. Finally, Charmute de Brotas, Natal África do Sul, Natal IAC, Natal Murcha and IPR Folha Murcha grouped together, because of their similarities on the evaluated traits. These selections were characterized to produce fruits of better juice quality based on maturity index and juice color, which favors their use for the processing resulted from the highest IPIs.



### 3.5 DISCUSSION

Trees of 19 late-season sweet orange selections were evaluated for vegetative growth at the seven-year of age, when the trees were well established in the field and stabilized in terms to vegetative growth and crop yield. Based on the results, Olinda Valencia trees were significantly ( $p \leq 0.001$ ) the most vigorous among the selections. This selection had the highest values for almost all vegetative measurements, except for the scion trunk diameter and the trunk diameter index. Olinda Valencia trees have been recognized as vigorous in previous works (HODGSON, 1967; CASTLE; BALDWIN, 2011), particularly young-grafted trees (TORRES; GARCÍA, 2012; SIMPSON et al., 2014). Further, several other Valencia selections had vigorous development in our field trial, e.g., Frost, Berry, Whit's Late, Chafeei Late, #121, Campbell 479 and Late Fla. These vigorous developments have also been described in previous study (HODGSON, 1967). On the other hand, Natal IAC, Natal Murcha, Folha Murcha IAC and IPR Folha Murcha selections had low vegetative growth in our field trial. These findings are in accordance with those previously reported by Stenzel et al. (2005) and Azevedo et al. (2015) for Folha Murcha and by Girardi et al. (2017) for Natal sweet orange.

Tree size plays an important role in the citrus orchard planning, field managements and other operations, including harvesting, pruning, irrigation and spraying to control insect pests and diseases (ALBRIGO et al., 2019). Indeed, vegetative growth may determine tree density, depending as well on the tree architecture of each cultivar (STUCHI, 2005). Currently, the demand for small to medium-sized trees, which favors high-density plantings (ALBRIGO et al., 2019), have increased in commercial citrus-growing areas, particularly under endemic occurrence of HLB, which has impacted the lifetime of the orchards and changed the whole production system (BOVÉ, 2006; MOREIRA et al., 2019; BASSANEZI et al., 2020; FERRAREZI et al., 2020; CARVALHO et al., 2021b; CARVALHO et al., 2021c; GIRARDI et al., 2021). High-density planting assures higher yield at the young tree stage under the HLB pressure, favored by the increase of the number of trees per area that optimizes the land use, and improving the returns of the initial investments (WHEATON et al., 1991; BASSANEZI et al., 2020). The smallest trees favored higher-density plantings and yields in the estimates study. Among them, we could include

Folha Murcha IAC, IPR Folha Murcha, Natal IAC, Natal Murcha and Campbell 479 Valencia (Table 3.4.5). On the other hand, the most vigorous selections allowed a limited number of trees per hectare and lower fruit yield, particularly Olinda Valencia, the most vigorous (Table 3.4.5; Figure 3.4.2).

Although significant differences were apparent between the genotypes, all evaluated sweet orange scions were graft-compatible with Rangpur lime. No overgrowth or tree decline was observed seven years after planting. Our results are in agreement with previous reports demonstrating a broad affinity of Rangpur lime with several sweet orange cultivars and their relatives (BASTOS et al., 2017; ALVES et al., 2021; CARVALHO et al., 2021a; CRUZ et al., 2021; CARVALHO et al., 2022).

Fruit yield is essential for the evaluation of citrus germplasm. Genotypes that have higher yields and fruit quality in addition to tolerance or resistance to biotic and abiotic stresses, are highly demanded by the citrus growers (CASTLE, 2010). Sweet orange trees usually start to produce fruits at two to five years of age (DI GIORGE et al., 1990; CASTLE et al., 2010). Currently, genotypes that show a tendency of early fruiting are desirable due to the HLB pressure (BOVÉ; AYRES, 2007; SPREEN et al., 2007).

Trees of Natal África do Sul and Whit's Late Valencia had higher fruit production at the early stages, with four to nine years of age. As these selections were very productive, they may be a good alternative for planting in new orchards to ensure higher fruit yields and early return of the investment under an HLB endemic scenario. Here, the most vigorous trees had low fruit load in the first years, which includes the Valencia selections Olinda, #121 and Frost (Table 3.4.1). Moreover, Olinda trees did not show any fruit yield in 2016, starting to bear fruits later than all other selections. Vigorous vegetative growth may limit fruit production in young trees (CARVALHO et al., 2021a), as the photoassimilates are directed to the vegetative growth at the expense of fruit growth and development (AGUSTÍ; PRIMO-MILLO, 2020).

Fruit and juice quality are of major importance for the evaluation of sweet orange selections. Quality standards are regulated by several agencies around the world (OECD, 2010; CEAGESP, 2011). In our study, the fruit physical traits, including size, shape, weight, and color, were significantly different ( $p \leq 0.05$ ) among the evaluated sweet orange selections. Despite of the significant differences, all sweet oranges produced fruits of large size ( $\geq 71$  mm) and higher grade (A), according to the fresh fruit standards of the domestic market (CEAGESP, 2011).

The number of seeds per fruit is another important attribute to be considered in the evaluation of sweet oranges, as seedless fruits, or with low number of seeds, are usually preferred by the fresh fruit market and juice processors (CARVALHO et al., 2019a; DONADIO, 1999). The number of seeds in the fruits can vary from zero for some cultivars to dozens (LADANIYA, 2008). In our study, all common sweet orange selections produced fruits with seeds, ranging from one to five seeds per fruit. However, they can be considered commercially as seedless because they had less than eight seeds per fruit (ALBRIGO et al., 2019), particularly Valencia selections Late 161, Olinda and Frost, and Charmute de Brotas, Natal IAC and IPR Folha Murcha. Our findings are in agreement with those reported in previous studies for Valencia (MESEJO et al., 2007; YILDIZ et al., 2013), Charmute de Brotas (NASCIMENTO et al., 2005), Natal (LADANIYA, 2008; COUTO et al., 2017) and Folha Murcha (STUCHI; DONADIO, 2000). The low number of seeds observed in Valencia sweet oranges were related to the weak parthenocarpy of this cultivar, resulted from some defect during the female gametogenesis (KOLTUNOW et al., 1995).

The color break is not a reliable sign of maturity of the sweet orange fruits (BERK, 2016; GUPTA et al., 2021), though, this attribute may be critical for the fresh consumption (LADANIYA, 2008). The fresh market usually requires fruits with full coloration and without peel damage (OECD, 2010; CEAGESP, 2011), while juice processing demands juice of intense and luminous coloration (GAMA; SYLOS, 2005). Juice color is not usually a concern with most mid- to late-season maturing sweet oranges (CASTLE; BALDWIN, 2011), as they achieve better coloration than the early-season maturing cultivars (FELLERS, 1990). In our study, the Charmute de Brotas, Natal IAC, IPR Folha Murcha and all Valencia selections produced fruits of better peel color. In regard to the juice color, Cutter Valencia, Charmute de Brotas, Natal África do Sul, Natal Murcha and Folha Murcha IAC fruits produced juices of improved coloration, as the CCI were higher indicating an intense yellow coloration. The lowest juice content recorded in this fruit selections may have increased the concentration of colored compounds, as carotenoids, favoring for a deeper yellow color. Fruit of Valencia sweet orange is recognized to produce juice of high quality and intense coloration (GAMA; SYLOS, 2005). However, our results show that Charmute de Brotas and some selections of Natal and Folha Murcha may also be potential alternatives for juice processing, as the color indices were more yellow-intense than those observed for most of the Valencia selections (yellowish green).

The juice content in the fruits is an important qualitative attribute in the evaluation of sweet orange cultivars. Further, this quality attribute may vary according to the region, due to climate and soil conditions, as well as to the rootstock, plant nutrition, irrigation, and tree age, among other factors (CASTLE et al., 2010; BERK, 2016; ALBRIGO et al., 2019). Sweet orange fruits should have at least 35% of juice content, according to the fresh market requirements (OECD, 2010; CEAGESP, 2011; LADANIYA, 2008) and 36 to 40% for juice processing (CHITARRA; CHITARRA, 2005; LADO et al., 2014; ALBRIGO et al., 2019). Fruits of almost all sweet orange selections included in this study had juice content commercially acceptable, higher than the threshold established for the fresh fruit market and processing, except Olinda Valencia fruits that barely scored 26% of juice content. Despite of being planted in several important citrus-growing areas worldwide (AL-JALEEL; ZEKRI, 2002; CASTLE; BALDWIN, 2011; TORRES; GARCÍA, 2012), this Valencia selection did not show reasonable horticultural performance in our study under the humid subtropical climate. On the other hand, the majority of the Valencia selections had high fruit juice content under the northwestern Paraná edaphoclimatic conditions, supporting the performance reported in previous studies (DOMINGUES et al., 2021).

Most of the sweet orange selections included in the study had TSS content values higher than 10 °Brix, which is the minimum value required by the fresh fruit market in Brazil and in the United States (ARPAIA; KADER, 2000; CEAGESP, 2011). The TSS contents were also above the international standard established by the Organization for Economic Co-operation and Development (OECD, 2010) and by the juice processing industry (LADANIYA, 2008), 8 °Brix. The lowest TSS content, 9.1°Brix, was for the Olinda Valencia fruits. This value was lower than the ones reported by Castle and Baldwin (2011) for the same selection grown in St. Cloud, FL, USA, for fruits from five years old trees, 12.7 °Brix. These variations may be related to tree age, soil-climate conditions, fertilization, and used rootstock.

The titratable acidity (TA) or acidity level, expressed as grams of citric acid per 100 mL of juice, ranged from 0.7 to 1.1 g 100.mL<sup>-1</sup> in juices of the sweet orange selections. This acidity level is above the minimum demanded by the fresh market (POZZAN; TRIBONE, 2005) and within the range for juice processing, from 0.7 to 1.2 g 100.mL<sup>-1</sup> (BERK, 2016). All sweet orange selections included in the study exhibited maturity index above the minimum international standards of the fresh market, 6.5:1 (OECD, 2010). Moreover, most sweet orange selections achieved the maturity value

of 12, which is the threshold value demanded by juice processing (POZZAN; TRIBONE, 2005), though they were inferior to 16, considered as the maximum threshold value for human consumption (JONES; CREE, 1965).

Based on our results, most sweet orange selections showed similar performance indices for the fresh fruit market. The exception was the Olinda Valencia that did not have a good performance in our field trial for the fresh and the industrial markets. For this reason, this cultivar was not included in the performance index estimates. On the other hand, all the other selections can be indicated for the fresh fruit market. Significant differences were found among the performance indices estimated for juice processing. Interestingly, Natal África do Sul and Charmute de Brotas had higher estimates, suggesting a better performance for the juice processing than some other tested selections.

Differences were found among the sweet orange selections for the incidence of citrus canker on harvested fruits. Valencia Frost and Valencia Late Fla. had the highest incidence of citrus canker on fruits, differing from all other selections. These two sweet oranges have been reported in previous studies as very susceptible to citrus canker (CARVALHO et al., 2015). Valencia Late Fla. showed a moderate resistance to citrus canker when trees were grown under protected conditions (AMARAL et al., 2010), but were highly susceptible when cultivated under field conditions (CARVALHO et al., 2015). The lowest incidence of citrus canker was found on fruits of IPR Folha Murcha, Natal Murcha, Charmute de Brotas and Valencia Chafeei Late, Campbell 479, Campbell 294, Olinda, Mutaç o and Whit's Late, in agreement with the results reported by Vargas et al. (2013) and Carvalho et al. (2015).

The incidence of citrus canker on harvested fruit progressed differently over the two seasons, 2020 and 2021 (Table 3.4.7). Most of the variations on canker incidence on fruits could be related to the environmental conditions for disease development, as higher temperature and intense rainfall are frequent during the rainy season in southern Brazil (Figure 3.3.1). Constant rainfalls and higher temperatures during the early stage of fruit development favors infection and dissemination of the citrus canker bacterium, as the *Xcc* dispersion is favored by wind-driven rain splash (BOCK et al., 2010). The penetration of the bacterium into the host tissue is facilitated by natural openings, as leaf stomata (GRAHAM et al., 2016), and wounds caused by citrus leafminer larvae (*Phyllocnistis citrella* Stainton), thorns, and soil particle abrasion,

particularly in citrus fruitlets that is more susceptible to Xcc infection (BOCK et al. 2010; BEHLAU et al., 2021b).

Citrus canker has become a serious problem for the Brazilian growers as the younger stage of the fruits coincides with the rainy season, September–March (AMARAL et al., 2010; LANZA et al., 2019). In 2020, moderate rainfall volume, 540 mm, and temperature were recorded during the rainy season comparing to 2021, 680 mm (Tables 3.4.7; Figure 3.3.1). A higher rainfall volume in 2021 may have contributed to increase the incidence of canker in fruits, favored by bacterial dispersion and further infection on fruits at the early stages of development. A longer rainy season may have promoted the progression and severity of the disease as previously noticed by Behlau et al. (2010). The highest canker incidence observed in the 2021 harvest may have impacted on fruit yield, as a reduction in production was found compared to the previous season (Table 3.4.2).

All tested sweet orange selections were susceptible to the infection by the CLas bacterium. Although there is no citrus cultivar immune to CLas (RODRIGUES et al., 2020), significant differences ( $p \leq 0.001$ ) were found within the sweet orange selections in relation to the cumulative incidence of HLB (Figure 3.4.1). Natal IAC and Folha Murcha IAC had the lowest frequency of CLas-infected trees (Figure 3.4.1). Further, previous studies have demonstrated that the orchard arrangement may play an important role in the dispersion of the CLas vector, the ACP (MARTINI et al., 2015), and consequently in the incidence of HLB-infected trees in the orchard. Trees of different size at the same area have showed differences in the ACP population dynamics (RODRIGUES et al., 2020). Larger trees may serve as a barrier preventing the ACP from landing on smaller trees. Further, smaller trees may be less affected due to the flushing pattern of these trees (RODRIGUES et al., 2020). Moreover, the presence of smaller trees surrounded by larger ones may reduce their exposure to wind and sun similar to the effect associated with windbreaks (BAUDRY et al., 2000; MARTINI et al., 2015). Windbreaks induce shading and temperature changes in orchards, which reduce the tree flushing and consequently the attractiveness by the ACP, decreasing the incidence of HLB (MARTINI et al., 2015).

Folha Murcha IAC and IPR Folha Murcha have showed lower incidence of HLB-affected trees compared to Pera sweet orange under the same conditions of our field trial (ALMEIDA et al., 2016). Lower HLB incidence in these two selections may be explained by the delayed bud break (CANTUARIAS-AVILÉS et al., 2011), the longer

period to produce new shoots in early spring (CARVALHO et al., 2021c) and the morphological characteristics of the curling leaves. Therefore, these phenological and morphological traits may favor the delay of new vegetative flushes, escaping from HLB bacterium transmission and consequent infection (CIFUENTES-ARENAS et al., 2018). Further, the ACP population typically peaks at late spring and early summer in Brazil and in some other important citrus-growing regions (PRIMO-MILLO; AGUSTÍ, 2020; CARVALHO et al., 2021c). Folha Murcha sweet orange usually produces shoots of shorter lengths, which limits the contact area for ACP feeding. Our results suggest that the variations in tree size, curled leaves, flushing pattern and time of flushes may have contributed to the response of these cultivars to the ACP preference and, consequently, to the HLB incidence. Stover and McCollum (2011) also observed similar situation evaluating the HLB incidence in different citrus cultivars. Therefore, further investigations may be necessary to better determine the agronomic performance under homogeneous orchards of the sweet orange selections that exhibited low incidence of HLB in our experimental trial.

Overall, the 19 assessed late-season sweet orange selections show significant differences for several horticultural traits, including vegetative growth, fruit yield and quality, row/tree spacing and yield estimates, performance for fresh fruit market and industrial processing, and incidences of citrus canker and cumulative HLB. IPR Folha Murcha, Natal IAC, Natal Murcha and Folha Murcha IAC had the smallest vegetative growth (Table 2; Figure 3), which favored the lowest tree/row spacings, and the highest tree density determined in the estimates study (Table 6). On the other hand, the majority of the Valencia selections had the most vigorous trees among the evaluated late-season sweet oranges, particularly Olinda that stayed alone from all other selections in the principal component analysis (Figure 3), requiring the largest spacings and the lowest tree density, similarly to Valencia #121 (Table 2 and 6). All evaluated sweet orange selections were graft-compatible with the Rangpur lime rootstock, as no scion/rootstock overgrowth or tree decline were observed across nine years of evaluation. Whit's Late Valencia and Natal África do Sul were the most productive trees among the tested selections, with excelled yields over six cropping seasons (Table 3). Most late-season sweet orange selections produced fruits of excellent quality, except Olinda Valencia. Fruits of Valencia selections had the highest juice content with improved peel color (Table 4 and 5). Fruits of Charmute de Brotas, Natal África do Sul, Natal IAC, Natal Murcha, IPR Folha Murcha and the Valencia

selections Frost, IAC, Campbell 479, Late 161, Late Fla., and #121 had juice of higher TSS contents. This favored an outstanding TSS.TA<sup>-1</sup> ratio and juice color in the cases of Natal Murcha, Charmute de Brotas, Natal África do Sul, and IPR Folha Murcha (Table 5). All assessed late-season sweet oranges exhibited similar performances for the fresh fruit market, while Natal África do Sul and Charmute the Brotas were more indicated for industrial processing (Table 7). Furthermore, all evaluated selections exhibited different levels of citrus canker incidence on harvested fruits and HLB incidence in trees (Table 8; Figure 2). IPR Folha Murcha had the lowest incidence of canker on fruits while Folha Murcha IAC and Natal IAC expressed lower cumulative HLB incidence than all other tested selections, evidencing their excelled horticultural performance in the humid subtropical region.

### 3.6 CONCLUSIONS

Based on our findings, Natal IAC, Folha Murcha IAC, IPR Folha Murcha, Natal Murcha, and the Valencia selections Campbell 479 and Late Fla. express better horticultural performance than other late-season selections under the humid subtropical climate. These selections have low HLB incidence, moderate incidence of citrus canker on fruits, outstanding yield efficiency, excellent fruit quality that attends the standards of the fresh fruit market and the juice processing industry, and low vegetative growth favoring higher tree density in new plantings. This optimizes the land use, field management and orchard operations, harvesting, yield, and faster return of the investments under an HLB endemic scenario. In contrast, the Valencia selections Olinda and Mutação are not recommended for planting in the Brazilian humid subtropical region or in regions with similar conditions, as these selections show poor horticultural performance. Together, our findings contribute to a more strategic and oriented recommendation of late-season sweet orange for the establishment of new plantings in the humid subtropical regions in the face of HLB pressure.

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## 4 ARTIGO B

# PHYSICOCHEMICAL QUALITY AND ANTIOXIDANT CAPACITY OF SWEET ORANGE FRUITS AND BY-PRODUCTS AND EFFECTIVENESS OF THE CARNAUBA WAX AND ROSIN RESIN-BASED COATING TO PROLONG FRUIT POSTHARVEST LIFE

### 4.1 ABSTRACT

Large amounts of by-products generated from the citrus processing are wasted every year, mainly due to the lack of appropriate practices. These products are rich in bioactive compounds such as phenolics, flavonoids, antioxidants, fibers, and vitamins. Moreover, coatings may be used in the citrus postharvest storage to preserve fruit quality and to prolong shelf life. Carnauba wax and rosin resin are derived from renewable resources and present numerous feasible applications, including fruit coating. In this paper, we report the evaluation of physicochemical quality and antioxidant capacity of the late-season Valencia Late and Natal IAC sweet orange fruits and their by-products (peel, pulp, and rag), as well as the effectiveness of the carnauba wax/rosin resin-based coating to prolong postharvest life under cold storage conditions. Mature Valencia Late and Natal IAC late sweet orange fruits were harvested in November of 2019 and 2020. In 2019, fruits and their by-products were only characterized based on the physicochemical attributes and antioxidant capacity. In 2020, fruit were treated with carnauba wax/rosin resin coating and stored for 0, 15, 30, 45 and 60 days in cold chamber at  $5 \pm 1$  °C and 60–70% RH. Fruit color index, weight loss, physicochemical quality and sensory profile of the fruit were monitored at harvest and during the cold storage period. Each evaluation was performed in triplicates of 10 fruits per treatment. Fruits of Valencia Late and Natal IAC sweet oranges showed adequate physicochemical quality in both years, attending the requirements for fresh fruit and processing. Flavedo and albedo tissues (peel) showed the highest total phenolic and flavonoid contents, as well as, the antioxidant activity, while the segment wall and the juice sacs (pulp and rag) showed the lowest values for these attributes. Carnauba wax/rosin resin coating treatment associated with cold storage was efficient to preserve fruit color and to retard weight loss for both sweet orange cultivars up to 60 days. Additionally, the physicochemical quality and the sensory attributes of the carnauba wax/rosin resin treated fruits were preserved during cold storage, while uncoated fruit had the lowest scores for most of the sensory attributes after 60 days of cold storage. Together, the by-products from Valencia Late and Natal IAC fruits contain high levels of healthy beneficial compounds. These compounds may be used as source of low-cost antioxidants. Furthermore, the wax-coating associated with cold storage was efficient to decrease weight loss and to preserve color development, and overall physicochemical and sensory quality of the fruit.

*Keywords:* *Citrus xsinensis* (L.) Osbeck; late-season cultivars; citrus by-products; cold storage; renewable fruit coatings.

## 4.2 INTRODUCTION

Fresh fruit consumption has increased worldwide, pushed by consumers concerned on high-quality and healthy food habits (LADANIYA, 2008). *Citrus* spp. are widely cultivated in the world, represented by a diversity of species grown in tropical, subtropical and in the Mediterranean regions. Brazil leads the production of sweet oranges [*Citrus x sinensis* (L.) Osbeck]. Nearly 70% of these fruits are processed to produce frozen concentrated orange juice (FCOJ) and not from concentrate juice (NFC) for exports (FAO, 2020). In this context, the citrus-processing industry generates large amounts of by-products every year, including peel (flavedo and albedo), pulp (juice sacs), rag (segment wall and central core) and seed residues (LERTWORASIRIKUL; SAETAN, 2010; ZARINA; TAN, 2013) that accounts for about half of the total fruit weight (LI et al., 2006). The citrus by-products may become waste and a possible source of environmental pollution if not processed for animal feeding and organic matter in agriculture (WANG et al., 2008; GAHNEM et al., 2012). However, these by-products could be a major source of phenolic and antioxidant compounds (MA et al., 2009). Therefore, better practices on citrus by-products application are essential to determine the potential of them as healthy beneficial products, such as outstanding low-cost antioxidant source (CASTRO-VAZQUEZ et al., 2016).

Although attending the needs of the internal fresh fruit market, the Brazilian citrus industry has a small share of the global fresh fruit market mostly due to phytosanitary problems associated with the lack of a developed postharvest processing structure. Under this context, the citrus fruit can have a long postharvest life if appropriate practices are adopted (LADANIYA, 2008). Proper postharvest practices for the citrus fresh fruit are essential to preserve quality, to extend shelf life and to avoid losses. Fruit losses are substantially high in low-income countries, because of poor postharvest management, involving limited refrigeration. In contrast, developed countries usually have good postharvest management, and the fruit undergo to a cold chain at the very early stage after harvest and remain under this condition up to consumption (TARANCÓN et al., 2021). The cold chain is responsible for maintaining the fresh fruit quality, as the cooling process retards the deterioration and losses associated with natural senescence by decreasing the metabolic rates (GOEDHALS-GERBER; KHUMALO, 2020). However, investments in cold chain and high-tech postharvest management practices are still a challenge for low-income

countries, where politics with efforts for nonhazardous and safe food management are taking place gradually.

The application of natural-based coating materials, involving waxes and resins, on fresh fruit are recognized as eco-friendly approaches and can be used by low-income countries where natural resources are available (GUTIÉRREZ et al., 2019). These materials are becoming very popular in the citrus postharvest practices as they can reduce losses by the differential permeability of CO<sub>2</sub>, O<sub>2</sub> and water vapor, decreasing the metabolic rate and water losses (LADANIYA, 2008; MIRANDA et al., 2021). Protective coatings may be extracted from different natural and renewable resources as carnauba palm (MOTAMEDI et al., 2018; DE FREITAS et al., 2019), sugarcane (JONGLERTJUNYA et al., 2014), soybean (FEI et al., 2021), candelilla (HAGENMAIER, 2000), coconut (BASWAL et al., 2020), wood (GAILLARD et al., 2011; BARMAN et al., 2019), and beeswax (BASWAL et al., 2020; NASRIN et al., 2020). Carnauba wax is derived from leaves of palm trees [*Copernicia prunifera* (Mill.) HE Moore], native from Brazil and found in the tropical rainforests (LORENZI et al., 2010). This wax is widely used in commercial fruit coating compounds to increase toughness and luster (BARMAN et al., 2019; DE FREITAS et al., 2019). Rosins, a.k.a. colophony, can also be applied as protective coatings on fresh fruits (GAILLARD et al., 2011). Rosins are residues left after distillation of the volatile fraction of pine oil and turpentine from the crude resin of the pine trees (GAILLARD et al., 2011; BARMAN et al., 2019). Furthermore, carnauba wax and wood resin are allowed as non-synthetic ingredients in coatings for organic citrus (LADANIYA, 2008).

The application of renewable coating materials on fresh fruit is an effective postharvest practice to preserve fruit quality and to prolong shelf life by improving visual quality and reducing postharvest losses (MIRANDA et al., 2021). Therefore, the objectives of this study were to evaluate the physicochemical quality and antioxidant capacity of the late-season Valencia Late and Natal IAC sweet orange fruits and their by-products, as well as the effectiveness of the carnauba wax and rosin resin-based coating to prolong the postharvest life of these fruits under cold storage condition.

#### 4.3 MATERIALS AND METHODS

##### 4.3.1 Fruit Harvest and Treatments

Fully mature fruit of Valencia Late and Natal IAC sweet oranges [*C. xsinensis* (L.) Osbeck] were harvested in mid-November 2019 for fruit characterization and mid-November 2020 for postharvest assay from an experimental orchard located in Guairaçá, state of Paraná, Brazil. The sweet orange trees were grafted onto Rangpur lime [*C. xlimonia* (L.) Osbeck] and were seven to eight-year-old. Three replicates of 20 fruits per cultivar were collected from 10 trees in 2019, while 500 fruits per cultivar were harvested in 2020. After harvest, fruits were immediately transferred to a cold chamber for pre-cooling for 12 h at  $5 \pm 1$  °C and 60–70% RH at the facility of the Instituto de Desenvolvimento Rural do Paraná – IAPAR/EMATER (IDR-Paraná) in Londrina, state of Paraná, Brazil. The fruits were hand-washed with neutral detergent under tap water and submerged in 1.0% NaCl solution. For the postharvest assay, half of each fruit batch (250 fruits) was coated with carnauba wax/rosin resin (10/1) at 18% total solids (Aruá, BR 18, Matão, SP, Brazil). The other half of the fruits were uncoated and used as control. Fruits were packed in industrial plastic boxes (40.8 kg capacity) and stored for 0, 15, 30, 45 and 60 days in a cold chamber at  $5 \pm 1$  °C and 60–70% RH.

#### 4.3.2 Fruit Analyses

Three replicates of 10 fruit per cultivar were evaluated in regard to physical characteristics, i.e., weight, length, diameter, shape index, flavedo and albedo thickness, color, juice content, and number of seeds; and chemical attributes, i.e., total soluble solids (TSS), titratable acidity (TA) and ratio (TSS.TA<sup>-1</sup>). Total phenolics, total flavonoids and the percentage of DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging in different tissues from fruits were determined using three replicates of 10 fruits per cultivar. The antioxidant analyses were performed at the Citrus Horticultural Lab of the Southwest Florida Research and Education Center (SWFREC – UF/IFAS) in Immokalee, FL, United States, whereas the fruit characterization and postharvest assay were conducted at the Plant Protection Lab of the Instituto de Desenvolvimento Rural do Paraná (IDR-Paraná) in Londrina, PR, Brazil.

Fruit samples were weighted (g) using a semi-analytical scale (Bel, M505, Piracicaba, SP, Brazil). Fruit length, diameter, flavedo and albedo thickness (mm) were measured with a digital Vernier caliper (Mitutoyo, ABS, Kawasaki, Kanagawa, Japan) and classified according to the fresh fruit standards (CEAGESP, 2011). Fruit shape index was calculated based on the relation between fruit length and diameter.

Fruit color was determined by three readings along the equatorial circumference of each fruit using a digital colorimeter (Konica Minolta Sensing Americas Inc., CR-400, Ramsey, MN, USA). This attribute was expressed as citrus color index (CCI) (JIMÉNEZ-CUESTA et al., 1981) based on the CIE  $L^*a^*b^*$  color scale (McGUIRE, 1992). The CCI is a comprehensive indicator of color impression with positive values for red, negative values for blue-green, and zero for an intermediate mixture of red, yellow, and blue green (ZHOU et al., 2010).

Fruit samples were juiced using a Croydon extractor (Croydon, ES4EA-B60000, Duque de Caxias, RJ, Brazil). Juice content was determined according to the following equation and expressed as percentage (%):

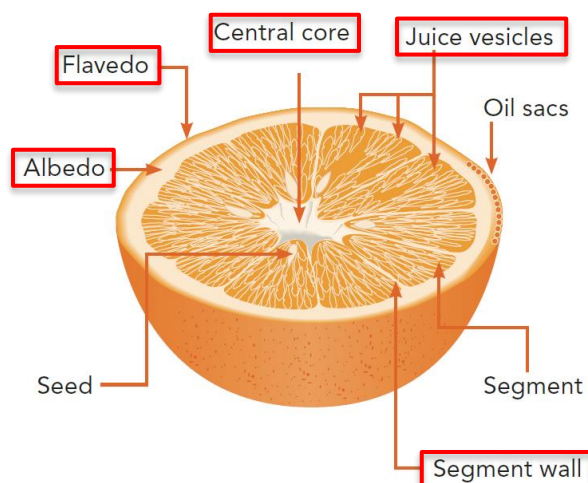
$$JC = \frac{JW}{FW} \times 100,$$

where,  $JC$  = juice content,  $FW$  = fruit weight (g), and  $JW$  = juice weight (g).

The number of seeds was manually counted. The TSS was measured in a drop of undiluted juice with a digital refractometer (Atago Co., Ltd, PAL-3, Tokyo, Kantō, Japan) at 20 °C. Total soluble solids were expressed in °Brix units. Titratable acidity was determined in 25 mL of diluted juice (5 mL of juice and 20 mL of distilled water), 0.1 N NaOH and phenolphthalein as indicator in a TitroLine easy titrator (Schott Instruments GmbH, TitroLine easy, Mainz, Rhineland-Palatinate, Germany). The results were expressed in grams of citric acid per 100 mL of juice ( $\text{g}\cdot 100\text{mL}^{-1}$ ) (AOAC, 2019). The ratio ( $\text{TSS}\cdot\text{TA}^{-1}$ ) was then calculated to determine fruit maturity.

The fruit tissues were manually separated into flavedo, albedo, juice sacs, segment wall and central core (Figure 4.4.1). Then, they were freeze-dried at  $-40$  °C under vacuum (Labconco Co., Freeze Drier 8, Kansas City, KA, USA) until total dehydration ( $\sim 50$  h). Prior to analysis, the fruit tissues were ground in liquid nitrogen using a mortar and pestle. Fifty milligrams of powdered tissue were extracted twice with 1 mL of absolute methanol for 30 min in the dark, at room temperature. After centrifugation at  $20,000 \times g$  (Centrifuge 5417 C, Eppendorf, Hamburg, Germany) for 5 min, supernatants were filtered through a  $0.45 \mu\text{m}$  syringe filter (Corning Inc., SFCA, Tewksbury, MA, USA) to obtain a clear solution which was dried in vacuum (Concentrator Plus/Vacufuge® Plus, Eppendorf, Hamburg, Germany) for 1.5 h at room temperature. The dried extracts were re-suspended in 1 mL Milli-Q water, vortexed

and centrifuged at 20,000 × g (Centrifuge 5417 C, Eppendorf, Hamburg, Germany) for 1 min. The supernatants were used to quantify total phenolics, total flavonoids and the percentage of DPPH scavenging by spectrophotometry (Molecular Devices, Spectramax 190, San Jose, CA, USA). All assays were conducted in triplicate.



**Figure 3.4.1** Different fruit tissues extracted from Valencia Late and Natal IAC, including flavedo, albedo, central core, juice sacs and segment wall.

The total phenolic content was determined according to Rahman et al. (2018). An aliquot of 12.5  $\mu\text{L}$  supernatant were diluted in 50  $\mu\text{L}$  Milli-Q water and maintained at room temperature for 1 h with 12.5  $\mu\text{L}$  of 1N Folin-Ciocalteu and 125  $\mu\text{L}$  of 7%  $\text{NaCO}_3$ . The absorbance was measured against a blank at 750 nm in a spectrometer (Molecular Devices, Spectramax 190, San Jose, CA, USA) and the total phenolic content determined by using a standard calibration curve developed with gallic acid (Sigma-Aldrich, Saint Louis, MO, USA). Phenolic concentrations were expressed as gallic acid equivalent ( $\text{mg GAE} \times 100 \text{ g dry tissue}^{-1}$ ).

The total flavonoids were determined using the method described by Wang et al. (2008) with some modifications. An aliquot of 25  $\mu\text{L}$  supernatant was mixed with 25  $\mu\text{L}$  of 5%  $\text{NaNO}_2$  and incubated for 6 min at room temperature in the dark. After, 25  $\mu\text{L}$  of 10%  $\text{AlCl}_3$  were added to the reaction and incubated again for 6 min. The reaction was stopped by adding 125  $\mu\text{L}$  of 4%  $\text{NaOH}$ . After 15 min in the dark at room temperature for color development, the absorbance was measured at 350 nm in a spectrometer (Molecular Devices, Spectramax 190, San Jose, CA, USA). The total

flavonoids were expressed as hesperidin equivalents through the calibration curve developed with hesperidin (Sigma-Aldrich, Saint Louis, MO, USA). The results were expressed as hesperidin equivalents (mg of hesperidin  $\times$  100 g dry tissue<sup>-1</sup>).

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the fruit tissues was measured using the method previously reported by He et al. (2012), with minor modifications. An aliquot of 150  $\mu$ L 0.2mM DPPH radical diluted in methanol was mixed with 50  $\mu$ L supernatant. Then, the mixture was vortexed for 30 s and incubated for 30 min in the dark at room temperature for color development. After incubation period, the absorbance of the reaction progress was recorded at 517 nm in a spectrometer (Molecular Devices, Spectramax 190, San Jose, CA, USA). The percentage of inhibition of DPPH radical was expressed and calculated according to He et al. (2012):

$$\% \text{ Antioxidant activity} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100,$$

where,  $Abs_{control}$  = absorbance of the control; and  $Abs_{sample}$  = absorbance of the sample

#### 4.3.3 Postharvest Assay

Carnauba wax/rosin resin coated, and uncoated fruits were evaluated periodically at 0, 15, 30, 45, and 60 days during cold storage for the following characteristics: fruit color index, weight loss, juice content, TSS, TA, ratio, and sensory profile. A triplicate of 10 fruits per treatment was evaluated each time. Fruit color, juice content, TSS, TA and ratio was determined as described previously. Fruits were weighted just after harvest and the weight loss was monitored after each period of cold storage using a semi-analytical scale (Bel, M505, Piracicaba, SP, Brazil). Total weight loss was expressed as a percentage according to the standard method of the AOAC (2019).

The sensory profile of Valencia Late and Natal IAC sweet orange fruits were assessed by 12-untrained panelists, five men and seven women, after each period of cold storage and wax coating treatment. Participants were asked voluntarily to take part of the sensory tasting sessions, without any compensation. Panelists and frequent sweet orange consumers were recruited from a pool of staff and graduate students, with ages ranging from 17 to 53 years old, from the department of Plant Protection of

the IDR-Paraná. The evaluation was performed in a standard testing room with white LED lights. Ten fruits per treatment were assessed during each session, at harvest and 15, 30, 45 and 60 days after cold storage. Prior to the sensory evaluations, fruit samples were equilibrated at room temperature for 1 h and then hand peeled. Fruits were separated in segments and sectioned into uniform pieces. Each panelist received one entire fruit and three peeled sectioned fruit segments prepared from different fruits of each treatment. The samples were placed on white disposable plates randomly arranged and identified by three-digit random codes (triads) to avoid potential biases during the sessions. Panelists were provided with a glass of water at room temperature for palate cleansing and instructed to rinse their mouth with water before and between samplings. After signing the consent form of the study, the panelists were instructed to rate the sensory attributes using a nine-point hedonic scale. Based on their preference, the panelists rated the following sensory attributes for the sweet orange fruits: color, texture, firmness, aroma, flavor, juiciness, and preference. The scores rated by the panelists were used to build the preference mapping to describe the sensory attributes.

#### 4.3.4 Statistical Analyses

The statistical design for both fruit characterization and postharvest assay were a complete randomized design. In the case of the postharvest assay, the experimental design was a complete randomized in a factorial arrangement, with four treatments (two cultivars × two coating treatments) and five storage periods (0, 15, 30, 45, and 60 days of cold storage). The data were tested for normal distribution and homogeneity at  $p \leq 0.05$ , submitted to the analysis of variance (ANOVA) and the differences between means were compared by the Tukey's test ( $p \leq 0.05$ ). All analyses were carried out using the packages *ExpDes* (HUSSON et al., 2017) and *ggplot2* (WICKHAM, 2016) in R v. 4.0.2 (The R Foundation for Statistical Computing, Vienna, Austria).

### 4.4 RESULTS

#### 4.4.1 Pre-storage Fruit Evaluation

Fruits of Valencia Late and Natal IAC were initially characterized according to their physicochemical quality attributes (Table 4.4.1) and antioxidant capacity (Figure 4.4.1). Valencia Late fruits were smaller and lighter than the ones of Natal IAC, considering fruit weight (Table 4.4.1). The fruit shape indices ranged from 0.93 for Valencia Late to 0.99 for Natal IAC, indicating a round or sub-globose shape. The citrus color index (CCI) was relatively higher for Natal IAC (2.11) than Valencia Late (1.57). The two tested cultivars showed similarities for flavedo and albedo thickness, as well as for the number of seeds per fruit. The lowest juice content in fruit was for Valencia Late with 36%, while the fruit of Natal IAC had higher juice content, >41%. Also, Natal IAC fruit had higher total soluble solids (TSS) and titratable acidity (TA). Similar sugar-acidity ratios (TSS.TA<sup>-1</sup>) were found for the fruit of the two cultivars, ranging from 11.2 for Natal IAC to 12.0 for Valencia Late.

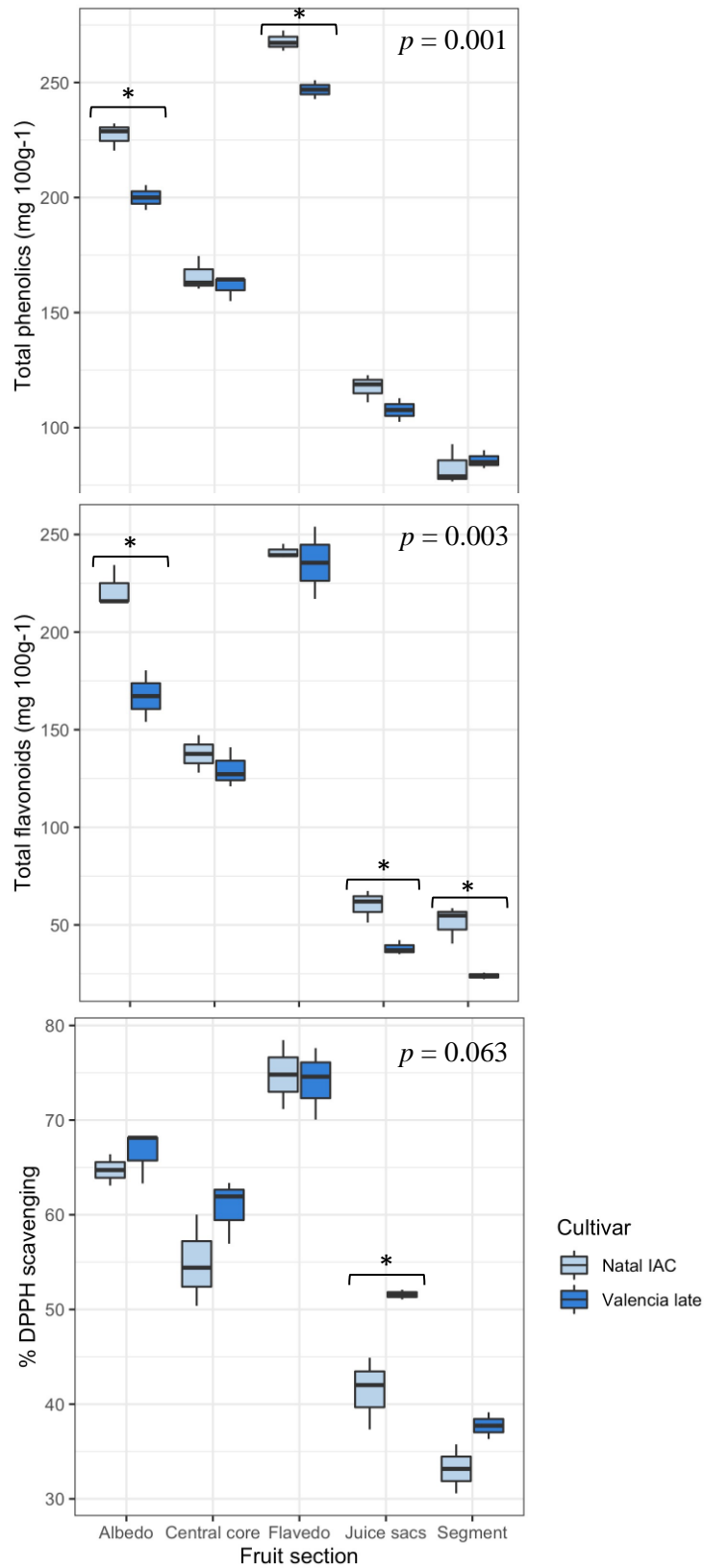
**Table 4.4.1** Physicochemical quality of Valencia Late and Natal IAC sweet orange fruits evaluated in the 2019 harvest. Each value represents the mean of triplicate samples (mean value ± standard deviation).

Fruit attribute	Valencia Late	Natal IAC	CV (%)	F value
Fruit length – FL (mm)	70.0 ± 1.17 a <sup>1</sup>	72.7 ± 2.95 a	3.15	2.21ns
Fruit diameter – FD (mm)	75.0 ± 1.18 a	73.4 ± 0.64 a	1.28	4.10ns
Fruit shape index (FL.FD <sup>-1</sup> )	0.93 ± 0.01 a	0.99 ± 0.05 a	3.49	4.36ns
Citrus color index – CCI	1.57 ± 0.34 b	2.11 ± 0.06 a	13.0	7.60*
Flavedo thickness (mm)	2.68 ± 0.30 a	2.74 ± 0.15 a	8.77	0.09ns
Albedo thickness (mm)	2.36 ± 0.25 a	2.33 ± 0.11 a	8.24	0.05ns
Fruit weight (g)	162 ± 2.25 a	150 ± 8.67 b	3.99	11.8*
Number of seeds	2 ± 0.92 a	2 ± 0.40 a	36.0	0.21ns
Juice content (%)	36.1 ± 2.91 a	41.3 ± 2.39 a	6.88	5.86ns
Soluble solids content – SST (°Brix)	11.2 ± 0.95 a	12.1 ± 0.64 a	6.95	1.98ns
Titratable acidity – TA (g.100 mL <sup>-1</sup> )	0.93 ± 0.13 a	1.08 ± 0.03 a	9.49	3.52ns
Ratio (SST.TA <sup>-1</sup> )	12.0 ± 0.65 a	11.2 ± 0.88 a	6.62	1.61ns

<sup>1</sup>Means followed by the same letter in the column do not significantly differ according to the Tukey's test. Significance level: \*,  $p \leq 0.05$ ; ns, non-significant.

The total of phenolics and flavonoids as well as the percentage of DPPH scavenging activity in the different fruit tissues including flavedo, albedo, central core,

juice sacs and segment wall of Valencia Late and Natal IAC sweet oranges were assessed (Figure 4.4.2). The highest content of phenolics and flavonoids as well as the DPPH scavenging activity were observed in the flavedo tissues for the fruit of both sweet orange cultivars while the juice sacs and the segment wall showed the lowest amount of these compounds. The albedo and central core tissues had intermediate values for the three compounds. Regarding the phenolic content, Natal IAC fruit achieved ( $p \leq 0.05$ ) higher level in flavedo ( $268 \pm 4.4$  mg GAE.100 g<sup>-1</sup>) and albedo ( $227 \pm 6.0$  mg GAE.100 g<sup>-1</sup>) tissues than Valencia Late fruit ( $247 \pm 4.1$  and  $200 \pm 5.4$  mg GAE.100 g<sup>-1</sup>, respectively). However, no such differences were observed for all other fruit tissues. Significant differences ( $p \leq 0.05$ ) were also found for the total flavonoids, as Natal IAC fruit had higher levels in the albedo, juice sacs and segment wall tissues ( $222 \pm 10.8$ ,  $60.2 \pm 8.2$ ,  $51 \pm 9.6$  mg of hesperidin per 100 g, respectively) than Valencia IAC ( $167 \pm 13.2$ ,  $38.0 \pm 3.7$ ,  $24 \pm 1.7$  mg of hesperidin per 100 g, respectively). In regard to the DPPH scavenging activity, juice sacs of Valencia Late fruit had significantly higher ( $p \leq 0.05$ ) antioxidant capacity ( $52 \pm 0.5\%$  of DPPH scavenging) than the Natal IAC juice sacs ( $41 \pm 3.8\%$  of DPPH scavenging).



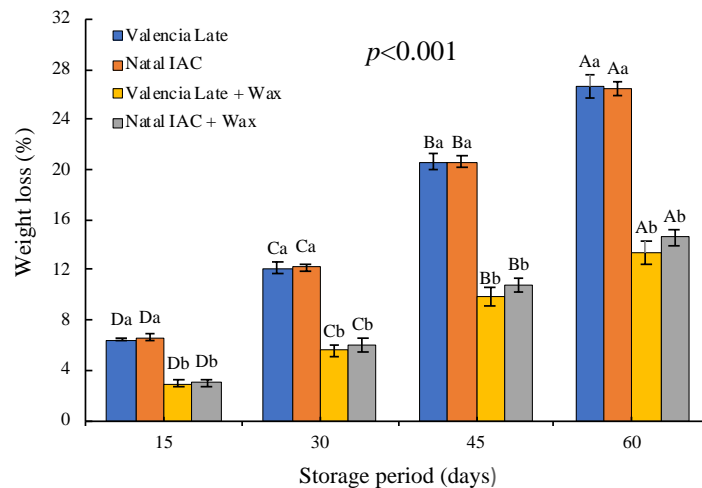
**Figure 4.4.2** Total phenolic content (mg GAE.100 g of dry tissue<sup>-1</sup>), total flavonoid content (mg hesperidin per 100 g of dry tissue), and percentage of DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity of different fruit tissues (flavedo, albedo,

central core, juice sacs and segment wall) of Valencia Late and Natal IAC sweet orange fruits. Significance level: \*,  $p \leq 0.05$ .

#### 4.4.2 Postharvest Fruit Evaluation

##### 4.4.2.1 Weight loss

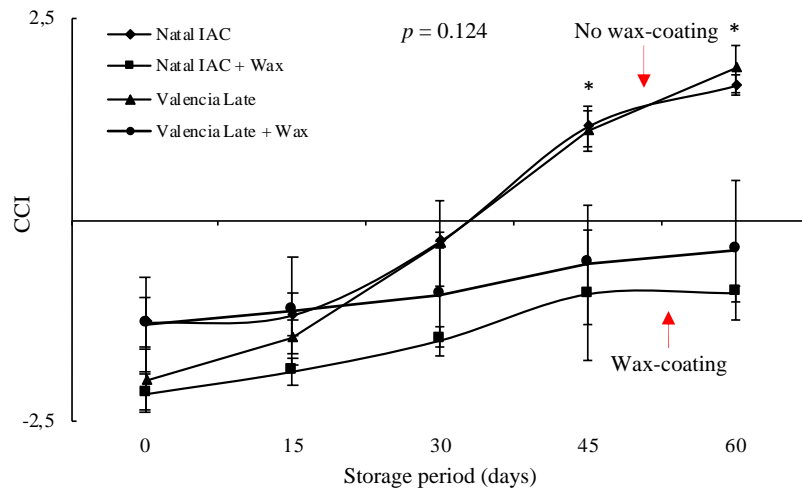
The weight loss of the fruit of the two sweet orange cultivars, Valencia Late and Natal IAC, increased significantly ( $p \leq 0.001$ ), particularly for those without wax coating treatment (Figure 4.4.3). Fruits of both sweet orange cultivars were similar for the same wax-coating treatment at each period of storage, but had differences in regard to the postharvest treatments, wax coating or non-coating. After 60 days of cold storage, fruit without coating treatment exceeded 25% of weight loss for both cultivars. On the other hand, the wax-coating fruit had a loss rate below 15%. For all four-storage periods, the non-coating fruit had twice the weight loss compared to the wax-coating ones, supporting the effectiveness of this postharvest treatment.



**Figure 4.4.3** Weight loss (%) of Valencia Late and Natal IAC sweet orange fruits treated with carnauba wax/rosin resin and maintained under different cold storage periods (15, 30, 45 and 60 days). Bars followed by the same capital or lowercase letter do not differ significantly in regard to the storage period and coating treatment, respectively, according to the Tukey's test ( $p \leq 0.05$ ).

#### 4.4.2.2 Fruit color index

The fruit color development of the fruit of the two assessed sweet orange cultivars were monitored for the entire storage period, from 0 (harvest) to 15, 30, 45 and 60 days (Figure 4.4.4). As main affects, the storage period and cultivar were highly significant ( $p \leq 0.001$ ), but no significant interaction of these two factors was found in the postharvest assay (Figure 4.4.4). The fruit color index progressed during the storage period for the uncoated treatment (control) of both cultivars. In contrast, no color progression was observed for the wax-coating treatment, regardless of the cultivar. Differences in the wax-coating treatments were observed after 30 days of cold storage but become evident ( $p \leq 0.05$ ) at 45 and 60 days of storage. Fruit of both cultivars had similar performance for color development.



**Figure 4.4.4** Citrus color indices (CCI) of Valencia Late and Natal IAC sweet orange fruits subjected to carnauba wax/rosin resin coating treatment after 0 (harvest), 15, 30, 45 and 60 days of cold storage. Significance level: \*,  $p \leq 0.05$ .

#### 4.4.2.3 Juice quality

The juice quality of the Valencia Late and Natal IAC fruits that received the uncoated and wax-coating postharvest treatments was evaluated at 0 (harvest), 15, 30, 45 and 60 days after cold storage (Table 4.4.2). No significant interactions ( $p \leq$

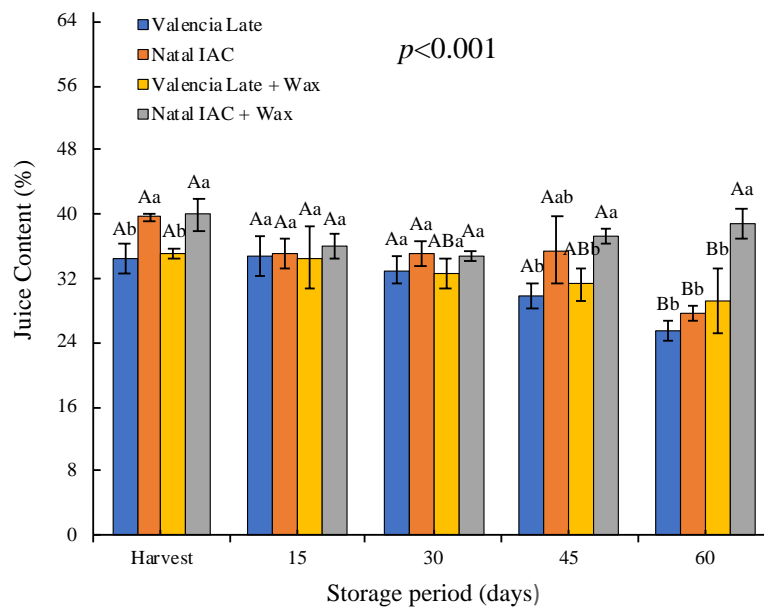
0.05) were found between the postharvest treatment and storage period. In contrast, significant differences ( $p \leq 0.05$ ) were observed for TSS and TSS.TA<sup>-1</sup> ratio for the postharvest treatments. The highest TSS content was found in wax-coated and uncoated fruits of Valencia Late and for uncoated Natal IAC fruits. Fruits of Valencia Late also displayed high TSS.TA<sup>-1</sup> ratio with an average of 18.7, diverging significantly from wax-coated fruit of Natal IAC. The acidity level was also similar among the treatments ranging from 0.62 to 0.75 g of citric acid per 100 mL of juice. In regard to the storage period, no significant differences were found among the treatments over the storage period.

**Table 4.4.2** Total soluble solids (TSS), titratable acidity (TA), and ratio (TSS.TA<sup>-1</sup>) of Valencia Late and Natal IAC sweet orange fruits subjected to carnauba wax/rosin resin coating treatment and different cold storage periods (mean value  $\pm$  standard deviation).

Source of variation	Total soluble solids (TSS) (°Brix)	Titratable acidity (TA) (g.100 mL <sup>-1</sup> )	Ratio (TSS.TA <sup>-1</sup> )
Treatment			
Natal IAC + wax-coating	10.4 $\pm$ 0.54 b <sup>1</sup>	0.71 $\pm$ 0.11ns	15.0 $\pm$ 3.48 b
Natal IAC	11.6 $\pm$ 0.89 a	0.74 $\pm$ 0.15	16.2 $\pm$ 2.57 ab
Valencia Late + wax-coating	12.1 $\pm$ 0.84 a	0.75 $\pm$ 0.07	16.8 $\pm$ 1.28 ab
Valencia Late	11.5 $\pm$ 1.13 a	0.62 $\pm$ 0.15	18.7 $\pm$ 2.58 a
Storage period			
0 days	11.1 $\pm$ 0.52ns	0.65 $\pm$ 0.01ns	17.7 $\pm$ 1.05ns
15 days	11.5 $\pm$ 0.49	0.70 $\pm$ 0.12	17.1 $\pm$ 2.20
30 days	11.4 $\pm$ 0.88	0.69 $\pm$ 0.09	16.9 $\pm$ 1.57
45 days	11.6 $\pm$ 1.29	0.73 $\pm$ 0.11	16.2 $\pm$ 2.27
60 days	11.4 $\pm$ 1.37	0.76 $\pm$ 0.12	15.4 $\pm$ 2.36
CV (%)	7.97	19.60	18.89
Treatment	9.97***	2.74ns	3.24*
Storage period	0.64ns	1.21ns	0.80ns
Treatment $\times$ Storage period	0.73ns	0.18ns	0.28ns

<sup>1</sup>Means followed by the same letter in the column do not significantly differ according to the Tukey's test. Significance level: \*,  $p \leq 0.05$ ; \*\*\*,  $p \leq 0.001$ ; ns, non-significant.

On the other hand, we found a highly significant ( $p \leq 0.001$ ) interaction between the postharvest treatments and days of cold storage period for juice content (Figure 4.4.4). Juice content fluctuated from 40% at harvest for fruit of both cultivars that were wax-coated to the lowest 25% at the end of the cold storage period for Valencia Late without any coating. Fruit of both cultivars showed a progressive decrease in juice content throughout storage period. However, no such tendency was observed for fruits of Natal IAC treated with carnauba wax and rosin resin

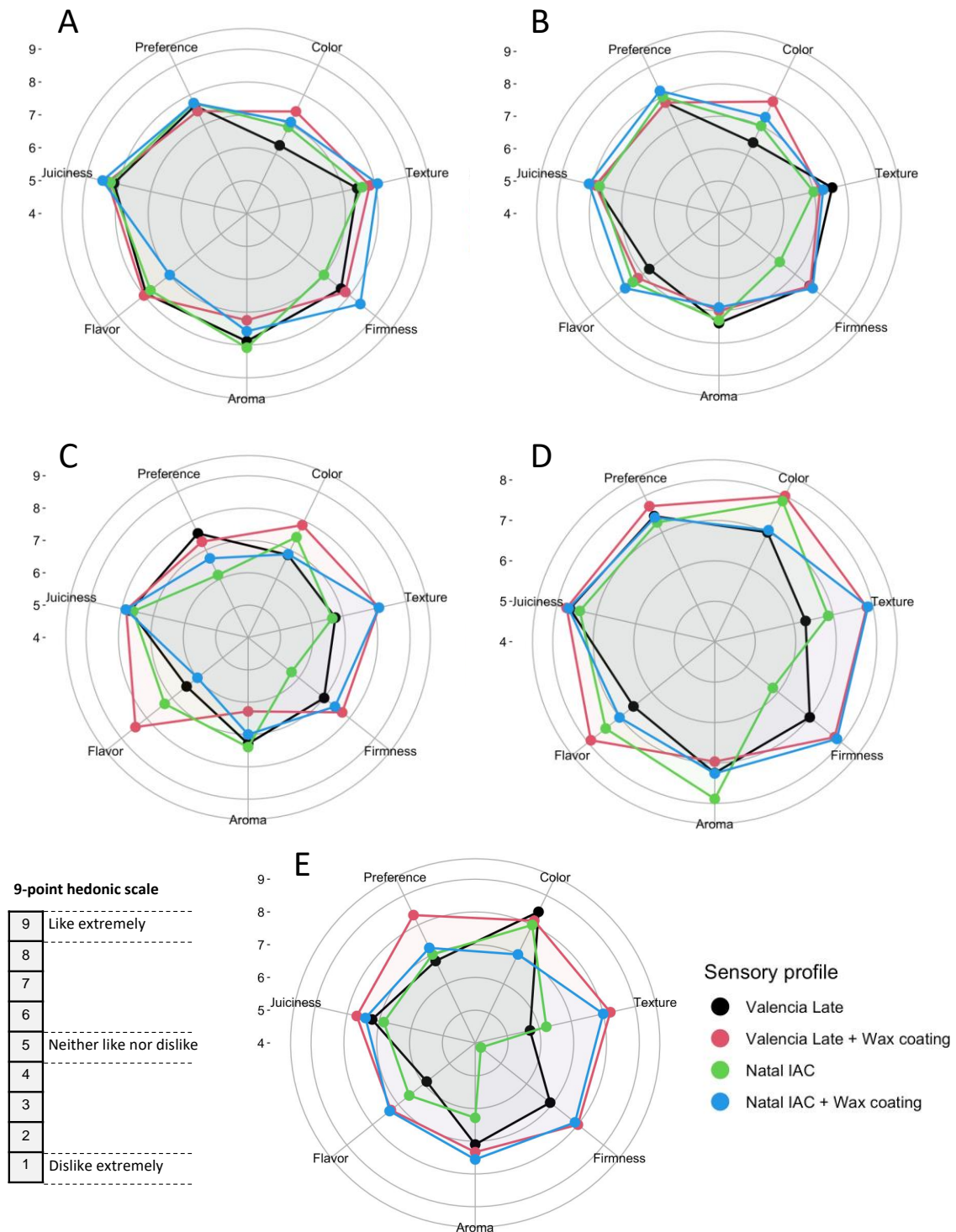


**Figure 4.4.5** Juice content of Valencia Late and Natal IAC sweet orange fruits subjected to carnauba wax/rosin resin coating treatment after 0 (harvest), 15, 30, 45, and 60 days of cold storage. Bars followed by the same capital or lowercase letter do not differ significantly in regard to the storage or coating treatment according to the Tukey's test ( $p \leq 0.05$ ).

#### 4.4.2.4 Sensory analysis

Based on a nine-point hedonic scale, the preference mapping of the sensory attributes was described for the postharvest treatments and cultivars throughout the storage period (Figure 4.4.6). Wax-coated fruits were more color-preferred by the panelists than uncoated fruits from the beginning of the postharvest assay, from harvest to 30 days of cold storage. However, no such tendency was observed after 30 days of cold storage when uncoated (control) fruits had an improvement in color

development and, consequently, preference by the panelists. Fruit firmness and texture scores were gradually reduced throughout the storage period. By contrast, no substantial differences were observed in aroma and taste of the fruits due to the postharvest treatments at most assessed periods, except at 60 days of cold storage. At that period, the uncoated fruits were mostly disliked by the panelist pool compared to wax-coated fruits. Regarding fruit juiciness, no variation was perceived by the panelists. Based on the overall preference, both cultivars and postharvest treatments did not differ at harvest, 15, 30 and 45 days of cold storage, but diverged after 60 days, as Valencia Late fruits were the most preferred by the panelists.



**Figure 4.4.6** Sensory evaluation for color, texture, firmness, aroma, flavor, juiciness, and preference of Valencia Late and Natal IAC sweet orange fruits treated/non-treated with carnauba wax/rosin resin coating at harvest (A) and after 15 (B), 30 (C), 45 (D), and 60 days (E) of cold storage.

#### 4.5 DISCUSSION

Based on the fruit characterization, the Valencia Late and Natal IAC fruits were considered as large-sized (>71mm) fruits with an optimal grade (A) according to the Brazilian standards for citrus fresh fruit (CEAGESP, 2011). Fruits of both cultivars were nearly round, as the fruit shape indices were very close to 1.0 that indicates a sub-globose or round shape (GOLDENBERG et al., 2018). Natal IAC fruit exhibited a deeper yellowish-orange coloration (2.11 CCI) compared to the Valencia Late ones (1.57 CCI).

The thickness of the flavedo and albedo, i.e. fruit rind or peel, was similar and very thin (< 3.0 mm) for the fruits of both cultivars (Table 4.4.1). However, there is a wide diversity in thickness of the citrus rind depending on scion-rootstock cultivar and soil and climatic conditions (EL-OTMANI et al., 2011; TADEO et al., 2020). Fruit rind plays an important role in maintaining fruit quality, as it regulates the water exchange between the fruit and the environment. Citrus fruits produced in cooler climates and high altitudes usually develop a compact and thin-skinned peel, whereas those from warmer humid climate have a thicker rind (LADANIYA, 2008). Based on these morphological traits, postharvest techniques involving coatings materials on the fruit surfaces are imperative to preserve fruit quality and to extend shelf life by reducing water loss and shrinkage (ALFEREZ et al., 2010; CARVALHO et al., 2020a). Moreover, the rind of citrus fruits from thinner albedo has a faster water potential adjustment compared to the one from thicker albedo (CRONJÉ et al., 2017), which may reduce problems of physiological disorders such as the postharvest peel pitting (ALFEREZ et al., 2005). Furthermore, the Valencia Late and Natal IAC fruits can be commercially classified as seedless, as they contained less than eight seeds per fruit (ALBRIGO et al., 2019).

Both sweet orange cultivars produced fruits with juice of excellent quality. The TSS content was superior to the commercial minimal standards (OECD, 2010; CEAGESP, 2011) and within the values described by Pozzan and Triboni (2005) for these cultivars. The level of citric acid was nearly to the range considered optimal for fresh consumption, which is 0.5 to 1.0 g.100 mL<sup>-1</sup> (PEREIRA et al., 2006). Similarly, the sugar-acidity ratios were substantially higher than those required by the international fresh citrus market, 6.5:1 (OECD, 2010).

The antioxidant capacity of the different fruit tissues was determined for both sweet orange cultivars (Figure 4.4.2). This is an important characteristic of the fruit potential as health promoter (BARROS et al., 2012). In our study, the total phenolics in the different fruit tissues were evaluated by spectrophotometry using the Folin–Ciocalteu reagent. The flavedo tissue had the highest amount of total phenolics. By contrast, the juice sacs and segment wall had the lowest levels for these compounds. Natal IAC fruits showed significantly ( $p \leq 0.05$ ) higher phenolics concentration than Valencia Late ones for the albedo and flavedo tissues. Similarly, Barros et al. (2012) reported higher total phenolics in the peel compared to the pulp for several Brazilian citrus cultivars, as Lima and Pera sweet oranges, Persian acid lime, Palestinian sweet lime and Ponkan mandarin. Several studies had also reported higher levels of phenolics in citrus peel compared to the pulp that includes juice sacs, central core, and segment wall (ABEYSINGHE et al., 2007; GUIMARÃES et al., 2010; RAHMAN et al., 2018). Phenolics may play a role as antioxidant and antifeedants compounds, contributing to plant pigmentation, attractants for pollinators, and protective agents against UV light, pathogens, herbivorous, and predators (SHAHIDI; NACZK, 2004). According to Barros et al. (2012), flavedo and albedo that cover the citrus fruits contain high levels of phenolics, because they are in the outer part of the fruit. Thus, they are more predisposed to the synthesis of these compounds due to external stresses. Fruit tissues with higher phenolic contents generally shows stronger antioxidant capacity (FANG et al., 2009), as confirmed in our study. However, the amount of total phenolics is variable in the citrus cultivars. Ramful et al. (2010), assessing the flavedo extracts of 21 cultivars of citrus fruits, reported a broad spectrum in the phenolic concentration, which varied from  $188 \pm 6.5$  to  $767 \pm 5.7$  mg GAE.100 g<sup>-1</sup>, depending on the citrus cultivar.

Flavonoids are among the major groups of phenolic compounds present in the citrus fruits (MANTHEY; GROHMANN, 2001). Further, these phytochemicals help to protect the plants against external stresses (COOK; SAMMAN, 1996). The main flavonoid found in the citrus fruits is hesperidin that represents a rich source in citrus by-products from peel and pulp (HOROWITZ, 1961; LAFUENTE et al., 2014). Flavonoids levels vary widely, depending on soil and climate conditions and species (CARIDI et al., 2007). The concentration of flavonoids ranged from  $32.7 \pm 1.0$  to  $49.2 \pm 1.3$  mg of rutin g<sup>-1</sup> in peels of eight citrus cultivars (WANG et al., 2008). In our study, the highest concentration of total flavonoids was found in the flavedo and albedo

tissues but with a higher content in the albedo of Natal IAC, which was also observed for pomelo fruits (CHANG; AZRINA, 2017).

The antioxidant capacity of DPPH scavenging assay was chosen to determine the total antioxidant capacity of different tissues of Valencia Late and Natal IAC sweet orange fruits (Figure 4.4.2). Similar to total phenolics and flavonoids, flavedo and albedo ranked higher for DPPH scavenging activity ( $\geq 65\%$ ) than all other fruit tissues, regardless of the cultivar. These findings were also confirmed by Rahman et al. (2018) that found a much higher amount of DPPH scavenging activity in flavedo than all other fresh fruit tissues. Juice sacs from Valencia Late contained a significant higher DPPH scavenging activity than Natal IAC juice sacs, showing that the same fruit tissue expresses a variation depending on the cultivar. The fruit tissues from both cultivars, recognized as residues (peel and pulp) or by-products of citrus processing, contain excellent antioxidant capacity and may be used in food products as active ingredients or substitutes for synthetic preservatives (GORINSTEIN et al., 2004; IGNAT et al., 2011), avoiding major losses along the food chain.

The major role of coating materials on fruit surfaces is to reduce water loss extending the fruit shelf life, which is expressed as weight loss (MIRANDA et al., 2021). In our study, the weight loss was influenced by the length of the storage period and wax-coating treatments but showed similar tendency between the two sweet orange cultivars (Figure 4.4.3). This result suggests that both late-season cultivars do not only share similarities in their physicochemical properties (Table 4.4.1), but also on their postharvest quality attributes (Figure 4.4.3). A significant ( $p \leq 0.001$ ) progressive increase in weight loss was observed over the storage period for both cultivars and wax-coating treatment. Uncoated fruits had the highest weight loss during the entire storage period. Both cultivars had the lowest weight after 60 days of cold storage. However, the wax-coating treated fruits had a weight loss below 15%, which supports the findings reported for Champagne and Salustiana sweet oranges stored for 60 days at  $3 \pm 1^\circ\text{C}$  and 80–90% RH (AGOSTINI et al., 2014; CARVALHO et al., 2020a). Similarly, Pereira et al. (2014) found significant variation in weight loss after 28 days of storage at  $24 \pm 2^\circ\text{C}$  and 35–45% RH for Valencia Delta sweet orange, testing carnauba wax treatment in comparison with non-coating. These authors noticed that Valencia Delta sweet orange fruits receiving wax-coating treatment lost around 14% of their initial weight compared to 26% for the fruits without any coating treatment. Lower weight loss rates were reported for some navel oranges, Navelina and

Washington, following 60 days of cold storage at  $3 \pm 1$  °C and 80–95% RH, with a range of 1 to 5% of weight loss (MALGARIM et al., 2007a; ÇANDIR et al., 2013). This indicates that each citrus cultivar has different postharvest behavior depending on the postharvest handling and the length of the storage period. Furthermore, in previous work, we suggested that these variations may be resulted from the genetic attributes of each cultivar associated with the storage conditions involving temperature, humidity, air movement and atmospheric gases (CARVALHO et al., 2020a). The water loss is detrimental to the physicochemical and organoleptic quality of the fruits. This is a major issue for the postharvest process and commercialization, particularly for the citrus fruits that contains around 80–90% of water (LADANIYA, 2008). The water loss not only affects the juice content but also modulates the overall appearance and firmness of the citrus fruits, which results in economic losses due to the reduction of the saleable weight (LADANIYA, 2008). Therefore, our results showed that fruits without any wax-coating treatment had an increase in weight loss by 67% compared to carnauba wax/rosin resin treatment following 60 days of cold storage, promoting decay on fruit firmness and depreciation due to the increase in the metabolic rate and water loss (CARVALHO et al., 2020a).

Fruit color directly influences consumer's perception and acceptance (LADO et al., 2014; GUPTA et al., 2021). The color development was quantitatively expressed as citrus color index, CCI (Figure 4.4.4). CCI has become an accurate and reliable parameter to measure color changes in citrus fruit, and widely applied by the citrus industry to establish harvest time and postharvest treatments, as degreening (JIMÉNEZ-CUESTA et al., 1981). According to the external coloration, the fresh fruit market requires sweet orange fruits that exhibit attractive coloration without any damage (OECD, 2010; CEAGESP, 2011). In our study, the uncoated treatment (control) induced a progressive color evolution in the flavedo, for both cultivars (Figure 4.4.4). By contrast, no color development was observed in the fruits with wax-coating treatment. This indicates that the wax-coating treatment may have retarded the chlorophyll degradation (ZHOU et al., 2010) and blocked the pathway of carotenoid synthesis (ROUSSOS et al., 2011; SUN et al., 2017) by reducing the metabolic rate and ethylene biosynthesis in the fruits. In most cases, coating delays the natural senescence process and the biosynthesis of colored pigments in the citrus fruit, resulting in a slower color change (NASRIN et al., 2020). Interestingly, the excessive water loss observed for uncoated fruits (Figure 4.4.3) may have favored the ethylene

biosynthesis that led to the chlorophyll degradation and color change, as reported by Nasrin et al. (2020).

The internal quality of the sweet orange fruits plays an important role on consumer's acceptance and marketability (LADANIYA, 2008). Sweet orange fruits must achieve certain quality standards for the fresh fruit market (OECD, 2010; CEAGESP, 2011). Significant differences were observed among the tested postharvest treatments for TSS content (Table 4.4.2). TSS concentration ranged from 10.4 to 12.1 °Brix. The lowest TSS content were found for Natal IAC without any coating treatment. However, the fruits of the cultivars included in the postharvest study attended the minimal TSS value required by the fresh citrus market, 10 °Brix (ARPAIA; KADER, 2000; CEAGESP, 2011), and were within the range described previously for these two cultivars (LEMOS et al., 2013; ZACARÍAS-GARCÍA et al., 2020). No such variation was observed among the treatments for the acidity level. The citric acid content was between 0.5 and 1.0 g.100 mL<sup>-1</sup>, considered as an optimal range to confer proper organoleptic quality to sweet oranges (PEREIRA et al., 2006). Natal IAC fruits combined with wax-coating had 15:1 for the sugar-acidity ratio. By contrast, non-coated Valencia Late fruit ranked higher for this ratio, above 18:1. These fruits produced juice of elevate sugar-acidity ratio as the acidity level remained constant and low (0.62 g of citric acid per 100mL) but high for TSS content (11.5 °Brix) over the storage period. All other treatments had intermediate values for sugar-acidity ratio. These indices indicate a suitable fruit maturity for both sweet orange cultivars and postharvest treatment, as the ratio were all above the minimal standard required by the fresh fruit market (OECD, 2010; CEAGESP, 2011). Sugar-acidity ratio may exceed 20:1 after harvest, depending on the cultivar and postharvest treatment, but the citric acid level cannot be below 0.4 g.100 mL<sup>-1</sup>, so that the sweet orange fruits do not taste too insipid with much less acidity, becoming inappropriate for fresh consumption (JONES; CREE, 1965; LADANYIA, 2008). Interestingly, the length of the storage period, up to 60 days, did not affect the internal quality of the fruits of both cultivars, regardless of the postharvest treatment. This is important as the internal quality of the fruits was preserved for the evaluated period. The postharvest treatment, carnauba wax/rosin resin, did not cause any depreciation or decay on the fruits after 60 days of cold storage at 5 ± 1 °C and 60–70% RH.

Juice content is an important characteristic that modulates fruit quality in sweet oranges. The use of this parameter as a commercial standard index is dependent on

the citrus cultivar and the market destination (LADO et al., 2014). According to the international fresh fruit market, sweet oranges must have at least 35% of juice (LADANIYA, 2008; OECD, 2010). Based on this requirement, the fruits of both cultivars had appropriate juice content in the first two periods of storage. At 30, 45 and 60 days of cold storage, the uncoated fruits had much less juice content than the minimum required by the fresh market (LADANIYA, 2008; OECD, 2010). Therefore, our results showed a progressive reduction in the juice content of the fruits when the storage period was extended. These results agree with previous studies that showed a reduction in juice content of the citrus fruits by increasing the length of the storage (LADANIYA, 2008; ÇANDIR et al., 2013). Interestingly, the juice content of Natal IAC fruits treated with carnauba wax and rosin resin did not change during the storage period (Figure 4.4.5). This may be due to the moderate water loss observed for the wax-coated fruits (Figure 4.4.3). Further, the morphological traits of this cultivar may have not affected the juice content, as the fruits of Natal IAC were moderately smaller than the ones of Valencia Late (Table 4.4.1). This may result in a low fruit-surface-area for water loss by transpiration. Davies and Zalman (2004) pointed out that larger sweet orange fruits have proportionately lower juice content than smaller ones supporting our findings.

The progression of the fruit color was also observed by the panelist group in the sensory analysis of uncoated fruit (Figure 4.4.6). Panelists expressed a high preference for wax-coated fruits at the beginning of the postharvest, by 30 days of cold storage, probably due to the fruit glossiness that turn them to look more attractive. Thereafter, uncoated fruits ranked high for this assessment as they exhibited a deeper yellow color, which was confirmed by the color measurements (Figure 4.4.4). It is worth to mention that fruit color is an important factor to determine purchase intent (LADANIYA, 2008). This evidence was reported in our study, as the panelists expressed their preference according to fruit attractiveness that involves glossiness and rich coloration.

Regarding fruit firmness, the panelists had less preference for uncoated fruits as storage progressed, particularly for Valencia Late fruits. This trend was also found for fruit texture. These characteristics were less preferred for uncoated fruits probably due to their excessive water loss (Figure 4.4.3). The cell vacuole progressively shrinks, and the turgor potential declined to zero after water loss, favoring cell collapse and consequent fruit shrinkage (CRONJÉ et al., 2017). The rates for aroma and taste

attributes remained constant up to 60 days of storage period. This agrees with the results found for juice quality in which no significant variations were observed (Table 4.4.2). However, the panelists indicated higher preference for wax-coated than uncoated fruits according to these sensory attributes after 60 days of cold storage, demonstrating the effectiveness of the carnauba wax/rosin resin in preserving fruit quality after a long storage period. The juiciness of the fruits was not differentially perceived by the panelist group over the storage period. However, the panelist expressed much higher preference for Valencia Late combined with wax-coating treatment after 60 days of storage, reinforcing the efficiency of this postharvest treatment. Therefore, the combination of both coating materials, carnauba wax and rosin resin, associated with the cold storage, brought beneficial results to the citrus fruits postharvest conservation, including the delay of water loss, maintenance of physicochemical and sensorial quality, and retarding of natural senescence and fruit color development.

#### 4.6 CONCLUSIONS

Fruits of Valencia Late and Natal IAC sweet oranges have excellent physicochemical quality and antioxidant capacity, attending the requirements of the fresh citrus market. Interestingly, the flavedo and albedo tissues contain the highest concentration of phenolics and flavonoids, as well as the antioxidant activity in the citrus fruits included in the study. Therefore, these fruit tissues, recognized as residues left from the citrus processing industry, can be used in food products as a remarkable low-cost antioxidant source.

Carnauba wax/rosin resin coating treatment combined with cold storage at  $5 \pm 1$  °C and 60–70% RH is efficient to decrease fruit color development and to prevent weight loss in fruits of both sweet orange cultivars up to 60 days. Moreover, the physicochemical and sensory quality of the carnauba wax/rosin resin coated fruits are preserved during cold storage, while uncoated fruits show decrease in juice content and lower preference as the storage period is prolonged, up to 60 days. The effect of carnauba wax and rosin resin on the shelf life extension of Valencia Late and Natal IAC fruits is positive by reducing weight loss, retarding senescence and color progression, and preserving the physicochemical and organoleptic fruit quality.

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