BRUNO MARTINS DALA PAULA

Efeitos do Huanglongbing (HLB) na composição química e características sensoriais de suco de laranja

Faculdade de Farmácia da UFMG

Belo Horizonte, MG

2017

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Tese apresentada ao Programa de Pós-Graduação em Ciência de Alimentos da Faculdade de Farmácia da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do grau de doutor.

Orientadora: Profª. Drª. Maria Beatriz Abreu Glória
Tutores no exterior: Dra. Anne Plotto e Dr. John A. Manthey

Faculdade de Farmácia da UFMG
Belo Horizonte, MG
2017

Dala Paula, Bruno Martins.

D136e Efeitos do Huanglongbing (HLB) na composição química e características sensoriais de suco de laranja / Bruno Martins Dala Paula. – 2017**.**

131 f. : il.

Orientadora: Maria Beatriz Abreu Glória.
Tutores: Anne Plotto, John A. Manthey.

Tese (doutorado) - Universidade Federal de Minas Gerais, Faculdade de Farmácia, Programa de Pós-Graduação em Ciência de Alimentos.

1. Suco de laranja – Indústria – Teses. 2. Alimentos – Análise – Teses. 3. Alimentos – Avaliação sensorial – Teses. 4. Alimentos – Composição – Teses. 5. Alimentos – Qualidade – Teses. 6. Frutas cítricas – Doenças e pragas – Teses. 7. Huanglongbing (HLB) – Doença – Teses. I. Glória, Maria Beatriz Abreu. II. Plotto, Anne. III. Manthey, John A. IV. Universidade Federal de Minas Gerais. Faculdade de Farmácia. V. Título.

CDD: 664

	UNIVERSIDADE FEDERAL DE MINAS GERAIS **PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA DE ALIMENTOS**	**PPGCA**

FOLHA DE APROVAÇÃO

EFEITOS DO HUANGLONGBING (HLB) NA COMPOSIÇÃO QUÍMICA E CARACTERÍSTICAS SENSORIAIS DE SUCO DE LARANJA

BRUNO MARTINS DALA PAULA

Tese submetida à Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIA DE ALIMENTOS, como requisito para obtenção do grau de Doutor em CIÊNCIA DE ALIMENTOS, área de concentração CIÊNCIA DE ALIMENTOS.

Aprovada em 14 de julho de 2017, pela banca constituída pelos membros:

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Belo Horizonte, 14 de julho de 2017.

À minha família, orientadora, tutores, e aos que estiveram ao meu lado dedico com muito entusiasmo e gratidão este trabalho.

AGRADECIMENTOS

Aos meus pais (Gilberto e Edite) pelo amor e suporte constante, além dos ensinamentos que foram a base para a formação de quem hoje eu sou. Às minhas irmãs (Roberta e Karina) e sobrinhos (Fernando e Guilherme) por compartilharem seus valiosos momentos e tornar meus dias mais prazerosos. À professora Maria Beatriz Abreu Glória, por acreditar em meu trabalho e assim contribuir para a reconstrução da minha autoestima, pelo altruísmo em compartilhar os seus conhecimentos e saberes. Por se um exemplo de profissional a ser seguido. Aos meus tutores no exterior Dra. Anne Plotto e Dr. John Manthey pela amizade e companheirismo dentro e fora dos laboratórios. Foram muitos os aprendizados e momentos de reflexões, muito obrigado! Ao Lucian Ferry, agradeço pelo companheirismo e por toda ajuda oferecida, principalmente com as inúmeras revisões da escrita no idioma inglês. Aos amigos de todas as horas que sempre me apoiaram e compreenderam as minhas ausências, muitas vezes motivadas por compromissos acadêmicos. Obrigado a: Abel, Alessandra Santos, Ana Lígia, Clayson Pereira, Christiano Moreira, Cristiano Araújo, Danielle Vasconcelos, Débora Figueiredo, Dhionne Gomes, Diogo Satiro, Emmanuel Almada, Fernanda Louro, Gustavo Medeiros, Hélio Navarro, Kênia Roberta, Luana Oliveira, Luiz Machado, Marco Polo, Nathália Botelho, Pollyana Castro, Sheyla França, Thiago Andrade, Tiago Sial, Valcir. Aos amigos e companheiros dessa aventura que é o mundo acadêmico, que conhecem bem as dificuldades e que nunca deixaram de me motivar com palavras de conforto e um sorriso no rosto: Adriana Correa, Aisa Del Rio, Andrezza Estevam, Arthur Magno, Cyntia Barreto, Caroline Paiva, Cecília Bandeira, Edneia Xavier, Fabiana Diniz, Flávia Custódio, Gisela Machado, Guilherme Reis, José Maria, Juliana Rigueira, Larissa Bomtempo, Laura Ciribelli, Patrícia Tette, Paula Santiago, Raquel Braga, Regina Adão, Regina Carvalho, Ricardo Byrro, Rummenigge Oliveira, Silvia Vieira, Tânia Silveira, Tarliane Silva e Warlley Evangelista. Aos professores que compartilharam os seus conhecimentos. Todos que contribuíram com o meu crescimento pessoal, acadêmico e profissional. Aos pesquisadores, técnicos e amigos do USDA, Fort Pierce pelo auxílio na pesquisa e suporte durante a minha estadia no USDA: Dr. Liz Baldwin, Dr. Jinhe Bai, Dr. Hong Chen, Carly Franko, Marcus Reis, Veronica Cook, Dave e Kimberly Wood, Dr. Chris Ference, Nancy Owen, Holly Sisson, Elena Branca, XiuXiu Sun. À minha mãe Americana, Jackie White e Bob pelo caloroso acolhimento e por serem exemplos de pessoas gentis e generosas. Aos amigos Sai Xu, Valentina Candia, Sajid Ali, Hernandes Burgos, Sheree Deal e Mathew por fazerem parte de um momento muito especial da minha vida. Aos funcionários da Faculdade de Farmácia por todo o auxílio prestado. Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Muito obrigado!

“One looks back with appreciation to the brilliant teachers, but with gratitude to those who touched our human feelings. The curriculum is so much necessary raw material, but warmth is the vital element for the growing plant and for the soul of the child” (Carl Jung)

SUMÁRIO

SUMÁRIO **6**

LISTA DE TABELAS **9**

LISTA DE FIGURAS **11**

LISTA DE ABREVIATURAS E SIGLAS **13**

RESUMO **14**

ABSTRACT **15**

INTRODUÇÃO GERAL **16**

REVISÃO DA LITERATURA **18**

1. CLASSIFICAÇÃO TAXONÔMICA DA LARANJA E PRINCIPAIS CULTIVARES PLANTADAS NO BRASIL 18
2. BREVE HISTÓRICO DO CULTIVO E PRODUÇÃO DE LARANJA 20
3. OCORRÊNCIA E EFEITO DA HUANGLONGBING NOS POMARES DE LARANJA 23
4. BREVE HISTÓRICO DA PRODUÇÃO DE SUCO DE LARANJAS 26
5. ETAPAS DO PROCESSAMENTO E COMERCIALIZAÇÃO DO SUCO DE LARANJA PRODUZIDO NO BRASIL 29
6. CONSUMO DA LARANJA *IN NATURA* E SEUS DERIVADOS 35
7. COMPOSIÇÃO QUÍMICA E NUTRICIONAL DO SUCO DE LARANJA 37
8. PROPRIEDADES FUNCIONAIS DA LARANJA 41

OBJETIVOS **43**

CAPÍTULO I - INFLUENCE OF HARVEST TIME ON QUALITY OF 'VALENCIA' ORANGES AND JUICE, SECOND SEASON **44**

ABSTRACT 44
1. INTRODUCTION 45
2. MATERIAL AND METHODS 46
   - 2.1 Fruit sampling 46
   - 2.2 Peel color and juice content analysis 46
   - 2.3 Sugar and acid analysis 46
   - 2.4 Secondary metabolite analysis 47
   - 2.5 Peel oil, pectin and pectinmethylesterase (PME) 47
   - 2.6 Volatile analysis 47
   - 2.7 Bioactive amines 48
   - 2.8 Statistical analysis 48

3. RESULTS AND DISCUSSION 48
3.1 Peel color and juice content 48
3.2 Peel oil and PME activity and pectin 49
3.3 Sugars and acids 50
3.4 Secondary metabolites 51
3.5 Bioactive amines 53
3.6 Volatiles 54
4. CONCLUSION 58

CAPÍTULO II - EFFECT OF HUANGLONGBING (GREENING DISEASE) ON ORANGE JUICE QUALITY, A REVIEW 59

ABSTRACT 59
KEYWORDS 59
1. INTRODCUTION 60
2. WORLDWIDE CONSUMPTION AND PRODUCTION OF FRESH ORANGES AND ORANGE JUICE 60
3. A BRIEF HISTORICAL BACKGROUND OF HUANGLONGBING INCIDENTS 61
5. SYMPTOMS OF HUANGLONGBING AND ITS IMPACT ON ORANGE TREES 68
6. FRESH ORANGES AND ORANGE JUICE QUALITY AFFECTED BY *CANDIDATUS* LIBERIBACTER ASIATICUS 72
6.1 Effects on physicochemical and biochemical characteristics 72
6.1.1 Peel color 72
6.1.2 Physicochemical characteristics 73
6.1.3 Sugars and organic acids 75
6.1.4 Secondary metabolites 76
6.1.5 Amino acids and bioactive amines 80
6.2 Effects on sensory characteristics 81
6.3 Effect on the levels and profile of volatile compounds 86
6.4 Huanglongbing control and mitigation of its symptoms 87
7. FINAL CONSIDERATIONS 88

CAPÍTULO III - ACTIVE TASTE COMPOUNDS IN JUICE MADE FROM ORANGES SYMPTOMATIC OF HUANGLONGBING (HLB) GREENING DISEASE 90

ABSTRACT 90
1. INTRODUCTION 91
2. MATERIAL AND METHODS 93
2.1 Juice samples 93
2.1.1 Sample preparation *93*

2.1.2 DNA extraction and qPCR detection of CLas from juice 93
2.2 Chemical analysis of COJ and HLBOJ 93
2.2.1 Titratable acidity and soluble solids 93
2.2.2 Total sugar, sucrose, glucose and fructose 94
2.2.3 Citric acid, malic acid and ascorbic acid analyses 94
2.2.4 Secondary metabolites analyses 95
2.3 Fractionation of phenolic compounds from orange juice 95
2.3.1 Preparation of phenolic compound extracts of COJ and HLBOJ 95
2.3.2 Fractionation of phenolic compound extracts by Fast Centrifugal Partition Chromatography (FCPC) 96
2.3.3 Chemical characterization of phenolic compounds by HPLC– MS ... 96
2.3.4 Sub-fractionation of fractions A and B by HPLC 97
2.4 Sensory evaluation 98
2.4.1 Comparative sensory analysis of COJ and HLBOJ 98
2.4.2 Descriptions of flavor attributes of fractions obtained from COJ and HLBOJ 98
2.5 Statistical analyses 100
3. RESULTS 100
4. DISCUSSION 108
5. CONCLUSION 110
CONCLUSÕES INTEGRADAS 111
REFERÊNCIAS BIBLIOGRÁFICAS 112
PRODUÇÃO CIENTÍFICA DURANTE O DOUTORADO 131

LISTA DE TABELAS

REVISÃO DA LITERATURA

Tabela 1. Características das principais cultivares de laranjas plantadas no Brasil 19

Tabela 2. Área destinada à colheita de laranja em hectares e quantidade produzida em mil toneladas no Brasil, durante os anos de 2010 a 2017*.....21

Tabela 3. Composição química representativa da laranja Valência *in natura* e do seu suco. 38

Tabela 4. Teores médios de flavonoides em casca, em vesícula de suco e em laranja Valência 39

CAPÍTULO I

Table 1. Effect of harvest time on volatile abundance in 'Valencia' orange juice (2012)[z] 55

CAPÍTULO II

Table 1. Worldwide distribution of Huanglongbing's bacteria and vectors. 63

Table 2. Effects on diameter, weight and juice content in fruit affected by Huanglongbing.....71

Table 3. Physicochemical characteristics of Valencia orange juice made with healthy fruit and fruit at different stages of HLB infection.....74

Table 4. Physicochemical characteristics of Hamlin orange juice made with healthy fruit and fruit at different stages of HLB infection.....75

Table 5. Sugars and acids of Valencia orange juice made with healthy fruit and fruit at different stages of HLB infection.....77

CAPÍTULO III

Table 1. Mobile phases gradients (1 and 2) used to separate fractions A and B: from healthy and huanglongbing (HLB)-affected Valencia orange juice into sub fractions. 97

Table 2. Descriptors and reference standards with suggested intensity for orange juice sensory descriptive panel, using a 16-point intensity scale* 99

Table 3. Sensory descriptors of each sub-fraction (SF) obtained from A-COJ and A-HLBOJ from healthy and huanglongbing (HLB)-affected Valencia orange juice, respectively. 105

Table 4. Sensory descriptors and concentration of each sub-fraction obtained from B-COJ and B-HLBOJ from healthy and huanglongbing (HLB)-affected Valencia orange juice, respectively 107

LISTA DE FIGURAS

REVISÃO DE LITERATURA

Figura 1. Esquema de uma planta de processamento comercial de suco de laranja.. 31

Figura 2. Exportações de FCOJ equivalente em toneladas por ano civil a partir do porto de Santos.. 34

Figura 3. Exportações de NFC equivalente em toneladas por ano civil a partir do porto de Santos.. 35

CAPÍTULO I

Figure 1. Changes of peel color (a*/b* ratio) and juice content of ‘Valencia’ orange fruit harvest from February to May 2012. 49

Figure 2. Changes of peel oil content, pectin methylesterase (PME) activity, and total pectin (galacturonic acid) content in ‘Valencia’ orange juice extracted from fruit harvested from February to May 2012. 49

Figure 3. Changes of soluble solids content (SSC), titrable acidity (TA), and SSC/TA ratio in ‘Valencia’ orange juice extracted from fruit harvested from February to May 2012. 50

Figure 4. Changes of citric, malic, and ascorbic acids in ‘Valencia’ orange juice extracted from fruit harvested from February to May 2012. 51

Figure 5. Changes of flavonoid glycosides (FGs, relative peak área) in ‘Valencia’ Orange juice extracted from fruit harvested from February to May 2012. Hesperedin-4’-glucoside; hesperidin; 6,8-di-C-glucosyl apigenin; isosakuranetin rutinoside; narirutin. 52

Figure 6. Changes of polymethoxylated flavones (PMFs, relative peak área) in ‘Valencia’ Orange juice extracted from fruit harvested from February to May 2012. Heptamethoxyflavone; quercetagetin hexamethylether; nobiletin; tetramethylscutellarein; sinesetin; and tangeretin. 52

Figure 7. Changes of limonoids (relative peak área) in ‘Valencia’ orange juice extracted from fruit harvested from February to May 2012. Obacunone glucoside;

nomilin glucoside; nomilinic acid glucoside; limonin glucoside; limonin; and nomilin. 53

Figure 8. Changes of putrescine, spermidine, and spermine in 'Valencia' orange juice extracted from fruit harvested from February to May 2012. 53

CAPÍTULO II

Figure 1. Countries currently affected by Huanglongbing – HLB.. 62

Figure 2. Development stages of Diaphorina citri Kuwayama (Hemiptera: Psyllidae) and Trioza etrytreae (Del Guercio) (Hemiptera: Triozidae) 67

Figure 3. HLB symptomatic orange leaves: symptomatic normal sized leaves with development of blotchy-mottle; and symptomatic small sized leaves. 69

Figure 4. Typical HLB symptomatic orange: asymmetric fruit containing small, brownish aborted seeds; normal change of color in a healthy orange, *C*Las (-); and inversion of colors in a typical HLB symptomatic orange, *C*Las (+). 70

Figure 5. Symptomatic and asymptomatic orange of HLB disease, *C*Las (+); and healthy oranges, CLas (-).....72

CAPÍTULO III

Figure 1 Sensory scores (average ± standard deviation for 12 trained panelists) for Valencia orange juice from healthy (COJ) or HLB-affected (HLBOJ) fruit.…101

Figure 2. Quality attributes (average ± standard deviation for four replicates) in Valencia orange juice from healthy (COJ) or HLB-affected (HLBOJ) fruit.…....102

Figure 3. Secondary metabolites (average ± standard deviation for three replicates) in Valencia orange juice from healthy (COJ) or HLB-affected (HLBOJ) fruit.....103

Figure 4. HPLC chromatograms of healthy (COJ) (a) or huanglongbing (HLB)-affected (HLBOJ) (b) Valencia orange juice from fraction A showing 10 sub-fractions with their respective lowest concentration at which taste was perceived. 104

LISTA DE ABREVIATURAS E SIGLAS

A-COJ	Fraction A of COJ
A-HLBOJ	Fraction A of HLBOJ
APTA	Agência Paulista de Tecnologia dos Agronegócios
AT	Acidez titulável
B-COJ	Fraction B of COJ
B-HLBOJ	Fraction B of HLBOJ
CDA	Coordenadoria de Defesa Agropecuária
*C*L	*Candidatus* Liberibacter
*C*Laf	*Candidatus* Liberibacter africanus
*C*Lam	*Candidatus* Liberibacter americanus
*C*Las	*Candidatus* Liberibacter asiaticus
COJ	Control juice
CVC	Clorose variegada dos citros
FCOJ	Frozen concentrated orange juice
FCPC	Fast centrifugal partition chromatography
FG	Flavonoid glycosides
Fundecitrus	Fundo de Defesa da Citricultura
GC-MS	Gas chromatography-mass spectrometry
HCA	Hydroxycinnamic acids
HLB	Huanglongbing
HLBOJ	HLB orange juice
HPLC	High performance liquid chromatography
IBGE	Instituto Brasileiro de Geografia e Estatística
MAPA	Ministério da Agricultura, Pecuária e Abastecimento
MG	Minas Gerais
MS	Mass-spectrometry
NFC	Not from concentrate juice
PCR	Polymerase chain reaction
PDA	Photodiode array
PME	Pectinmethylesterase
PMF	Polymethoxylated flavones
SIDRA	Sistema IBGE de Recuperação Automática
SP	São Paulo
SPME	Solid phase microextraction
SSC	Soluble solids content
SS	Sólidos solúveis
TA	Titratable acidity

RESUMO

Huanglongbing (HLB) é a mais severa doença em citros no mundo. Sintomas dessa doença incluem ramos com folhas amareladas, queda precoce e alteração das características sensoriais dos frutos, desfolhamento e morte prematura da árvore. Este trabalho teve como objetivos determinar as alterações físico-químicas, na composição química e nas características sensoriais no suco de laranja acometido pelo HLB, assim como investigar as causas do amargor no suco de laranja, além daquelas provenientes da limonina e nomilina. Foi realizado um estudo avaliativo das características físico-químicas e bioquímicas de sucos de laranjas Valência colhidas em diferentes meses (fevereiro, março, abril e maio) numa mesma safra (2012). Confirmaram-se as alterações nos parâmetros estudados, sendo as colhidas no meio da estação mais adequadas ao processamento. Em seguida foi realizada uma revisão de literatura sobre os efeitos do HLB nos parâmetros físico-químicas, composição química e características sensoriais de suco de laranja, assim como a determinação laboratorial das mesmas. Tanto a revisão quanto as análises químicas demonstraram aumento da acidez titulável (AT), conteúdos de ácido cítrico, limonina e nomilina, dentre outros metabólitos secundários e redução dos sólidos solúveis (SS), *ratio*, açúcares e teores de ácido málico. Os sucos provenientes de laranjas sintomáticas para o HLB apresentaram maior intensidade dos seguintes atributos sensoriais: sabores amargo, ácido, metálico e umami; aromas de toranja, de casca de laranja e de suco envelhecido; e das sensações bucais de adstringência, queimação e formigamento. Foi realizado um fracionamento dos compostos não-voláteis do suco de laranja, com subsequente descrição sensorial dos mesmos. Verificou-se sabor amargo em uma fração onde a limonina e nomilina estavam presentes, mas também em outras com predomínio de ácidos hidroxicinâmicos e ausência dos limonoides. Essa é uma evidência de que outro(s) composto(s), possivelmente algum ácido hidroxicinâmico, esteja(m) envolvido(s) com o típico amargor conferido pelo HLB.

PALAVRAS-CHAVE: Huanglongbing. Suco de laranja. Amargor. Qualidade de alimentos. Análise sensorial

ABSTRACT

Huanglongbing (HLB) is the most severe citrus disease in the world. Symptoms of HLB include branches with yellow leaves, premature dropping and changes in fruit sensory quality, loss of leaves and premature death of the tree. The objectives of this work were to evaluate the physicochemical, chemical composition and sensory changes in juice made with HLB symptomatic oranges, as well as to investigate the causes of the bitterness found in HLB orange juice, other than the bitter limonoids, limonin and nomilin. The physicochemical caracteristics and chemical composition were evaluated in juice made with Valencia oranges harvested throughout the season (February, March, April and May 2012). This study confirmed the changes of some chemical composition over the harvest season, with better attributes for processing in the mid-season. Then, a literature review and laboratory analysis evaluating the effects of HLB on physicochemical, chemical composition and sensory characteristics were conducted. Both, review and laboratory analysis, revealed increases of titratable acid (TA), levels of citric acid, limonin and nomilin, among other secondary metabolites, and a decrease of soluble solid contents (SSC), SSC/TA, sugars and malic acid contents. The juice from HLB symptomatic oranges had higher values for the sensory attributes: bitterness, sourness, metallic and umami tastes; flavors of grapefruit, orange peel and staleness; and astringent, burning and tingling month-feels compared to healthy juice. Non-volatile compounds were extracted from Valencia orange juice and fractionated and sensorially described. Bitterness was detected in the fraction containing limonin and nomilin as well as in fractions which did not contain these limonoids, but prevalence of hydroxycinnamic acids. This evidences the existence of other compounds, possibly hydroxycinnamate acids, involved with the typical HLB-bitterness.

KEYWORDS: Huanglongbing. Orange juice. Bitterness. Food quality. Sensory evaluation.

INTRODUÇÃO GERAL

O suco de laranja é a bebida mais consumida no mundo dentre os sucos de frutas, com participação de 45% no mercado (MARKESTRAT, 2016). O Brasil é o maior produtor mundial de laranjas e possui destaque no mercado internacional da fruta *in natura*, assim como de seu suco. No entanto, no período de 2011 a 2017, o país apresentou redução de aproximadamente 13,2% na safra de laranjas, e tendência de queda na área destinada ao cultivo da fruta, apesar do incentivo da demanda internacional pela laranja brasileira nos anos de 2016 e 2017, em decorrência do acentuado declínio da produção norte-americana da fruta, em especial no estado da Flórida (IBGE/SIDRA, 2017; USDA/FAS, 2017).

No entanto, atualmente os produtores citrícolas brasileiros e norte-americanos estão enfrentando sérios problemas com a incidência da doença *Huanglongbing* (HLB), também conhecida como "*greening"* dos citros em suas plantações. Esta doença teve o seu primeiro relato na China no fim do século XIX, no entanto, em 1966 foi realizado um estudo retrospectivo da situação dos citros na Índia, sendo o HLB apontado como uma das principais causas do gradual declínio das árvores cítricas ocorrido durante o século XVIII (FRASER et al., 1966). A doença foi notificada na África do Sul no início do século XX, estando atualmente presente em diversos países. Até 2004 não havia relatos nas áreas mediterrâneas da Europa e nas Américas, quando a doença foi detectada em vários pomares de São Paulo, sendo também notificada no ano seguinte nos Estados Unidos - EUA (RAITHORE et al., 2015; TEIXEIRA et al., 2005a; BOVÉ, 2006).

O HLB é mundialmente considerado a doença mais grave que acomete os citros e, consequentemente, um sério problema para a indústria de processamento dos mesmos. A doença está associada com a presença da bactéria gram negativa do gênero *Candidatus* Liberibacter (*C*L), sendo no continente Americano transmitida pelo psilídeo *Diaphorina citri.* Existem relatos da presença do psilídeo no Brasil desde o ano de 1942, embora sem

notificações da doença em citros no período (LIMA, 1942). Os frutos sintomáticos das laranjeiras infectadas apresentam coloração inadequada, são pequenos, geralmente amolecidos, possuem forma assimétrica e as suas características sensoriais têm sido associadas aos atributos negativos referente ao suco de laranja, incluindo: elevação do amargor, acidez, sabor metálico, fermentado, salgado/umami, adstringência, redução da doçura e sabor cítrico (PLOTTO et al., 2008; PLOTTO et al., 2010; DALA PAULA et al., 2017a; 2017b). Além disso, o HLB pode causar a devastação de grandes plantações e consequente prejuízo econômico aos agricultores, indústria de alimentos e exportadores da fruta e seus derivados (BOVÉ, 2006; BASSANEZI et al., 2009; COSTA, 2011; BALDWIN et al., 2017).

O aroma do suco de laranja se deve à complexa combinação de vários odores e sabores derivados de seus componentes. Os álcoois, aldeídos, ésteres, cetonas, hidrocarbonetos e metabólitos secundários, a exemplo dos compostos fenólicos e dos limonoides amargos (limonina e nomilina) têm sido amplamente investigados em suco de laranjas acometidas pelo HLB (PLOTTO et al., 2008; 2010; BALDWIN et al., 2010; DAGULO et al., 2010; SLISZ et al., 2012; MASSENTI et al., 2016). Porém, as causas do típico armargor em suco de laranja acometidas pelo HLB ainda não foram completamente elucidadas. Os artigos disponíveis na literatura científica relacionam a limonina e a nomilina como os principais compostos responsáveis pelo amargor, sendo que em alguns desses estudos, os teores desses metabólitos em suco de laranja acometidas pelo HLB estavam abaixo do limiar para a sua percepção, entretanto a análise sensorial indicou percepção do sabor amargo nas amostras estudadas (BALDWIN et al., 2010; DAGULO et al., 2010; DEA et al., 2013).

REVISÃO DA LITERATURA

1. CLASSIFICAÇÃO TAXONÔMICA DA LARANJA E PRINCIPAIS CULTIVARES PLANTADAS NO BRASIL

As frutas cítricas de maior relevância comercial no mundo são do gênero *Citrus,* pertencentes à família Rutaceae. Nesse gênero estão incluídas as laranjas doces, tangerinas, laranjas azedas, pomelos, toranjas, limas ácidas, limas doces, limões, cidras e outros tipos incluindo híbridos naturais (EMBRAPA, 1998). A classificação taxonômica desse gênero é complexa devido ao elevado grau de hibridação ocorrido, e ainda, as laranjeiras amargas apresentam características botânicas semelhantes às das laranjeiras doces (MATTOLI et al., 2005). Existem algumas divergências em sua classificação, no esquema taxonômico mais tradicional reconhecido por Swingle e Reece (1967), a laranja doce é classificada como *Citrus sinensis* (L.) Osbeck, como uma espécie separada. Mas em uma classificação mais recente, de acordo com Penso (1997), laranjas amargas e doces são consideradas como subespécies ou cultivares *Citrus aurantium* L. (Rutaceae). Essas são denominadas respectivamente, *C*. *aurantium* L. var. *amara* e *C*. *aurantium* L. var. *sinensis* (MATTOLI et al., 2005). Tendo em vista o predomínio da classificação taxonômica das laranjas, proposta por Swingle e Reece (1967), nos artigos da área de Ciência de Alimentos, esta também será empregada no presente estudo.

As laranjeiras são árvores de porte médio e copa esférica, podem ser classificadas em função do fruto em quatro subgrupos: comum, do grupo Navel ou laranjeiras de umbigo, sanguíneas e as de baixa acidez. As cultivares representantes de cada um dos quatro sub-grupos podem apresentar especificidades quanto à maturação, podendo ser precoce, meia-estação ou tardia. As principais cultivares plantadas e comercializadas no Brasil estão representadas na Tabela 1. Pera Rio, Valência, Hamlin, Natal, Valência Folha Murcha e Valência Americana são as principais cultivares plantadas e

destinadas ao processamento do suco (CAPUTO, 2012; EMBRAPA, 2014, FUNDECITRUS, 2017).

Tabela 1. Características das principais cultivares de laranjas plantadas no Brasil

Cultivar	Planta		Fruto				Mercado
	Porte*	Copa	Maturação	Semente	Teor de suco	Acidez	
Pera Rio	médio	ereta	A.T.	ausente	alto	baixa	I.M.
Valência	alto	arredondada	tardia	ausente	alto	média	I.M.
Natal	alto	compacta	tardia	ausente	alto	média	I.M.
Folha murcha	médio	arredondada	tardia	ausente	alto	baixa	I.M.
Hamlin	médio	arredondada	precoce	presente	baixo	alta	I.M.
Bahia	alto	arredondada	M.E.	ausente	baixo	média	mesa
Baianinha	alto	arredondada	M.E.	ausente	baixo	média	mesa
Lima	médio	arredondada	M.E.	ausente	baixo	baixa	mesa
Rubi	alto	arredondada	precoce	ausente	médio	média	mesa
Westin	baixo	semiereta	M.E.	ausente	médio	média	mesa

*são consideradas de porte alto cultivares acima de 5,0 m; de porte baixo, menores de 1,5 m e de porte médio, entre essas duas medidas. Leg.: A.T.: ano todo; I.M.: indústria e mesa; M.E.: meia-estação. Fonte: EMBRAPA, 2014.

Para atender ao mercado que se destinam (indústria ou consumo *in natura*), as laranjas devem apresentar determinadas características, tais como: intensa e uniforme coloração da casca, ausência ou reduzida quantidade de sementes, epicarpo ou casca com espessura fina, redimento de suco superior a 35 mL.100 g^{-1}, teores de sólidos solúveis (SS) aproximados à 10 °Brix, faixa de acidez entre 0,5 e 1,0 g.100 mL^{-1}, destacando que para o consumo *in natura* o *ratio* deve ser acima de 14 e para a produção de suco, acima de 8. As laranjas de mesa ou consumidas *in natura* podem ser subdivididas em três grupos confome a acidez, incluindo: laranjas de baixa acidez, entre 0,005 e 0,1 g.100 mL^{-1} (Lima e Piralima); laranjas-de-umbigo, com acidez entre 0,92 e 0,94 g.100 mL^{-1} (Bahia e Baianinha); e laranja comum, aquelas que possuem 0,95 a 1,0 g.100 mL^{-1} de acidez (Pera Rio, Natal, Folha Murcha e Valência) (EMBRAPA, 2014).

2. BREVE HISTÓRICO DO CULTIVO E PRODUÇÃO DE LARANJA

A laranja é originária do sudeste da Ásia. A subespécie doce é amplamente cultivada no mundo, enquanto a amarga é principalmente produzida em determinadas regiões, como na Espanha (Sevilha e Málaga), Itália (Sicília), Líbia (Trípoli) e Malta. A laranjeira amarga do norte da Índia parece ter sido introduzida na África Oriental, Arábia e Síria, de onde os árabes, a partir das Cruzadas, trouxeram para a Europa em meados de 1200 anos DC. No Brasil, há evidências de que a laranja doce foi introduzida na Bahia pelas primeiras expedições colonizadoras. Um fato que reforça a hipótese é a presença da fruta ao longo do litoral brasileiro desde meados do século XVI (MATTOLI et al., 2005).

O cultivo de laranjas em grande escala teve início aproximado em 1950 em Limeira, São Paulo, e na década seguinte a citricultura expandiu para as cidades de Bebedouro e Araraquara, ambas também localizadas no estado de São Paulo (TOLEDO & CASTILLO, 2008). Ao analisar a produção nacional de laranjas ao longo dos últimos oito anos (2010-2017), percebe-se uma contínua redução a partir de 2012. A estimativa da produção de laranjas determinada pelo Sistema IBGE de Recuperação Automática (SIDRA) em maio de 2017 para a safra do ano corrente foi de 14.673.412 t (Tabela 2). Caso a estimativa se concretize, o valor da produção será 7,8% inferior à safra de 2016 (15.917.673 t). A produção de laranjas em 2016 apresentou uma redução de 19,6% e 4,9% em relação à 2011 (19.811.064 t) e 2015 (16.746.247 t), respectivamente (Tabela 2). O elevado estoque de suco, nacional e internacional, a crise econômica europeia e mundial, os bloqueios alfandegários nos EUA a partir de 2012, além do longo período de depreciação nos preços da laranja configuraram-se como importantes fatores de desestímulo à produção citrícola nos anos de 2013 e 2014, principalmente. Além dos fatores mencionados, em São Paulo, no Triângulo Mineiro e no Norte e Nordeste do Paraná, persistem os problemas fitossanitários como a Clorose Variegada dos Citros (CVC), a pinta-preta, a leprose, o cancro cítrico e principalmente a doença HLB, com consequente impacto negativo na produção de laranjas. Dessa forma,

além da produção de laranjas, a área a ser colhida e o rendimento médio por hectare também vem apresentando reduções a partir de 2012 (IBGE, 2013). Dentre os 43 produtos cultiváveis presentes no Levantamento Sistemático da Produção Agrícola realizado pelo IBGE/SIDRA (2017), distribuídos entre cereais, leguminosas, oleaginosas, frutas e legumes em geral, a área destinada ao cultivo da laranja foi equivalente a cerca de 0,93% da área total destinada ao cultivo dos produtos listados (IBGE/SIDRA, 2017).

Tabela 2. Área destinada à colheita de laranja em hectares e quantidade produzida em mil toneladas no Brasil, durante os anos de 2010 a 2017*.

Local	**Área destinada à colheita (hectares)**							
	2010	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**
Brasil	851.142	818.685	762.765	719.360	689.047	668.189	741.133	738.658
SP	605.432	563.952	500.549	456.818	430.906	412.861	471.200	457.453
MG	33.092	33.000	36.610	39.567	42.951	44.071	47.082	40.672
	Quantidade produzida (mil toneladas)							
Brasil	18.503	19.811	18.013	17.549	16.928	16.746	15.918	14.673
SP	14.269	15.293	13.366	13.019	12.291	12.279	11.628	10.296
MG	817	824	864	894	940	987	961	900

*os números são referentes às safras iniciada no ano em questão com término no início do ano seguinte. Leg.: SP: São Paulo, MG: Minas Gerais. Fonte: IBGE/SIDRA, 2015 e IBGE/SIDRA, 2017 (adaptado).

Em 2016, os produtores brasileiros sentiram-se encorajados a expandir seus investimentos nos pomares, devido ao repentino aumento no preço da caixa de laranja (IBGE, 2017). Ainda hoje, o estado de São Paulo é considerado o de maior importância para o cultivo da fruta. Em 2016, São Paulo contribuiu com 73,1% do total de laranjas colhidas no país, sendo sua safra equivalente a 11.628.150 t. Conforme tendência nacional, o estado de São Paulo também apresentou um declínio da produção estimada para o ano de 2017, assim como na contribuição do total colhido no país, sendo previsto o percentual de 70,6% da safra de 2017 (IBGE, 2017).

As áreas plantadas com laranjas no Brasil e em especial no estado de São Paulo sofreram consecutivas reduções e mudanças no perfil do produtor. Em 2017 a previsão da área destinada ao cultivo de laranja foi de aproximadamente 457 mil hectares, correspondente à 75,6% da área registrada em 2010 (IBGE, 2017). Apesar da tendência de redução das áreas destinadas à colheita, em 2016 foi registrado um aumento de 14% em relação a 2015 (Tabela 2).

Os pomares com dimensões menores estão perdendo espaço e geralmente acabam absorvidos pelo cultivo da cana-de-açúcar. Isso devido às desleais condições de competição entre os pequenos agricultores e aqueles inseridos no agronegócio e também pelos problemas fitossanitários. A queda da produtividade do pequeno produtor o torna pouco competitivo no mercado, principalmente quando sua atividade é exclusivamente a produção de laranja e as dificuldades financeiras impedem a renovação do pomar com a formação moderna dentro dos padrões que garantam alta produtividade. Com este comportamento, a citricultura brasileira, que tradicionalmente era composta de pequenos produtores, está mudando o perfil, diminuindo consideravelmente o número de produtores, ao mesmo tempo em que o cultivo em maiores áreas torna-se mais expressivo. Assim, 1% (251) dos produtores que produziam 45% da laranja passou a produzir mais de 60% no estado de São Paulo. O aumento na participação se deu pelo estabelecimento dos novos plantios, a maioria de forma adensada, onde o maior número de pés de laranja por unidade de área tem aumentado a produtividade (CONAB, 2011).

O estado de Minas Gerais, representado especialmente pelo triângulo mineiro vem aumentando sua área destinada à colheita da fruta, embora ainda represente aproximadamente 9 a 10% da área destinada à colheita da laranja pelo estado de São Paulo. Minas Gerais apresentou um aumento da produção de laranjas ao longo de 2011 a 2015. Após esse período, registrou-se uma consecutiva queda em sua produção (Tabela 2) (IBGE, 2017).

A citricultura brasileira é considerada uma atividade moderadamente rentável em longo prazo, mas que envolve consideráveis riscos: de mercado, de insumos, de produto e os riscos climáticos. Assim a citricultura tem sua

produção influenciada por inúmeros fatores, tais como: genótipo da laranja, solo, idade do pomar, pragas, doenças e manejo cultural. Todos esses fatores interferem na rentabilidade do negócio de citros, pois afetam custos e receitas, fazendo-os divergir do esperado pelos produtores. Para essa atividade no Brasil, a principal fonte de risco é a perda de produtividade causada pelo ataque de pragas e doenças, principalmente o HLB. As condições climáticas também são fundamentais, visto que são determinantes no desenvolvimento de inúmeras doenças, assim como no processo fisiológico de desenvolvimento dos frutos. As condições climáticas correspondem a fatores de riscos mais acentuados para o estado da Flórida, nos EUA, uma vez que a região é alvo constante de tempestades tropicais e furacões. Essa percepção do risco se deve ao fato de que ao longo da história, a cultura de laranja foi constantemente atacada por pragas e doenças, sendo que, atualmente, considera-se que existam 300 pragas e doenças afetando a citricultura paulista (ADAMI, 2010).

3. OCORRÊNCIA E EFEITO DA HUANGLONGBING NOS POMARES DE LARANJA

Nesse tópico serão aborados os aspectos econômicos envolvidos com a presença do HLB em pomares de laranjas, enquanto no Capítulo II serão apresentados os agentes causadores e vetores, a incidência dos mesmos e do HLB ao redor do mundo, os efeitos da doença nos parâmetros físico-químicos, composição química e característica sensoriais de laranjas e de seu suco, assim como nas folhas, raízes e em suas árvores. O HLB é uma doença de difícil manejo devido ao prolongado período de latência da bactéria *Candidatus* Liberibacter asiaticus (*C*Las) na árvore, distribuição irregular do patógeno na planta, efeitos do ambiente (em especial da temperatura) sobre a expressão dos sintomas e, possivelmente, sobre a multiplicação da bactéria e variações potenciais de resistência à bactéria tanto pelas espécies cítricas quanto pelo inseto vetor.

A tentativa do controle da doença tem sido realizada, principalmente, a partir do emprego de mudas sadias produzidas em ambiente protegido do

contato com o psilídio, vetor da doença, da erradicação das árvores doentes e do controle químico do vetor. Dessa forma, com o controle químico tem-se aumentado a utilização de inseticidas em pomares, causando sérios impactos ambientais, econômicos, sociais e na saúde dos trabalhadores rurais e dos consumidores, o que sugere a insustentabilidade dessa prática (BOVÉ, 2006; GOTTWALD et al., 2012). Os impactos sociais também podem ser percebidos devido às mudanças no sistema de produção e também à substituição dos pomares de laranjas por outras culturas que não requerem trabalho intensivo como a produção de frutas (MIRANDA et al., 2012). O HLB pode matar ou debilitar uma árvore cítrica em dois a dez anos, sendo que ainda hoje não existem métodos curativos eficazes para a doença que possam ser usados em pomares comerciais, permitindo assim que a fitopatologia cause grande devastação da cultura (BASSANEZI et al., 2009; BALDWIN et al., 2010).

O HLB foi reportado pela primeira vez no sul da China em 1919 e atualmente sabe-se que está presente em 50 diferentes países da Ásia, África, Oceania e Américas do Sul, Central e do Norte (CABI, 2017; EPPO, 2017), mas somente a partir de 2004 surgiu no estado da Flórida, EUA, e no Brasil. Segundo Costa (2011), enquanto os países da América não possuíam o registro da doença, o crescimento da produção foi superior ao observado naqueles países com registros históricos da doença. Com o início da incidência do HLB nos pomares de citros do Brasil e dos EUA, verificou-se uma retração na taxa de crescimento anual para o período de 2000 a 2008 da produção dos países das Américas. Concomitantemente, houve aumento na taxa anual de crescimento da produção de alguns países africanos e asiáticos, principalmente China e Indonésia.

Segundo Miranda et al. (2012), o estado de São Paulo (SP) tem enfrentado um aumento da incidência do HLB, o que coloca em risco a sustentabilidade da produção de laranjas. No Brasil, a doença foi relatada pela primeira vez em março de 2004, em Araraquara, na região central de SP. Mais tarde, em outubro de 2004, a infecção havtia alcançado em média 3,4% dos blocos de pomares no estado. Consideraram-se como pomares comerciais, aqueles com população de duzentas ou mais plantas cítricas, da mesma

espécie, idade, sob os mesmos tratos culturais, plantadas no mesmo espaçamento e sem ruas ou corredores que dividam o bloco de plantas. Em 2010, a amostragem do FUNDECITRUS indicou 38,8% de blocos com pelo menos uma planta sintomática e 1,9% de árvores infectadas em SP. No último levantamento, em agosto de 2011, os blocos e as árvores infectadas haviam atingido, respectivamente, 53,4 e 3,78%.

Em SP, as ações de defesa sanitária vegetal para o controle da doença vêm sendo executadas pela Coordenadoria de Defesa Agropecuária (CDA) em conjunto com o Ministério da Agricultura, Pecuária e Abastecimento (MAPA) e a Agência Paulista de Tecnologia dos Agronegócios (APTA). Cerca de um ano após a primeira constatação do HLB no Brasil, foi publicada a Instrução Normativa/MAPA nº 10, de 2005, posteriormente substituída pela Instrução Normativa/MAPA nº 32, de 2006, que determinou a eliminação de plantas cítricas sintomáticas e comprovadamente infectadas pelas bactérias causadoras do HLB. Atualmente, está em vigor a Instrução Normativa/MAPA nº 53, de 2008, que garante uma maior agilidade no processo de fiscalização, atribuindo novos deveres a todos os produtores de citros, como a elaboração de um relatório semestral informando a incidência do HLB nas fazendas e determinando a eliminação de plantas sintomáticas e assintomáticas do mesmo talhão[1], quando a incidência da doença for superior a 28%. Houve ainda a publicação da Portaria CDA-21, de 15/12/2011, na qual todo o estado de SP fica delimitado e oficializado como área sob vigilância fitossanitária visando o controle do HLB (MAPA, 2005; MAPA, 2006; MAPA, 2008; RUIZ et al., 2010; CDA, 2011).

O sucesso do controle do HLB depende da ação conjunta de todos os citricultores. Desse modo as ações governamentais que objetivam eliminar as fontes de inóculos oriundas de propriedades que não cumprem a legislação específica para a doença, são justificadas, pois se não forem feitas, podem colocar em risco a sanidade dos pomares das propriedades circunvizinhas (RUIZ et al., 2010). No entanto, o prejuízo associado à eliminação das laranjeiras sintomáticas ou mesmo de toda a cultura tem desestimulado muitos

[1] Talhão: fração ou parcela de uma propriedade separada por ruas, estradas, carreadores ou outro meio qualquer, geralmente, com largura superior ao espaçamento entre linhas (FUNDECITRUS, 2017).

citricultores em atender a Instrução Normativa/MAPA nº 53, de 2008. Essa ação compromete o controle do HLB pelo risco de deslocamento da doença para outros pomares vizinhos onde a doença não foi diagnosticada e regiões brasileiras ainda livres (MAPA, 2008; MIRANDA et al., 2012).

Além da incidência do HLB, outros fatores também contribuíram para o aumento dos custos da produção e distribuição de laranjas. Os custos de colheita e transporte da fruta até as fábricas sofreram impactos relevantes no período entre as safras de 2003/2004 e 2009/2010. A média dos custos operacionais de produção da laranja dos pomares próprios das indústrias colocadas no portão das fábricas para esse intervalo de tempo sofreu um acréscimo, em dólares, de 202%. O custo saltou de US$ 1,31 para US$ 3,96 por caixa de 40,8 Kg. Nesses cálculos estão inclusos os custos de colheita e transporte dos frutos, porém, está excluído o cálculo de depreciação e amortização do capital investido. Contribuiu também para a escalada dos custos agrícolas, uma forte inflação de custos de mão de obra, insumos agrícolas e incremento de tratamentos fitossanitários contra o cancro cítrico, HLB e pinta preta (NEVES et al., 2009).

4. BREVE HISTÓRICO DA PRODUÇÃO DE SUCO DE LARANJAS

O sucesso da produção nacional de laranjas pode ser explicado pelas excelentes condições climáticas do Brasil para o cultivo da fruta, assim como pela instalação das grandes indústrias de suco concentrado na região Sudeste do país, em especial, no estado de SP em meados da década de 60. Estas proporcionaram o desenvolvimento do maior parque citrícola do mundo, que, desde a sua criação, teve como principal escoamento de produção o mercado internacional (TOLEDO & CASTILLO, 2008).

Desde 1962, quando começaram as primeiras exportações, a citricultura tem contribuído de forma definitiva para o desenvolvimento do Brasil. Em 2009, as exportações do complexo citros somaram 2,9 milhões t, sendo 1,129 milhão t de suco de laranja concentrado e congelado (do inglês *Frozen Concentrated Orange Juice* - FCOJ), 939 mil t de suco de laranja não concentrado (do inglês

Not from Concentrate Juice - NFC), e 851 mil t de subprodutos (NEVES et al., 2009). Em 2014 o Brasil produziu 1,831 milhão t de suco de laranja, no entanto, apresentou uma redução de aproximadamente 12,1% de sua produção quando comparado a 2013, cuja produção foi de 2,084 milhões t (CONAB, 2017). A safra que se iniciou em 2015 gerou uma das piores quedas de produtividade de FCOJ já registrada na história da citricultura paulista – cerca de 25,7% menor que o equivalente obtido na safra iniciada em 2014. Uma das principais causas naturais levantadas para o ocorrido foi o aumento, acima da média, das chuvas em decorrência do fenômeno *El Niño* durante os principais meses de colheita. As consequências afetaram diretamente as indústrias de suco de laranja uma vez que recebeu sua matéria prima com reduzido percentual de suco na fruta, aumento do percentual de casca e polpa e redução do teor de SS de 12 °Brix na safra 2014 para 10,2 °Brix na safra de 2015 (CITRUSBR, 2016a). Ainda, segundo os dados da Associação Nacional dos Exportadores de Sucos Cítricos (CITRUSBR, 2016b) fornecidos ao Fundo de Defesa da Citricultura (Fundecitrus), a produção de suco de laranja provenientes da safra de 2015 (finalizada em abril de 2016) pelos produtores associados ao CitrusBR no Cinturão Citrícola Paulista e Triângulo Mineiro foi de 795.463 t FCOJ. Considerando o mesmo rendimento industrial médio aplicado das indústrias associadas ao CitrusBR aos processadores não associados, pode-se inferir uma produção aproximada de 70.000 toneladas de FCOJ. Esses números foram maiores que aqueles apresentados recentemente para a safra de 2016 (finalizada em abril de 2017). A produção de FCOJ pelos produtores associados ao CitrusBR do Cinturão Cintrícola Paulista e Triângulo Mineiro foi de 648.004 t, já para os não associados foi de aproximadamente 54.000 t (CITRUSBR, 2017).

A citricultura norte-americana ocupa a quarta posição mundial em volume de produção do fruto, sendo o estado da Flórida a grande região produtora. Entretanto, diferentemente do Brasil, a maior parte da produção americana é destinada ao abastecimento do mercado interno, e não à exportação. O impacto da safra americana na exportação do suco brasileiro se deve ao fato do Brasil ficar na dependência dos resultados da produtividade dos pomares da Flórida, para que os EUA comprem o suco brasileiro, com o objetivo de suprir a

demanda interna. Caso a produção americana seja suficiente para o seu abastecimento, as indústrias brasileiras ficam com grande quantidade de suco sem um mercado certo para exportação, gerando um estoque ocioso, impulsionando assim a queda do preço do produto. Para se tornarem menos suscetíveis à influência americana, os citricultores brasileiros vêm buscando maior diversificação no mercado.

A citricultura brasileira conseguiu uma boa eficiência na cadeia citrícola. Desde mudas e viveiros certificados, plantio e cultivo da laranja, produção do suco de laranja até a distribuição internacional em sistemas integrados a granel com caminhões-tanques, terminais portuários e navios dedicados que levam ao consumidor europeu, norte-americano e asiático produtos citrícolas com dezenas de especificações e misturas (*blends)* para as mais variadas aplicações. Além disso, é responsável por cerca de metade do suco de laranja do planeta cujas exportações trazem de US$ 1,5 bilhão a US$ 2,5 bilhões por ano ao país. Grande parcela do suco distribuído é feito por empresas multiprodutos, nas quais o suco de laranja integral corresponde a apenas mais um item de seu vasto portfólio de bebidas, como néctares e refrescos de outros sabores, água, refrigerantes, energéticos, lácteos e demais bebidas não alcoólicas, que invariavelmente canalizam mais investimentos de marketing. Estas empresas dão mais atenção e prioridade de produção àquelas categorias de bebidas que estão em alta e oferecerem maior margem de lucro, mesmo sendo algumas dessas de qualidade nutricional inferior ao suco integral de laranja (NEVES et al., 2009).

Com o mercado altamente competitivo, as indústrias de bebidas apostam na diversificação de sua linha de produtos. Além do suco (concentrado, integral e reconstituído), o néctar é outra opção de bebida à base de laranja. Por possuir menor teor de suco (ingrediente de maior custo), o preço final dos néctares é menor que os preços praticados de sucos integrais e sucos reconstituídos. Neste aspecto, os néctares vêm ganhando espaço entre os consumidores (FIGUEIRA et al., 2010).

Nas safras, de 1995/96 a 2009/10, a queda na produção mundial de suco foi de 13% (equivalente a 308 mil toneladas). As maiores reduções aconteceram

na Flórida em 295.000 t e no cinturão citrícola de SP e Triângulo Mineiro em 31.000 t. O surgimento do HLB em citros em meados de 2004 no Brasil e 2005 nos EUA possivelmente contribuiu com a diminuição da produção de suco de laranja. Apesar da queda notificada, essas regiões continuam liderando a produção mundial de suco de laranja, com 81% de toda a produção (NEVES et al., 2009).

5. ETAPAS DO PROCESSAMENTO E COMERCIALIZAÇÃO DO SUCO DE LARANJA PRODUZIDO NO BRASIL

A laranja é considerada uma fruta de padrão de maturação não-climatérica, assim como os demais citros. Não há incremento na produção de etileno ou na taxa de respiração associado com a maturação. Assim, para o processamento do suco de laranja, essa deve ser colhida após a maturidade fisiológica e quando o produto apresentar as características de qualidade adequadas para o consumo ou para a comercialização (CHITARRA & CHITARRA, 2005).

O processo de produção de FCOJ consiste em várias operações industriais de grande escala, além do suco, há vários subprodutos obtidos durante o seu processamento da laranja, conforme ilustrado na Figura 1. Após a colheita, as laranjas são transportadas, geralmente em caminhões ou carretas, até o pátio das empresas. Durante o descarregamento dos frutos, uma amostra representativa da carga é coletada e destinada ao laboratório de controle de qualidade, a fim de se avaliar a cor, defeitos e extração do suco visando monitorar o rendimento e o teor de SS, AT e *ratio*. As laranjas passam por procesoss de lavagem e sanitização, sem que a superfície da fruta seja danificada, a higienização usualmente é realizada por um sistema de aspersão de água quente e clorada, com escovas rotativas. Em seguida as laranjas são descarregadas em plataformas inclináveis e levadas por meio de esteiras para as mesas de seleção manual. Durante o trajeto, os frutos que apresentarem ferimentos nas cascas, que estiverem excessivamente danificados por ácaros ou contendo sujidades, não apresentarem dimensões adequadas ou apresentarem

em estágio avançado de senescência são rejeitados. Os resíduos e os descartes da seleção dos frutos são pesados e enviados à fábrica de ração para serem transformados em farelo de polpa cítrica, a partir do processo de secagem da matéria prima. Será formada uma forragem concentrada que serve de ração para alimentação animal.

As laranjas sadias são classificadas automaticamente por tamanho a fim de permitir o ajuste dos copos das extratoras em função do seu tamanho. Os frutos são transportados por elevadores de canecas aos silos de armazenagem, onde ficarão até serem encaminhados para a etapa de extração (CETESB, 2005; MACHADO, 2010). A etapa de extração é a principal etapa do processo de obtenção do suco diretamente da laranja, e tem por finalidade separar o suco do bagaço, da casca e da semente. Nessa etapa ocorre a separação do suco de laranja, da emulsão que dará origem ao óleo essencial, do bagaço e da casca que darão origem à polpa cítrica e da polpa que poderá ser readicionada ao suco conforme solicitação do cliente. Na indústria de cítricos há várias extratoras acopladas em série que são projetadas para extrair o máximo de suco, evitando incorporar componentes da casca e óleo essencial. Geralmente as extratoras de suco de laranja são formadas por copos que se interpenetram comprimindo a fruta inteira e separando as frações de interesse comercial (CETESB et al., 2005; RODRIGUES & FERRI, 2012).

A quantidade de suco extraído da laranja pode variar em uma faixa de 35 a 60 mL.100 g^{-1} dependendo das condições climáticas, da cultivar, do tamanho do fruto e das condições de extração. Durante a etapa de extração, ocorre o rompimento das células de óleo essencial presentes na casca que posteriormente será recuperado e utilizado na produção de compostos para bebidas, cosméticos e produtos químicos. O D-limoneno ou terpeno cítrico é o principal componente do óleo da casca da laranja, sendo utilizado como matéria-prima para a fabricação de resinas sintéticas e adesivos pelas indústrias de plásticos (CITRUSBR, data de publicação não informada).

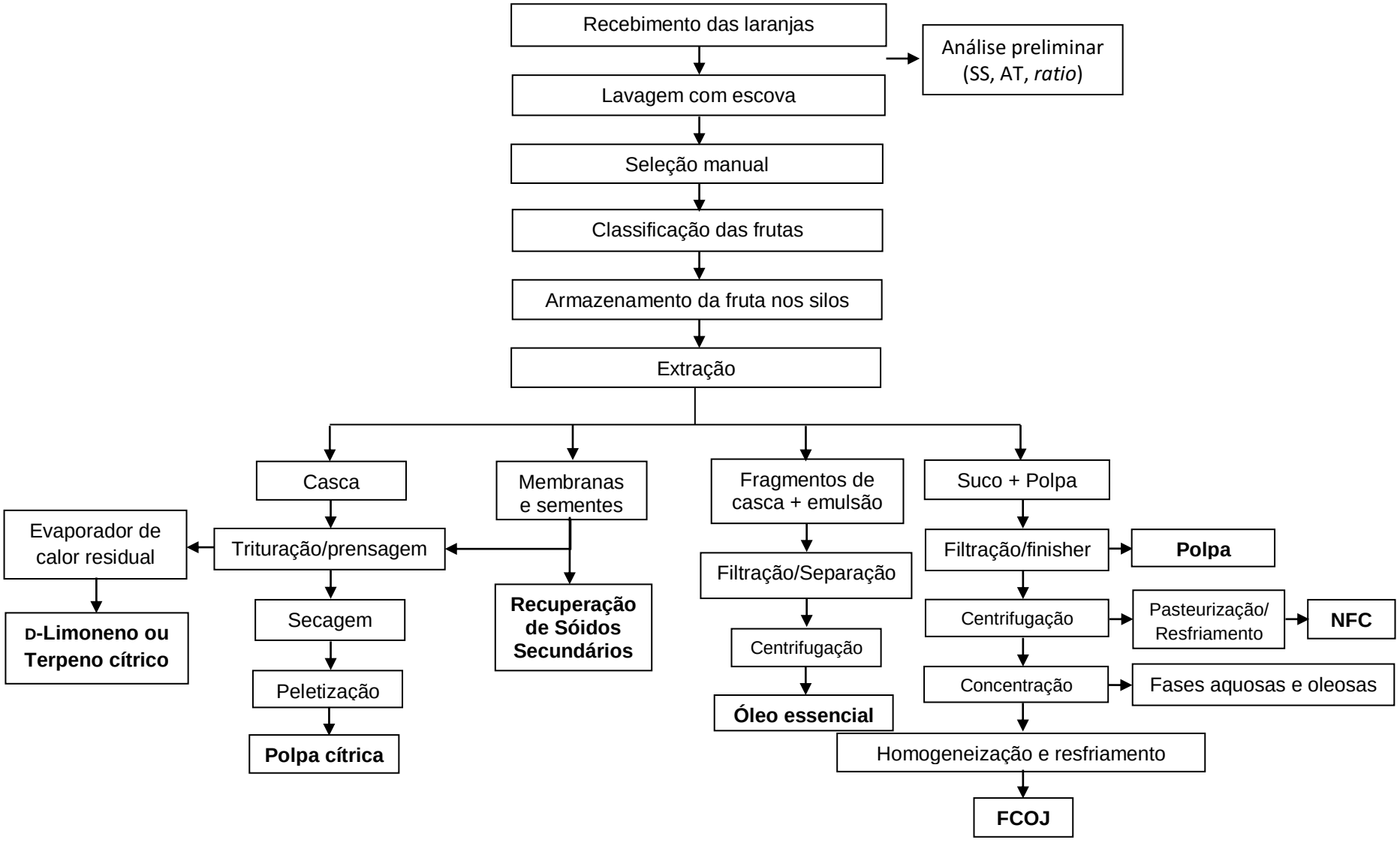


Figura 1. Esquema de uma planta de processamento comercial de suco de laranja. Fonte: JBT, 2015 (modificado).

Após a extração, o suco passa pela etapa de clarificação, uma vez que ainda contém polpa e resíduos de bagaço que são removidos por centrifugação ou em equipamentos denominados *finishers* (despolpadeiras), os quais separam a polpa do suco por filtração. Este processo consiste numa operação na qual o suco é tranportado por uma rosca sem fim que aplica uma pressão contra uma peneira (cuja malha é de 0,64 a 1,27 mm) separando assim os sólidos. Em geral, o teor de polpa do suco fica em torno de 4%. A polpa pode ser utilizada na produção de outros produtos, como por exemplo, o suco obtido da polpa (*pulp wash*) (RODRIGUES & FERRI, 2012).

Antes do processo de concentração, os sucos são pasteurizados, dessa forma, ocorre a inativação de micro-organismos responsáveis pela degradação do suco de laranja e da pectinesterase, enzima responsável pela desesterificação da pectina, produzindo metanol e pectina com baixo teor de metoxilação. A pectina com baixo teor de metoxilação pode se ligar a íons de cálcio presentes e precipitar-se, afetando assim a qualidade do suco. Com isso a pectina que antes auxiliava na estabilização da turbidez, perde o seu papel, causando além da redução da turbidez, a alteração do sabor e do aroma (TRIBESS, 2003; CETESB, 2005; MACHADO, 2010, CITRUSBR, data de publicação não informada).

A concentração do suco de laranja consiste na extração da água de constituição do suco, reduzindo assim sua atividade de água. As temperaturas utilizadas para concentrar o suco são de 90 a 95 ºC, em evaporadores à vácuo (RODRIGUES & FERRI, 2012). O SS inicial do suco, geralmente na faixa de 10 a 11 °Brix, aumenta ao final do processo para 65 °Brix, padrão de qualidade fo FCOJ. Em seguida, o concentrado é armazenado a - 6,6 ºC ou temperatura inferior até que seja envasado para a venda. É possível armazenar o FCOJ durante vários anos, desde que em temperaturas adequadas. Assim, a utilização desse método de conservação favorece a produção de suco de laranja reconstituído. Sendo a via de produção de suco, a partir do reconstituído, a mais empregada em todo o mundo (VIEIRA et al., 2010).

Do processo de evaporação do suco de laranja pode-se obter a essência, formada por componentes polares e apolares, dissolvidos em uma fase aquosa

e outra oleaginosa. Esse subproduto pode ser readicionado ao suco, assim como pode ser usado para outros fins nas indústrias de bebidas e alimentos (CETESB, 2005; CITRUSBR, data de publicação não informada).

A indústria de suco concentrado tem se esforçado para reduzir o volume de sucos cítricos por eliminação do conteúdo de água. Esta redução é importante por duas razões principais, primeiro, por facilitar o transporte marítimo reduzindo o tamanho dos *containers.* Em segundo lugar, por ser vantajoso do ponto de vista de conservação, possibilitando o seu consumo fora do período de colheita. A especificação FCOJ se refere ao suco de laranja com teor de SS igual ou superior a 42,0 °Brix (VIEIRA, 2006; FLORIDA DEPARTMENT OF AGRICULTURE AND CONSUMER SERVICES, 2016).

O suco de laranja originário do Brasil é conhecido por sua elevada qualidade; além disso, o país é o maior produtor e exportador mundial de FCOJ. O volume de FCOJ exportado pelo Brasil no período de 2014 a 2016, a partir do porto de Santos, apresentou um discreto aumento de 4,17%, sendo o volume de exportação em 2016 equivalente a 1.080.448 t. No entanto, a exportação de FCOJ durante os meses de janeiro a abril de 2017 sofreu uma queda considerável quando comparado com o ano anterior (Figura 2).

Para produzir o FCOJ exportado em 2014, 2015 e 2016 as indústrias processadoras de suco do Cinturão Citrícola Brasileiro utilizaram aproximadamente 83%, 87% e 86%, respectivamente, do total de laranjas produzidas no Estado de SP. Esses valores estão próximos à porcentagem média utilizada entre os anos de 2004 a 2011, equivalente a 88,8%, porém discrepante da porcentagem utilizada em 2012, equivalente a 79% (FLORIDA DEPARTMENT OF CITRUS, 2016). Em 2012 o mercado brasileiro de citros sofreu embargos alfandegários dos EUA, além da redução das vendas à União Europeia proporcionado por reflexos da crise econômica.

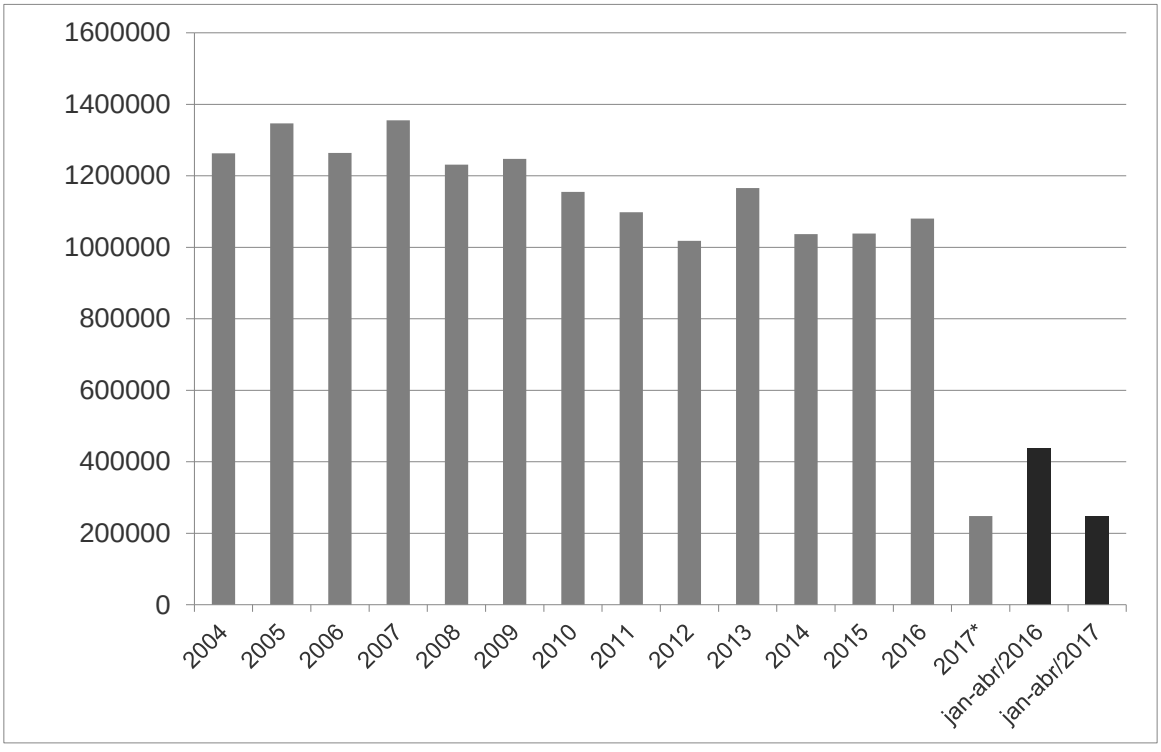


Figura 2. Exportações de FCOJ equivalente em toneladas por ano civil a partir do porto de Santos. Leg: *até abril de 2017. Fonte: CITRUSBR, 2016b.

O tipo de suco produzido é estabelecido pelo comportamento do consumidor em mercados de mais alto poder aquisitivo, que nos últimos anos passou a preferir o NFC ao FCOJ, por ser um produto de paladar mais agradável, com sabor mais aproximado ao suco extraído na hora e por ter a imagem de uma bebida mais saudável. As primeiras produções de NFC no Brasil começaram em 1999/2000 ainda em caráter experimental, e em 2000 foram realizadas as primeiras exportações (NEVES et al., 2009).

O volume de NFC exportado pelo Brasil tem aumentando continuamente ao longo dos anos, com destaque para 2016, que ultrapassou a quantidade de 1,4 milhões de toneladas (CITRUSBR, 2016b). Assim como o FCOJ, as exportações para os meses de janeiro a abril de 2017 foram inferiores ao do mesmo período de 2016 (Figura 3).

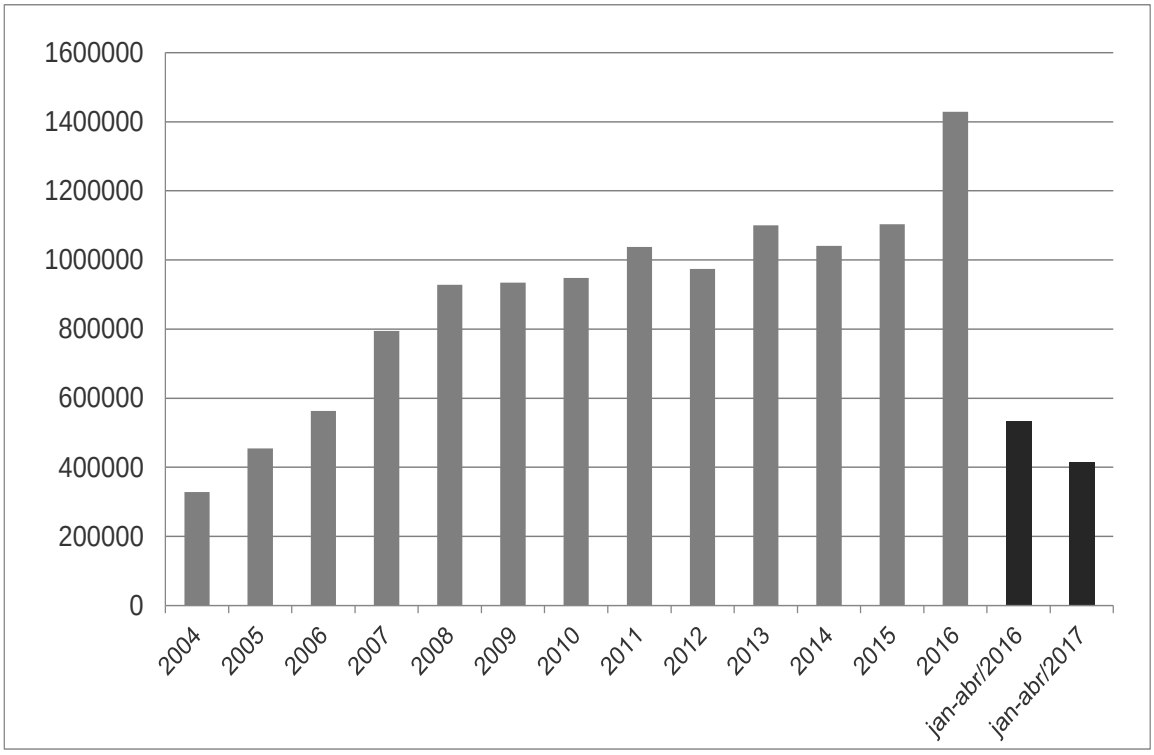


Figura 3. Exportações de NFC equivalente em toneladas por ano civil a partir do porto de Santos. Fonte: CITRUSBR, 2016b.

6. CONSUMO DA LARANJA *IN NATURA* E SEUS DERIVADOS

O consumo interno da laranja *in natura* é crescente e recebe apoio a partir da prática comum do preparo de suco nas residências, em padarias e restaurantes, além do mercado de suco pasteurizado produzido em fábricas com atuação regional. Uma laranja média pode gerar cerca de 90 g de suco. O mercado doméstico de laranja *in natura* se tornou um grande consumidor da produção brasileira uma vez que mais de 100 milhões de caixas de laranja (40,8 kg/caixa), equivalente a aproximadamente 30% da produção nacional, são consumidas pelo povo brasileiro que tem à sua disposição uma fruta nutritiva a um preço competitivo (NEVES et al., 2009; NEVES & TROMBIN, 2011).

O suco de laranja é um produto de grande importância econômica para as exportações brasileiras. O agradável sabor, além das atrativas propriedades nutricionais torna essa bebida muito apreciada e consumida por populações de

diferentes culturas e hábitos alimentares. Dentre os sucos de frutas, o de sabor laranja é o mais consumido no mundo. Em 2016, o consumo de suco integral de laranja representou 45% dentre o total de sucos de frutas consumidos pelos 40 principais países que representam 99% do consumo mundial da bebida (MARKETSTRAT, 2016). Apesar do destaque do suco integral de laranja dentre os demais sabores, o seu consumo em nível mundial vem apresentando redução ao longo dos anos. Para o período compreendendo entre 2003 a 2015, a redução foi equivalente a 2.608 milhões de litros. Os sete principais consumidores mundias de suco de laranja são: EUA, Alemanha, França, China, Canadá, Reino Unido e Brasil.

A situação de mercado para o FCOJ não é diferente daquela do NFC. Analisando o período de 2003 a 2015, o consumo reduziu 19,4%, sendo os EUA, Alemanha, França, China e Canadá os países responsáveis pelas quedas mais expressivas. Em 2015 o consumo aproximado de FCOJ pelos EUA foi equivalente a 613.000 t e no Brasil, 63.000 t (CITRUSBR, 2016a; MARKETSTRAT, 2016).

Em 2010 alguns fatos surgiram como promessa de melhoria para o mercado mundial de FCOJ. O consumo durante o ano de 2010 voltou a crescer na média de 1% em comparação ao de 2009. O incremento desse consumo foi observado nos países emergentes, ainda com mercado pequeno, mas também houve recuperação em alguns mercados tradicionais europeus. Em apenas um ano, estes consumiram 42 mil t a mais de FCOJ. A soma destes crescimentos em 2010 compensou a queda observada nos EUA (NEVES & TROMBIN, 2011). No entanto, o ano de 2013 refletiu as perdas na citricultura paulista, verificadas em 2012. A redução do fluxo dos estoques de suco dificultou a comercialização das frutas e até mesmo o seu apodrecimento nos pomares. A crise no Mercado Europeu e as barreiras alfandegárias impostas pelos EUA foram consideradas as principais responsáveis pelos prejuízos à citricultura brasileira no ano de 2013. A safra nacional, de 400,6 milhões de caixas, apresentou decréscimo de 14,6%, em relação à safra colhida em 2012. A laranja para indústria, em SP, fechou o mês de setembro de 2013 com o preço da caixa de laranja sendo comercializada a R$ 7,66, considerado baixo pelos produtores (IBGE, 2013). Em

contrapartida, os preços atuais (fevereiro e março de 2017) recebidos pelos produtores tiveram aumento de 107,4% em SP, 60,0% em Minas Gerais e 197,4% na Bahia, se compardos ao relativo mês de fevereiro de 2016. Segundo os dados da conjuntura mensal para a produção da laranja Pera Rio publicados pela Companhia Nacional de Abastecimento (CONAB), em fevereiro de 2017, os preços recebidos pelos produtores por uma caixa de laranja nos seguintes estados foram: SP (R$ 30,96), Minas Gerais (R$ 24,00) e Bahia (R$ 23,05). Sendo o preço no atacado equivalente a: R$ 79,97; R$ 75,07 e R$ 31,82, respectivamente, para os estados citados anteriormente (CONAB, 2017).

7. COMPOSIÇÃO QUÍMICA E NUTRICIONAL DO SUCO DE LARANJA

Os principais componentes do suco natural de laranja são os carboidratos, que constituem mais de 70% dos SS. Em segundo lugar são os ácidos orgânicos, principalmente o cítrico e o málico, que representam até 10% dos SS. O restante é composto por aminoácidos livres, bases nitrogenadas (6%), íons inorgânicos (aproximadamente 3%), vitaminas (2,5%), lipídeos (1,2%) (Tabela 3), flavonoides (1,2%) e outros (VIEIRA, 2006; VIEIRA et al., 2010; TACO, 2011).

Aproximadamente 70% dos compostos nitrogenados são aminoácidos livres e o restante do nitrogênio encontra-se na forma de proteínas, enzimas, aminas, nucleotídeos, ácidos nucleicos, fosfolipídios e vitaminas. A prolina é o aminoácido predominante no suco de laranja, representando 50% do total de aminoácidos livres (VIEIRA, 2006). Slisz et al. (2012) identificaram os principais aminoácidos presentes no suco de laranja: alanina, arginina, asparagina, aspartato, histidina, isoleucina, leucina, fenilalanina, prolina, treonina e valina, com prevalência da prolina, seguida pela arginina, asparagina, aspartato e alanina. O teor calórico da fruta *in natura* é proveniente em 90% dos carboidratos, 6% da proteína e 4% dos lipídeos, enquanto no suco, os carboidratos representam 92% do teor calórico total, seguido de 5.5 % das proteínas e 2.5 % dos lipídeos.

Tabela 3. Composição química representativa da laranja Valência *in natura* e do seu suco.

Componentes	**Fruta (por 100 g)**	**Suco (por 100 g)**
Calorias (Kcal)	46	37
Proteínas (g)	0,8	0,5
Lipídeos (g)	0,2	0,1
Carboidratos (g)	11,7	8,6
Fibra alimentar (g)	1,7	0,4
Magnésio (mg)	14	10
Cálcio (mg)	34	9
Manganês (mg)	0,06	0,03
Fósforo (mg)	20	17
Ferro (mg)	0,1	Tr
Potássio (mg)	158	143
Cobre (mg)	0,04	0,04
Tiamina (mg)	0,07	Tr
Riboflavina (mg)	0,04	Tr
Piridoxina (mg)	0,03	0,03
Vitamina C (mg)	47,8	73,3*

Tr = traços. *valor referente ao suco da laranja Pera Rio, uma vez que a fonte utilizada não informa o seu teor no suco de laranja Valência. Fonte: TACO, 2011.

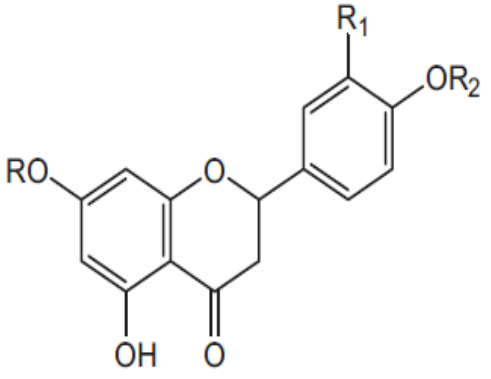
Os citros contêm uma gama de flavonoides, em especial os compostos pertencentes ao grupo das flavanonas, geralmente glicosilados por um disscarídeo no carbono de posição 7. As flavanonas são encontradas em alta concentrações nas frutas cítricas quando comparadas aos outros vegetais, um litro de suco de laranja pode conter entre 200 a 600 mg de hesperidina, 15 a 85 mg de narirutina (MANACH et al., 2004) e 8 a 30 mg de didimina (GATTUSO et al., 2007). Um simples copo de suco de larnaja pode conter entre 40 a 140 mg de flavanonas glicosiladas (MANACH et al., 2004). A Tabela 4 contém a concentração média de algumas flavononas, flavonas e flavonas metoxiladas em laranja Valência, assim como em sua casca e vesículas de suco. As estruturas

químicas dos flavonoides determinados na Tabela 4 estão representadas na Figura 4 (NOGATA et al., 2006). Além dos flavonoides, a laranja também é fonte de ácidos hidroxicinâmicos, a exemplo do *p*-cumárico encontrado em laranjas numa faixa de 17,8 a 18,1 (PEI et al., 2016) e dos ácidos ferúlico e sinápico (DALA PAULA et al., 2017a; 2017b). As estruturas químicas dos ácidos hidroxicinâmicos mencionados estão representadas na Figura 4.

Tabela 4. Teores médios de flavonoides em casca, em vesícula de suco e em laranja Valência

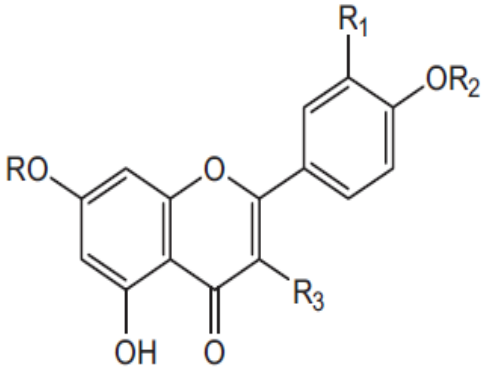
Flavonoides	**Laranja**	**Casca**	**Vesícula de suco**
Flavanonas (mg.100 g^{-1} peso fresco)*			
Eriocitrina	15,9	5,9	19,7
Neoeriocitrina	2,7	0,0	5,5
Narirutina	166	66,5	54,3
Naringina	0,0	0,0	0,0
Hesperidina	962	1410	93,2
Neohesperidina	0,0	0,0	0,0
Neoponcirina	57,1	42,1	2,9
Poncirina	0,0	0,0	0,0
Flavonas (mg.100 g^{-1} peso fresco)*			
Rutina	10,8	0,0	21,6
Isorhoifolina	0,3	1,1	0,0
Rhoifolina	1,5	5,8	0,0
Diosmina	1,4	5,5	0,0
Neodiosmina	7,7	3,0	0,0
Flavonas polimetoxiladas (mg.100 g^{-1} peso fresco)*			
Sinensetina	8,8	34,0	0,0
Nobiletina	5,0	18,1	0,6
Tangeritina	2,2	8,5	0,0
Heptametoxiflavona	0,5	2,0	0,0

*Cada valor se refere a média de quatro replicatas. Fonte: NOGATA et al., 2006.



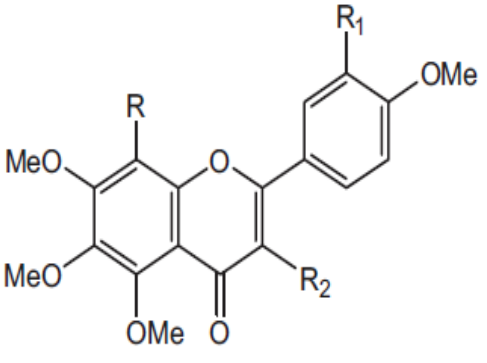
Flavanonas

Eriocitrina	(R=rutinose, R_1=OH, R_2=H)
Neoeriocitrina	(R=neohesperidose, R_1=OH, R_2=H)
Narirutina	(R=rutinose, R_1=R_2=H)
Naringina	(R=neohesperidose, R_1=R_2=H)
Hesperidina	(R=rutinose, R_1=OH, R_2=CH_3)
Neohesperidina	(R=neohesperidose, R_1=OH, R_2=CH_3)
Neoponcirina	(R= rutinose, R_1=H, R_2=CH_3)
Poncirina	(R=neohesperidose, R_1=H, R_2=CH_3)



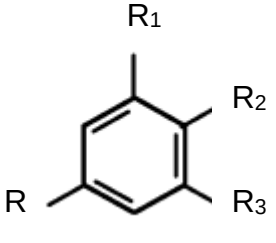
Flavonas

Rutina	(R=H, R_1=OH. R_2=H, R_3=O-rutinose)
Isorhoifolina	(R=rutinose, R_1=R_2=R_3=H)
Rhoifolina	(R=neohesperidose, R_1=R_2=R_3=H)
Diosmina	(R=rutinose, R_1=OH, R_2= CH_3, R_3=H)
Neodiosmina	(R=neohesperidoside, R_1=OH, R_2=CH_3, R_3=H)



Flavonas polimetoxiladas

Sinensetina	(R=H, R_1=OCH_3, R_2=H)
Nobiletina	(R=R_1=OCH_3, R_2=H)
Tangeretina	(R=OME, R_1=R_2=H)
Heptametoxiflavona	(R=R_1=R_2=OMe)



Ácidos hidroxicinâmicos

Ferúlico	(R= CH_2=CH_2-COOH, R_1=H, R_2=OH, R_3=OCH_3)
Sinápico	(R =CH_2=CH_2-COOH, R_1=R_3=OCH_3, R_2=OH)
p-Cumárico	(R=CH_2=CH_2-COOH, R_1=R_3=H, R_2=OH)

Figura 4. Estruturas química de compostos fenólicos encontrados em laranja. Fonte: NOGATA et al., 2006; BENAVENTE-GARCÍA & CASTILLO, 2008).

8. PROPRIEDADES FUNCIONAIS DA LARANJA

O consumo do suco de laranja fresco e pasteurizado interfere na constituição da microbiota intestinal. A administração de ambos os sucos contribui para o aumento de *Lactobacillus* spp. e *Bifdobacterium* spp., reduzindo a população de enterobactérias. Essa alteração da microbiota, por sua vez, aumenta a atividade antioxidante intestinal, eleva a produção de ácidos graxos de cadeia curta, importante substrato energético aos colonócitos, e reduz a produção de amônia. Dessa forma, fica claro o potencial do consumo do suco de laranja como alimento prebiótico (DUQUE et al., 2016).

O suco de laranja é considerado de elevado valor benéfico por conter antioxidantes naturais, dentre eles, ácido ascórbico, carotenoides, poliaminas (espermina e espermidina), fenilpropanoides e os flavonoides, especialmente a hesperidina e a naringenina. Estudos epidemiológicos associam o consumo regular de suco de laranja com a redução dos riscos aos danos oxidativos ocasionados pelos radicais livres e a diminuição da prevalência de doenças como os diferentes tipos de câncer, doenças cardiovasculares e neurológicas (GIL-IZQUIERDO et al., 2002; FRANKE et al., 2005; VIEIRA et al., 2007; 2010; GALAVERNA et al., 2008;).

Com relação aos carotenoides, o suco de laranja constitui-se em uma rica fonte, podendo ser encontrado o maior número dessas substâncias quando comparado aos outros sucos de frutas. Estes apresentam grande diversidade estrutural, assim como importantes funções para a saúde humana, alguns são pró-vitamina A (β-caroteno, α-caroteno, β-criptoxantina) e outros tais como a zeaxantina e a luteína estão associados com a preservação da degeneração macular e com a diminuição da ocorrência de catarata, ambos relatados com o avançar da idade (GAMA & SYLOS, 2007). No entanto, é importante observar que no suco de laranja, os teores de vitamina C, compostos fenólicos e carotenoides podem variar conforme os procedimentos adotados para a sua produção. Gil-Izquierdo et al. (2002) pesquisaram a interferência do processamento industrial e extração do suco de laranja realizado a partir de diferentes técnicas quanto aos teores de compostos fenólicos e vitamina C no

suco da fruta. Os autores observaram que a técnica de extração utilizada industrialmente contribuiu com o aumento de 22% de compostos fenólicos e 25% quando comparado com a extração manual. Também perceberam que a pasteurização foi capaz de degradar vários compostos fenólicos. Gama e Sylos (2007) estudaram a alteração nos teores de pigmentos carotenoides em suco de laranja Valência após a pasteurização (95 a 105 ºC durante 10 s) e observaram redução ($p < 0,05$) nos teores de violaxantina e luteína em 38% e 20%, respectivamente, e na etapa de concentração do suco houve perda de 17% de luteína. A β-criptoxantina tornou-se o carotenoide mais concentrado nos sucos pasteurizados. No entanto, com relação aos teores de β-caroteno, α-caroteno, β-criptoxantina e zeaxantina, que são considerados importantes compostos ativos contra a degeneração macular e catarata, os autores não observaram diminuição significativa após as etapas pasteurização e concentração.

Nas cascas e nos frutos imaturos de laranjas amargas, assim como no suco de laranja, podem ser encontradas aminas fenólicas, tais como a N-metiltiramina, a octopamin e a sinefrina. Esta última apresenta grande interesse farmacológico por ser um agente simpatomimético. A sinefrina possui atividades relacionadas à vasoconstrição e relaxamento da musculatura brônquica, podendo ser utilizada como descongestionante das vias superiores. E também pode interferir no metabolismo humano, sendo responsável pela redução da massa de gordura em humanos obesos uma vez que estimula a lipólise e aumenta a taxa metabólica e a oxidação de gordura a partir de uma maior termogênese (MATTOLI et al., 2005; VIEIRA et al., 2010).

OBJETIVOS

O presente trabalho teve como objetivo geral determinar as alterações na físico-químicas, na composição química e nas características sensoriais responsáveis pelo típico sabor de laranjas sintomáticas para o HLB.

Os objetivos específicos foram:

i) Investigar a influência da época de colheita em uma mesma safra nas características físico-químicas, bioquímicas e sensoriais do suco de laranja Valência;

ii) Realizar uma revisão da literatura científica sobre as alterações físico-químicas, na composição química e nas características sensoriais de suco de laranjas acometidas pelo HLB;

iii) Avaliar as características físico-químicas, a composição química e as características sensoriais dos sucos provenientes de laranjas Valência sintomáticas para o HLB (laranjas colhidas em árvores infectadas pela bactéria *Candidatus* Liberibacter asiaticus); e saudáveis (livres da infecção de bactéria *Candidatus* Liberibacter asiaticus).

Cada um destes objetivos específicos foi atendido e apresentado na forma de capítulos:

I. Influence of harvest time on quality of ‘valencia’ oranges and juice, second season
II. Effect of Huanglongbing (greening disease) on orange juice quality, a review
III. Active taste compounds in juice made from oranges symptomatic of Huanglongbing (HLB) greening disease.

CAPÍTULO I - INFLUENCE OF HARVEST TIME ON QUALITY OF 'VALENCIA' ORANGES AND JUICE, SECOND SEASON

ABSTRACT

Valencia' oranges were harvested from February to May 2012 in the Indian River area of Florida, and the effect of harvest time on fruit and juice quality was investigated. This was a follow-up study to one done in 2007, where the fruit were harvested from southern Florida from February to June. Peel color became less green and more orange over the season, and juice content in fruit declined as the season progressed. For sugars, the solids/acid ratio increased over the season, and titratable acidity, citric acid, and total ascorbic acid declined. Phenolic compounds generally increased, whereas they had fluctuated in the previous study. Limonoids generally increased as the season progressed except for the bitter compound nomilin which remained steady. Alkaloids increased throughout the season. Hydroxycinnamates all decreased over the season. The polyamines spermidine and spermine increased, while putrescine remained constant. For volatiles, terpenes, aldehydes, esters, and ketones increased steadily or in the last months of the season. Alcohols (aliphatic and terpene alcohols) did not change over the harvest season. This study confirms changes of some chemicals over the harvest season, while other secondary metabolites are more dependent on the climatic conditions during fruit formation and at harvest.

KEYWORDS: Flavor. Secondary metabolites. Maturity. Juice content.

Artigo publicado:

BAI, J.; BALDWIN, E.; MCCOLLUM, G.; MANTHEY, J.; PLOTTO, A.; DALA PAULA, B.M.; GLORIA, M.B.A.; WIDMER, W.; LUZIO, G.; CAMERON, R.; NARCISO, J. Influence of harvest time on quality of 'Valencia' oranges and juice, second season. Proceedings of the Florida State Horticultural Society, v. 126, p. 232-238, 2013.

1. INTRODUCTION

'Valencia' is the predominant orange cultivar grown in Florida and is mainly used for juice. This cultivar has good color and flavor and is favored by the juice industry. The harvest season for 'Valencia' juice oranges is generally a 4-month period (March to June) after they first reach acceptable maturity (SOULE et al., 1967). There is a gradual decrease in titratable acidity (TA) by decomposition of citric acid, and a slight increase in soluble solids content (SSC) and a consistent increase of SSC/TA ratio (CHEN et al., 1990; HUTTON & LANDSBERG, 2000). Florida maturity indices for oranges harvested between 16 Nov. to 31 July are: SSC ≈ Brix > 8.5%, TA > 0.4%, SSC/TA ratio > 10.25, and juice content > ~45 mL.100 g^{-1} (4.5 gal per 1.6-bu box) (RITENOUR et al., 2004).

Fresh oranges as well as orange juice are popular worldwide for their flavor and nutrition. Orange flavor is a complex mixture of volatile compounds of which some 200 have been identified (JOHNSON et al., 1996). The most important volatiles are esters, aldehydes, and terpenes, followed by alcohols, ketones, and hydrocarbons (NISPEROS-CARRIEDO & SHAW, 1990; PLOTTO et al., 2004; 2008; SHAW, 1991).

The health benefits of oranges are linked to the secondary metabolites, including numerous flavonoids (ROUSEFF, 1980; LEE & AEDIN, 2006; GATTUSO et al., 2007; TRIPOLI et al., 2007), limonoids (MAIER et al., 1980; MILLER et al., 1989; GUTHRIE et al., 2000; MANNERS et al., 2003), hydroxycinnamates (KROON & WILLIAMSON, 1999; MANTHEY & GROHMANN, 2001) and the polyamines spermine and spermidine (SANTIAGO-SILVA, LABANCA & GLORIA, 2011). Secondary metabolites in oranges may also contribute to fruit and juice quality in many ways, influencing the appearance, the taste as well as the possible health benefits (BALDWIN, 1993). It has been noticed previously that bitterness and the limonin content, a major bitter compound in oranges, decreased during the harvest season (MAIER et al., 1980). However, very little attention has been given to the seasonal changes of other secondary metabolites. Since information is lacking concerning development of flavor volatiles, nutrients and phytonutrients in citrus fruit during ripening (BALDWIN, 1993), a follow up to a previous study

conducted in 2007 (BAI et al., 2009) was done where ‘Valencia’ oranges were harvested and evaluated over the season for physical and chemical quality characteristics.

2. MATERIAL AND METHODS

2.1 Fruit sampling

Fruit were harvested from four trees grown in a commercial orchard located in the Indian River area of Florida on February, March, April, and May 2012. At each harvest time, 20 fruits were picked from each replicate tree. After measuring fruit weight and peel color, the fruit were cleaned with JBT Fruit Cleaner 395 (JBT, Lakeland, FL), juiced using a fresh juicer (JBT Fresh’n Squeeze) and frozen at -20 °C until analysis.

2.2 Peel color and juice content analysis

Peel color was evaluated using a Minolta Chromameter (Model CR-300, Minolta, Tokyo, Japan) measuring a* and b* values for red/green and yellow/blue color, respectively, and expressed as a*/b* ratio. Juice content was measured and expressed as milliliters per 100 g of fresh fruit.

2.3 Sugar and acid analysis

TA was determined by titrating to pH 8.1 with 0.1 N NaOH using an autotitrator (Metler Toledo DL50, Daigger & Company, Vernon Hills, IL) and SSC using a refractometer (Atago RX-5000 α, Tokyo, Japan).

For analysis of individual acids, approximately 40 g of juice was extracted using 70 mL 80% ethanol solution (BAI et al., 2010). The mixture was boiled for 15 min, cooled and centrifuged at 10,000 × *g* for 15 min. The supernatant was brought to 100 mL with 80% ethanol. Ten milliliters of the solution were then filtered through a C-18 Sep-Pak (Waters/Millipore) followed by a 0.45 µm Millipore filter (BALDWIN et al., 1991). Organic acids, including ascorbic acid, were analyzed using an Altech OA 1000 Prevail organic acid column (Altech Corp., Flemington, NJ) with a flow rate of 0.2 $mL.min^{-1}$ at 35 °C and a mobile phase of 0.01 N H_2SO_4. The injection volume was 20 µL using a

Perkin Elmer Series 200 autosampler, a Spectra System P4000 pump and a Spectra System UV 6000 LP detector (Shimadzu) was used for the analysis.

2.4 Secondary metabolite analysis

For sample preparation, 2 mL juice was added to 11 mL methanol in a Teflon gasket screw-top test tube and shaken for 18 h with an orbital shaker (VSOS-4P, Pro Scientific, Oxford, CT) at 120 rpm at 25 °C. The mixtures were centrifuged at 10,000 × *g* for 15 min. The total volume of supernatant was adjusted to 12 mL by methanol. Then 1 mL butanol was added, and the sample was taken to dryness using a Savant centrifugal evaporator. Methanol (2 mL) was added, and each sample was vortexed for 2 min. Samples were then passed through a 0.45 µm PTFE filter. The filter was washed with an additional 1.5 mL methanol, and the total volume was adjusted to 4 mL prior to analysis by HPLC-MS (BALDWIN et al., 2010).

2.5 Peel oil, pectin and pectinmethylesterase (PME)

Peel oil content was determined by the Bromate Titration Method (SCOTT & VELDHIUS, 1966) and total pectin, measured as galacturonic acid, was determined using a microplate reader as described in Bai et al. (2010). For PME, juice 30 mL per sample was homogenized using a Brinkmann PT 10/35 homogenizer (Swizerland) at speed 4 for 45 s. PME activity was determined titrimetrically with 0.5% citrus pectin (BAI et al., 2010).

2.6 Volatile analysis

Juice (6 mL) was pipetted into a 20 mL vial, and then the vials were crimp capped with Teflon/silicone septa. Juice samples were incubated for 30 min at 40 °C. A 2-cm solid phase microextraction (SPME) fiber (50/30 µm DVB/Carboxen/PDMS; Supelco, Bellefonte, PA) was then exposed to the headspace for 60 min at 40 °C. After exposure, the SPME fiber was inserted into the injector of a gas chromatography-mass spectrometry (GC-MS) (Model 6890, Agilent, Santa Clara, CA) to desorb the extract for 15 min at 250 °C. The GC-MS equipment and settings were described in Bai et al. (2011).

Volatile compounds were identified by comparison of their mass spectra with library entries (NIST/EPA/NIH Mass Spectral Library, version 2.0d;

National Institute of Standards and Technology, Gaithersburg, MD), as well as by comparing RIs with published RIs (KONDJOYAN & BERDAGUÉ, 1996; ADAMS, 2007).

2.7 Bioactive amines

The juice samples were centrifuged at 11,180 x *g* at 4 °C for 20 min and filtered through 0.45 µm HAWP membranes (Millipore Corp, Milford, MA). HPLC analysis of the extract was performed by ion pair reverse phase HPLC, post-column derivatization with *o*-phtalaldehyde and fluorimetric detection at 340 and 445 nm excitation and emission, respectively (VIEIRA et al., 2010).

2.8 Statistical analysis

SAS Version 9.1 (SAS Institute, Gary, NC) was used to analyze the data, using analysis of variance (PROC ANOVA). Mean separation was determined by Tukey's test at the 5% level.

3. RESULTS AND DISCUSSION

3.1 Peel color and juice content

The a*/b* ratio (a* is a measure of redness/greenness and b* is a measure of yellowness) serves as an indicator of quantitative development of orange color (AYERS & TOMES, 1966). A greater a*/b* ratio is a sign of deeper orange color, and a negative value shows more green than orange. The a*/b* values increased from February to May, indicating that the fruit did not undergo re-greening as can often happen later in the season (RITENOUR et al., 2004). The season ended early (May) however, which eliminated the potential for fruit re-greening in June (Fig. 1A).

Juice content declined over the season (Fig. 1B), but was above the Florida orange juice standard of 45 mL.100 g^{-1} (RITENOUR et al., 2004). The results are similar to previous results (BAI et al., 2009) although there was no re-greening in this season.

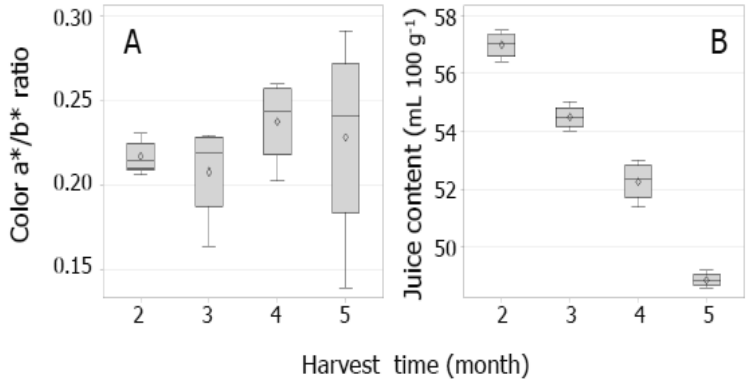


Figure 1. Changes of peel color (a*/b* ratio) (A) and juice content (B) of 'Valencia' orange fruit harvest from February to May 2012.

3.2 Peel oil and PME activity and pectin

Peel oil remained steady over the season with the exception of an increase in the month of April (Fig. 2A). PME is an enzyme that demethylates pectin in cell walls and can destabilize the cloud in orange juice (VERSTEEG et al., 1980; CAMERON et al., 1998; ACKERLEY et al., 2002; BALDWIN et al., 2012). PME activity mildly fluctuated over the season (Fig. 2B) while galacturonic acid held steady in the entire harvest season (Fig. 2C) (BAI et al., 2009).

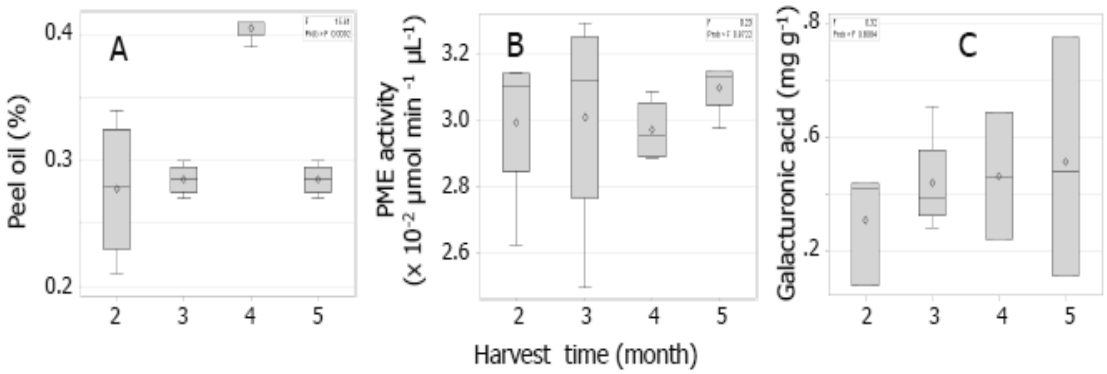


Figure 2. Changes of peel oil content (A), pectin methylesterase (PME) activity (B), and total pectin (galacturonic acid) content (C) in 'Valencia' orange juice extracted from fruit harvested from February to May 2012.

Galacturonic acid is the main component of pectin. This is in contrast to Sinclair and Jolliffe (1958; 1961) and Rouse et al. (1962) who observed that in

maturing oranges, total pectin and water-soluble pectic substances decreased in the peel and pulp, in both California and Florida 'Valencia' fruit.

3.3 Sugars and acids

SSC (°Brix) of the juice increased from 15.5% to 16.5% over the harvest season (Fig. 3A). However, TA content decreased consistently from over 1.4% to under 1.0% (Fig. 3B). Consequently, SSC/TA ratio increased from 11 in February to just over 17 in May (Fig. 3C). All juices passed Florida juice standard (RITENOUR et al., 2004). A high quality juice has a SSC/TA ratio between 12.5 and 19.5 (MATTHEWS, 1994). In this study, early harvested high acid fruit had a SSC/TA ratio of 11, out of the best quality range.

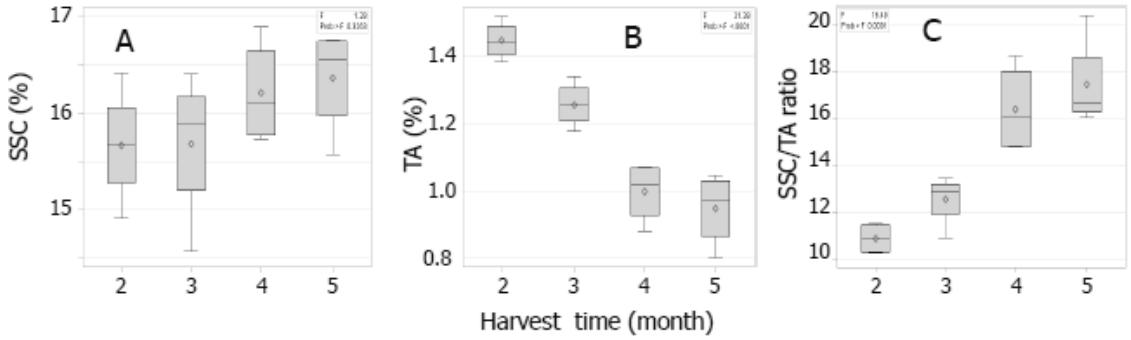


Figure 3. Changes of soluble solids content (SSC) (A), titrable acidity (TA) (B), and SSC/TA ratio (C) in 'Valencia' orange juice extracted from fruit harvested from February to May 2012.

Citric acid, the principal organic acid, decreased throughout the harvest season (Fig. 4A), similar to our previous study (BAI et al., 2009); however, malic acid (9% to 15% of total organic acids) slightly decreased in the first month and then increased from March to May (Fig. 4B). In mature orange juice sacs, both aconitase and citrate lyase activities were absent (ECHEVERRIA & VALICH, 1988). The regulation of citrate formation may be by decreasing synthesis of oxaloacetate, the precursor of citrate, during maturation (BRUEMMER, 1989), explaining the decrease in citric acid.

Total ascorbic acid content decreased consistently during harvest season (Fig. 4C), which is in agreement with Harding et al. (1940) and Rygg and Getty (1955) and our last study (BAI et al., 2009).

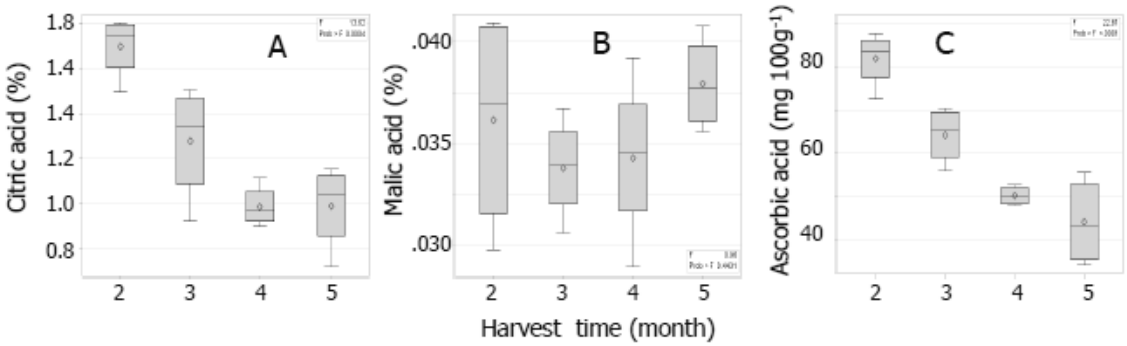


Figure 4. Changes of citric (A), malic (B), and ascorbic (C) acids in 'Valencia' orange juice extracted from fruit harvested from February to May 2012.

3.4 Secondary metabolites

Several classes of secondary metabolites were measured in the 'Valencia' orange juice between February and May 2012. These classes of compounds consisted of: phenolic compounds, limonoids, and alkaloids. The phenolic compounds included the flavonoid glycosides (FGs), polymethoxylated flavones (PMFs) and hydroxycinnamic acids (HCAs). All five FGs, hesperidin-4'-glucoside, hesperidin, 6,8-di-*C*-glucosyl apigenin, isosakuranetin rutinoside and narirutin, showed gradual increases through May (Fig. 5A-E); PMFs including heptamethoxyflavone, quercetagetin hexamethylether, nobelitin, tetramethylscutellarein, sinesetin, and tangeretin, increased steadily (Fig. 6A-F); Nine of HCAs were detected without further identification, and the contents decreased steadily (data not shown).

Five out of six limonoids (all the limonoid glucosides) increased, including obacunone glucoside, nomilin glucoside, nomilinic acid glucoside, and limonin glucoside (Fig. 7A-D). Of the two aglycones measured, limonin increased (Fig. 7E), while nomilin levels were steady (Fig. 7F). Feruloyl putrescine and an unknown alkaloid increased after April (data not shown).

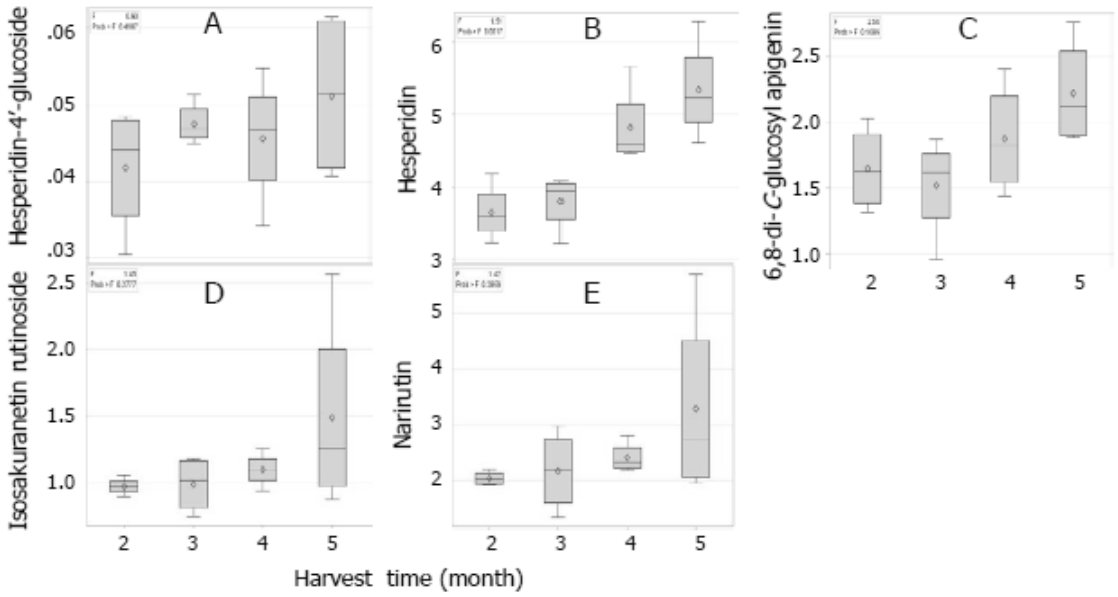


Figure 5. Changes of flavonoid glycosides (FGs, relative peak área) in 'Valencia' Orange juice extracted from fruit harvested from February to May 2012. (A) Hesperedin-4'-glucoside; (B) hesperidin; (C) 6,8-di-C-glucosyl apigenin; (D) isosakuranetin rutinoside; (E) narirutin.

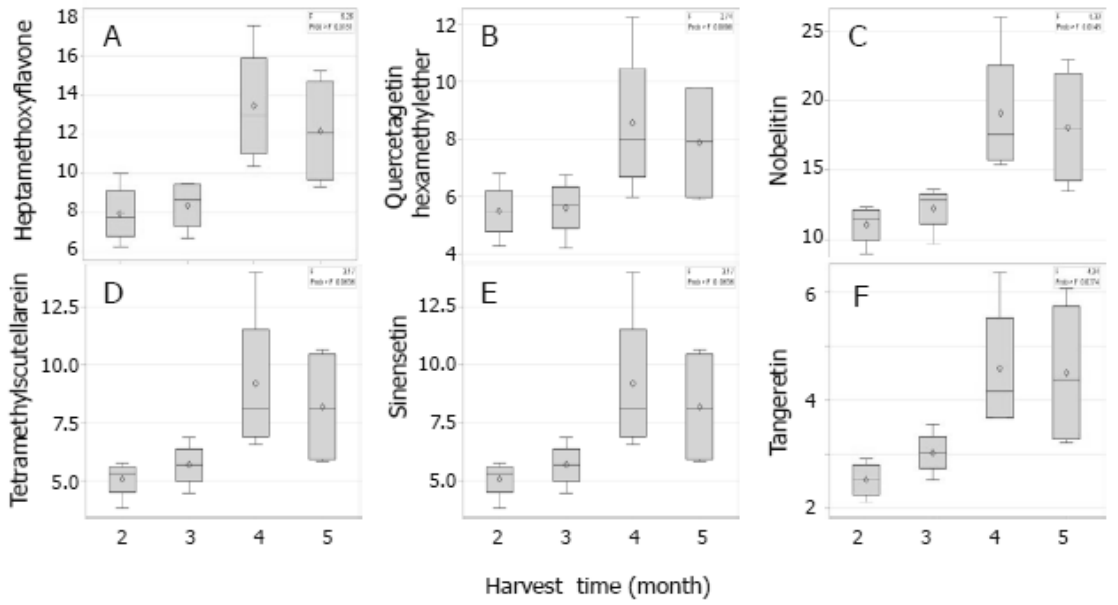


Figure 6. Changes of polymethoxylated flavones (PMFs, relative peak área) in 'Valencia' Orange juice extracted from fruit harvested from February to May 2012. (A) heptamethoxyflavone; (B) quercetagetin hexamethylether; (C) nobiletin; (D) tetramethylscutellarein; (E) sinesetin; and (F) tangeretin.

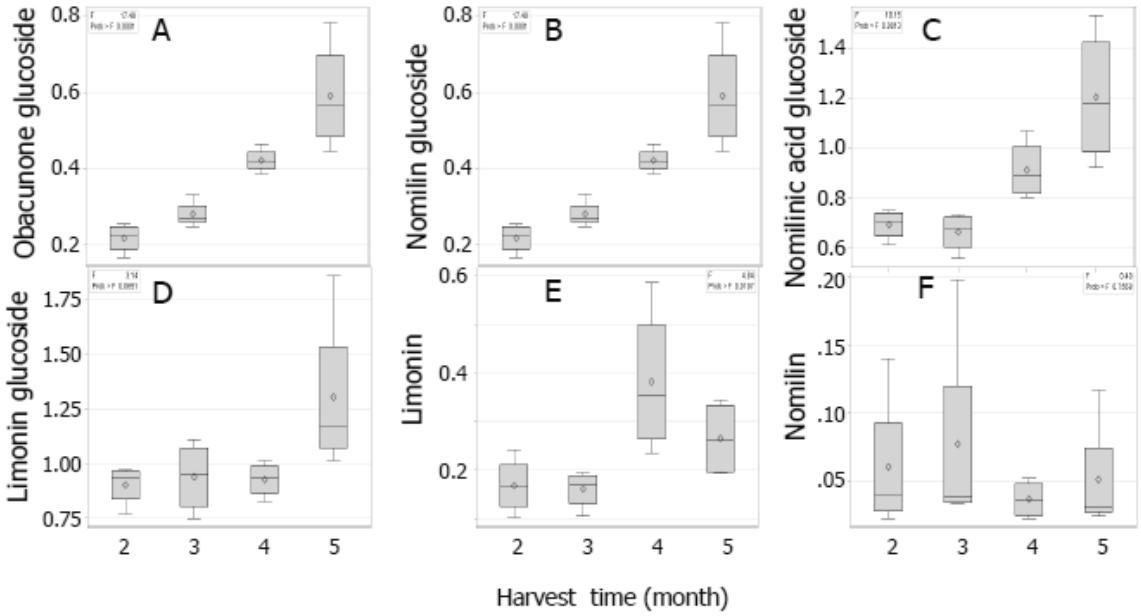


Figure 7. Changes of limonoids (relative peak área) in 'Valencia' orange juice extracted from fruit harvested from February to May 2012. (A) Obacunone glucoside; (B) nomilin glucoside; (C) nomilinic acid glucoside; (D) limonin glucoside; (E) limonin; and (F) nomilin.

3.5 Bioactive amines

Among 10 amines (putrescine, agmatine, spermine, spermidine, cadaverine, serotonin, histidine, tyramine, tryptamine, phenylethylamine) investigated, the polyamines spermidine, spermine, and putrescine were detected in the samples. Putrescine was the prevalent amine, followed by spermidine and spermine (Fig. 8).

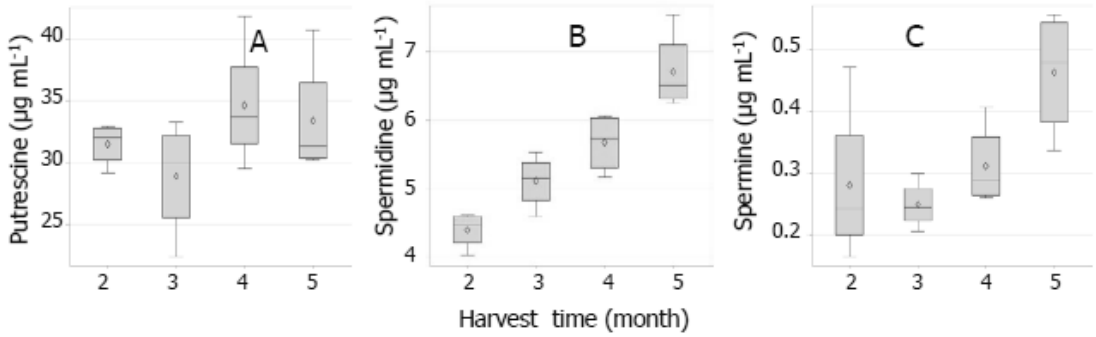


Figure 8. Changes of (A) putrescine, (B) spermidine, and (C) spermine in 'Valencia' orange juice extracted from fruit harvested from February to May 2012.

No changes were observed for putrescine; however, there was an increase in spermidine and spermine levels with harvest time (Fig. 8B). An increase in spermidine levels during ripening was reported by Tassoni et al. (2004) for Brasiliano NL92 orange, followed by a decrease in over ripened oranges.

3.6 Volatiles

Instead of direct headspace method used in the last study, this research used SPME extraction, and thus detected more volatile compounds. In the total 97 compounds, there were 18 monoterpene hydrocarbons, 22 sesquiterpene hydrocarbons, 9 aliphatic esters, 4 terpene esters, 12 aliphatic aldehydes, 3 terpene aldehydes, 4 aliphatic alcohols, 5 terpene alcohols, 4 ketones and 1 acid, with the rest being minor or unidentified components (Table 1).

Limonene was the major component, representing 85% of total volatiles, throughout the entire harvest season. The concentration decreased in April and recovered in May. Most of groups and chemicals had higher concentrations in April and/or May than earlier in the season, agreeing with the results observed in the last study (BAI et al., 2009). However, both aliphatic and terpene alcohols did not have differences between harvest time as a group

Table 1. Effect of harvest time on volatile abundance in 'Valencia' orange juice (2012)[z]

Compound	RI[y]	AO[x]	Volatile abundance (total ion current x 10^7)			
			Feb	Mar	Apr	May
Monoterpene hydrocarbons						
limonene	1046	1	1411 ab	1382 ab	1113 b	1649 a
β-myrcene	997	5	15.04 b	12.68 b	26.39 a	21.18 ab
α-pinene	953	8	8.92	7.47	5.86	10.52
α-terpinene	1031	14	3.32	2.94	3.34	4.57
γ-terpinene	1069	15	1.59 b	1.61 b	5.54 a	4.28 a
α-phellandrene	1022	18	3.79	1.03	1.26	4.17
β-phellandrene	1048	22	0.00 c	0.00 c	5.40 a	2.13 b
p-cymene	1039	30	1.61	1.51	0.83	0.62
δ-3-carene	1020	34	0.94 ab	0.77 bc	1.02 a	0.59 c
α-thujene	942	39	0.82	0.76	0.49	0.81
mt[w]1197	1197	44	0.37 b	0.34 b	0.33 b	0.94 a
isolimonene	1081	49	0.37	0.36	0.44	0.51
sabinene	990	59	0.31 b	0.28b	0.34 ab	0.41 a
1,3,8-*p*-menthatriene-	1041	67	0.36	0.17	0.15	0.16
p-mentha-6,8-dien-2-ol	1146	74	0.00b	0.00b	0.60 a	0.00 b
p-mentha-2,4(8)-diene	1098	75	0.24	0.09	0.19	0.07
β-ocimene	1238	80	0.00 b	0.09 b	0.10 b	0.31 a
mt1332	1348	91	0.08	0.00	0.00	0.11
total (excluding limonene)			37.78 b	30.10 b	52.28 a	51.38 a
Sesquiterpene hydrocarbons						
valencene	1536	2	86.50 b	78.65 b	142.81 a	114.83 ab
st[w]1526	1526	6	11.50 b	10.28 b	21.44 a	11.63 b
st1526	1574	12	4.55	5.41	1.58	4.82
α-selinene	1542	13	1.71 b	1.17 b	10.53 a	1.96 b
α-cadinene	1554	16	2.09 b	3.01 ab	3.30 ab	3.80 a
st1454	1454	17	2.40	2.13	3.63	3.42
st1405	1405	21	1.19 b	1.40 b	2.60 a	2.56 a
α-copaene	1397	23	3.71	0.48	3.30	0.00
st1548	1548	24	0.84 b	1.24 b	3.38 a	1.13 b
cedrane-V6	1518	26	1.27 b	4.42 a	0.25 ab	0.19 b
α-caryophllene	1479	28	0.00	0.00	3.66	1.83
α-farnesene	1507	29	1.07 b	1.01 b	1.05 b	1.50 a
β-panasinsen	1563	37	0.79	0.81	0.87	0.050
β-cubebene	1358	51	0.46	0.42	0.50	0.23

Table 1. Effect of harvest time on volatile abundance in 'Valencia' orange juice (2012)[z] (continuation…)

Compound	RI[y]	AO[x]	Volatile abundance (total ion current x 10^7)			
			Feb	Mar	Apr	May
Sesquiterpene hydrocarbons						
β-selinene	1740	55	0.37	0.35	0.35	0.40
β-curcumene	1532	60	0.24	0.23	0.47	0.40
st1500	1500	63	0.89	0.00	0.41	0.00
st1348	1332	81	0.14	0.12	0.13	0.10
st1577	1577	85	0.07	0.04	0.09	0.12
β-pamasinsene	1513	86	0.00	0.00	0.18	0.11
alloaromadendrene	1462	88	0.00	0.00	0.03	0.18
β-humulene	1475	92	0.00 b	0.00 b	0.18 a	0.00
total			119.80 b	111.17 b	200.73 a	149.73 ab
Aliphatic esters						
ethyl butanoate	801	3	31.72 b	37.81 b	41.36 ab	49.92 a
ethyl pentanoate	883	9	6.12 b	5.63 ab	5.78 ab	8.35 a
ethyl 3-hydroxyhexanoate	1124	19	1.87 ab	2.22 b	2.31 ab	2.85 a
ethyl acetate	600	31	0.89 b	1.03 ab	1.12 ab	1.39 a
ethyl 2-methylbutanoate	854	40	0.00 b	0.49 b	2.36 a	0.00 b
ethyl octanoate	1184	42	1.09 b	0.00 ab	1.03 ab	0.00 a
methyl hexanoate	929	54	0.25	0.30	0.35	0.59
methyl butanoate	715	57	0.31	0.32	0.41	0.31
Z-5-dodecen-1-y1 acetate	1596	62	0.31	0.41	0.22	0.37
total			42.56 b	48.23 b	54.92 ab	63.78 a
Terpene esters						
neryl acetate	1347	25	0.93 b	0.99 b	2.42 a	2.24 a
citronellyl acetate	1337	35	0.61 b	0.59 b	0.66 b	1.24 a
terpinyl acetate	1202	47	0.65	0.31	0.37	0.39
carvyl acetate	1328	76	0.00 c	0.17 b	0.03 c	0.34 a
Total			2.19 bc	2.07 c	3.47 ab	4.21 a
Aliphatic aldehydes						
Z-3-hexenal	863	7	7.26	9.02	14.68	12.47
hexanal	804	11	2.33	0.49	8.48	5.54
E,E-2,4-decadienal	1270	20	1.50 b	1.67 ab	2.17 ab	2.52 a
octanal	1010	38	0.00 b	0.60 b	0.60	1.71 a
decanal	1200	41	0.52	0.57	0.53	0.60
nonanal	1104	45	0.23 b	0.30 b	0.83 a	0.61 ab
acetaldehyde	466	53	0.00 b	0.24 ab	1.06 a	0.29 ab

Table 1. Effect of harvest time on volatile abundance in 'Valencia' orange juice (2012)[z] (continuation…)

Compound	RI[y]	AO[x]	Volatile abundance (total ion current x 10^7)			
			Feb	Mar	Apr	May
Aliphatic aldehydes						
heptanal	911	68	0.34	0.16	0.13	0.16
E-2-octenal	1062	78	0.35 a	0.21 ab	0.20 ab	0.00 b
E-2-heptenal	968	84	0.19	0.08	0.15	0.09
pentenal	672	93	0.00 b	0.00 b	0.33 a	0.00 b
Z-dodec-5-enal	1378		0.04	0.03	0.10	0.00
total			12.76 b	13.38 b	29.26 a	24.00 a
Terpene aldehydes						
perilla aldehyde	1288	43	0.27 b	033 b	0.61 a	0.79 a
geranial	1263	73	0.12	0.10	0.21	0.20
neral	1236	90	0.12 a	0.00 b	0.03 ab	0.04 ab
total			0.52 bc	0.43 c	0.85 ab	1.03 a
Aliphatic alcohols						
ethanol	487	4	17.87	17.87	17.83	22.50
undecanol	1364	56	0.05	0.20	1.14	0.00
hexanol	873	66	0.28	0.23	0.11	0.29
2-methyl-decanol	1322	70	0.00	0.18	0.14	0.40
total			18.19	18.48	19.21	23.19
Terpene alcohols						
linalool	1100	10	5.69	6.08	4.45	3.93
terpinen-4-ol	1191	32	0.78 b	0.75 b	1.07 ab	1.51 a
E-carveol	1413	33	0.49	0.52	1.39	1.14
citronellol	1217	65	0.12 b	0.18 ab	0.54 a	0.26 ab
nerol	1220	95	0.00	0.00	0.00	0.12
total			7.07	7.53	7.46	6.97
Ketones						
2-pentanone	676	27	1.30	1.22	1.93	1.15
β-ionone	1426	46	0.63 a	0.47 ab	0.46 ab	0.29 b
geranylacetone	1436	83	0.08	0.12	0.11	0.03
nootkatone	1892	50	0.00 b	0.00 b	1.63 a	0.00 b
total			2.01 b	1.81 b	4.13 a	1.47 b
Other						
hexanoic acid	965	72	0.00	0.00	0.33	0.33
ri[w]923	923	36	0.55	0.45	0.96	1.07
benzene	1496	48	0.30	0.41	0.67	0.32

Table 1. Effect of harvest time on volatile abundance in 'Valencia' orange juice (2012)[z] (continuation…)

Compound	RI[y]	AO[x]	Volatile abundance (total ion current x 10^7)			
			Feb	Mar	Apr	May
Other						
ri1071	1071	52	0.27	0.24	0.52	0.58
ri1743	1743	58	0.22 c	0.27 bc	0.50 a	0.35 b
ri1251	1251	61	0.37	0.34	0.16	0.46
tetradecane	1389	64	0.34	0.21	0.36	0.30
ri1317	1317	71	0.38	0.13	0.17	0.00
ri1300	1300	77	0.14	0.12	0.19	0.08
ri1936	1936	79	0.00 b	0.00 b	0.19 a	0.33 a
ri1711	1711	82	0.00 b	0.00 b	0.23 a	0.00 b
ri1391	1391	87	0.00 b	0.00 b	0.23 a	0.00 b
ri1732	1732	89	0.00	0.00	0.13	0.08
ri1724	1724	94	0.00 b	0.00 b	0.16 a	0.00 b
ri1619	1619	96	0.00 b	0.00 b	0.11 a	0.00 b
ri1697	1697	97	0.00 b	0.00 b	0.09 a	0.00 b
total			2.57 b	2.23 b	5.05 a	3.89 b
total (excluding limonene)			202 b	192 b	311 a	269 a

[z]Values followed by different letters in the same compound (row) are significantly different at P=0.05 using Tukey`s test. [y]RI: retention index. [x]AO: abundance order from compound with the highest amount. [w]nonidentified monoterpene (mt), sesquiterpene (st) and other compounds (ri) followed by the RI values.

4. CONCLUSION

'Valencia' oranges are harvested generally from March to June. Because of unusually warm weather, the 2012 harvest season was started in February and ended in May (rather than normally March to June). The mid season harvests are preferred for the optimum SSC, TA, SST/TA, reduced bitterness. Later-harvested 'Valencia' fruit had higher color, soluble solids, solid/acid ratio, volatiles, flavonoids, spermidine and limonoid glycoside contents while having reduced juice content, acids (including ascorbic acid) and limonin and nomilin (in May).

CAPÍTULO II - EFFECT OF HUANGLONGBING (GREENING DISEASE) ON ORANGE JUICE QUALITY, A REVIEW

ABSTRACT

Huanglongbing (HLB) or citrus greening is one of the most severe citrus diseases in the world. It is associated with the presence of the gram-negative bacterium *Candidatus* Liberibacter asiaticus which is transmitted by the psyllid *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) and also the African species, *Candidatus* Liberibacter africanus, which is transmitted by the insect *Trioza erytreae* (Hemiptera: Triozidae). Fruits from a tree infected with HLB's bacteria can be either symptomatic or asymptomatic. Symptomatic fruits are small in size, lopsided, and asymmetrical with a green peel. The disease has a negative impact on fruit quality and results in off-flavored orange juice. Symptomatic fruits show higher total acidity (TA) and lower soluble solids content (SSC), SSC/TA, total sugar content and malic acid levels compared to healthy and asymptomatic fruit. The disease also causes an increase in secondary metabolites, including: hydroxycinnamic acids; bitter limonoids (limonin and nomilin), narirutin and hesperidin in the orange juice, peel and pulp. Typical HLB orange juice is described as being distinctly bitter, sour, salty/umami, metallic, pungent, musty, and lacking in sweetness and citrusy flavor. However, the changes in volatile compounds have not been well established, except for the increase in numerous limonene and linalool degradation compounds, as well as a decrease in some aldehydes. The most studied orange cultivar is Valencia, followed by Hamlin. HLB remains without a cure and its management is difficult due to an unpredictable latency time of the bacteria after the tree has been infected. The scientific literature is still lacking scientific information covering the effects of HLB on orange juice quality, as well as investigations on cultivars other than Valencia and Hamlin.

KEYWORDS*:* Huanglongbing. *Candidatus* Liberibacter asiaticus. Orange juice. Valencia. Hamlin. Quality.

Artigo submetido a publicação no Journal of the Science of Food and Agriculture.

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1. INTRODCUTION

Huanglongbing (HLB) is a citrus disease that may bring an end to the citrus industry if the disease continues to spread throughout the various citrus growing regions of the world (GOTTWALD et al., 2012). Practically all commercial citrus species and cultivars are vulnerable to HLB. The disease has an array of symptoms which can be present anywhere from the roots to the citrus fruit itself, changing the chemical characteristics and sensory attributes of the fruits (BOVÉ, 2006; BALDWIN et al., 2010; DALA PAULA et al., 2017a; 2017b). In this review, the effects of HLB on orange juice quality is described based on research available in the scientific literature.

2. WORLDWIDE CONSUMPTION AND PRODUCTION OF FRESH ORANGES AND ORANGE JUICE

Orange juice is the most widely consumed fruit juice in the world, with a consumption of 7,574 out of 16,988 million L in 2015, representing 45% of total fruit juice consumption (MARKSTRAT, 2016). Brazil is the world's largest orange producer, and reached production levels of approximately 14,350 metric tons in the harvest of 2015/2016. China is the second largest producer with 7,000, followed by the European Union – 6,055, the United States – 5,371 and Mexico – 3,535 metric tons (USDA-FAS, 2017). In the 2015-2016 harvest, Brazilian and American commercial orange production occupied approximately 741,133 and 223,143 hectares, respectively (IBGE-SIDRA, 2017, USDA-NASS, 2017). While the majority of the orange production in Brazil is processed and exported, the U.S. consumes practically all of its production and imports juice from other countries, including Brazil, in order to meet its market demand. Florida accounts for nearly 60% of U.S. production, with California accounting for the remaining 40%. Around 90% of the oranges produced in Florida are processed (USDA-FAS, 2017).

Currently, citrus producers in many countries are facing serious problems with the emergence of the plant pathology HLB (TEIXEIRA et al., 2008; BASSANEZI et al., 2009; 2011; SPREEN & ZANSLER, 2015), which means

yellow dragon disease in Chinese and is also known as citrus greening (HALBERT & MANJUNATH, 2004). HLB is considered one of the most severe citrus diseases in the world and, consequently, a serious problem for the citrus processing industry. The disease can affect almost all kinds of citrus, with sweet oranges, tangelos and mandarins being the most susceptible and limes, sour oranges and trifoliate oranges being the least (ABDULLAH et al., 2009).

3. A BRIEF HISTORICAL BACKGROUND OF HUANGLONGBING INCIDENTS

It is difficult to determine where exactly the HLB disease originated. However, there is evidence suggesting that HLB was responsible for India's "dieback" problem during the 18th century and for Yellow shoot in China in the 1890s (CAPOOR, 1963; ZHAO, 1981; GRAÇA, 2008). Initially, some researchers believed that the tristeza virus was the leading cause for the citrus "dieback" in India, however contradictory evidence supported other arguments. For example, many of the affected species that died in India are tolerant of tristeza when they are grown in other countries. After a three-month survey conducted in India by Fraser et al. (1966), HLB was determined to be the primary cause of the "dieback" (FRASER & SINGH, 1968; GRAÇA, 2008). In 1937, the disease was described for the first time in South Africa (VAN DER MERWE et al., 1937), and it was later linked to chromium and manganese toxicity. It was also associated with the leaf mottling citrus disease in the Philippines in the 1960's (FRASER et al., 1966; McCLEAN & SCHWARZ, 1970). Currently, the disease has spread out to more than 50 countries (Figure 1) in Africa, Asia, Oceania and the Americas (South, North, Central American and the Caribbean) (CABI, 2017; EPPO, 2017).

The first case of this century-old disease in America was reported in the state of São Paulo (SP), Brazil in 2004 (COLLETTA-FILHO et al., 2004; TEIXEIRA et al., 2005a). However, in a survey conducted in SP, just six months after HLB had been reported in Brazil, 46 cities stated having infected trees, suggesting that HLB had been present for almost ten years (Bové 2006). A year later, in August 2005, symptoms of the disease were recognized in Florida, U.S.; in 2007 in Cuba; in 2008 in the Dominican Republic; and in 2010 in

Mexico (COLLETA-FILHO et al., 2004; HALBERT, 2005; LLAUGER et al., 2008; MATOS et al., 2009; NAPPO, 2010). Currently, HLB is present in all Florida citrus-growing counties (BALDWIN et al., 2010), in California, Georgia, Louisiana, South Carolina and Texas (CABI, 2017; EPPO, 2017). As the severity of HLB increases, premature fruit drop becomes a growing problem, which has contributed to declining yields in Florida, especially during the last few years (CHEN et al., 2016). In Brazil, SP, Minas Gerais and Paraná have reported the presence of HLB, with SP being the most affected state. In India and China, HLB has spread to around 25 and 11 states, respectively (Table 1) (CABI, 2017; EPPO, 2017).

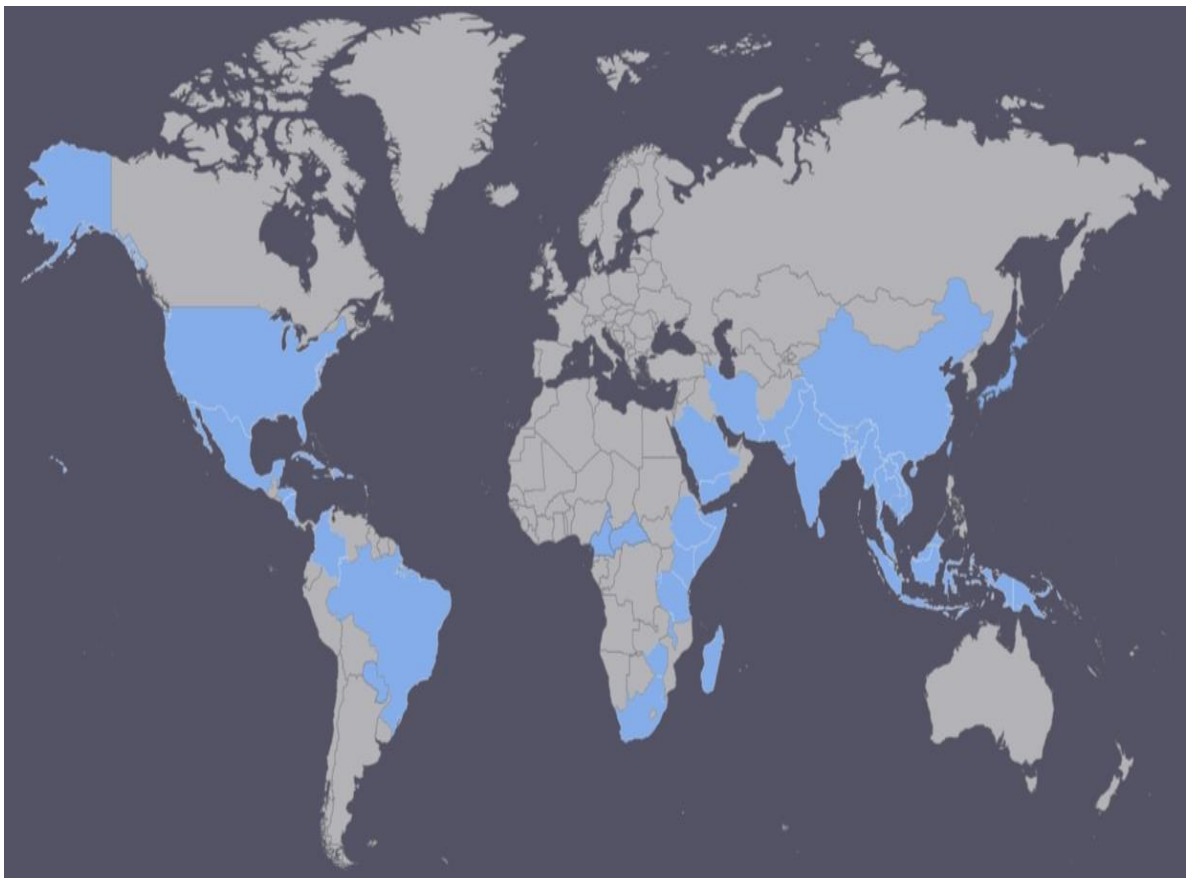

Figure 1. Countries currently affected by Huanglongbing – HLB. (CABI, 2017; EPPO, 2017).

Table 1. Worldwide distribution of Huanglongbing's bacteria and vectors.

Country	***Bacteria***	***Status***	***Vector***	***Status***
Asia				
Afghanistan	*-*	*-*	*Diaphorina citri*	*present*
Bangladesh	*C*Las	*present*	*Diaphorina citri*	*present*
Bhutan	*C*Las	*present*	*Diaphorina citri*	*present*
Cambodia	*C*Las	*present*	*Diaphorina citri*	*present*
China	*C*Las	*present (Fujian, Guangdong, Guangxi, Guizhou, Hainan, Hunan, Jiangxi, Schuan, Yunnan, Zhejiang); few occurrences (Xianggang - Hong Kong)*	*Diaphorina citri*	*present (Aomen, Fujian, Guangdong, Guizhou, Hainan, Henan, Hunan, Jiangxi, Sichuan, Yunnan, Zhejiang); widespread (Xianggang); restricted distribution (Guangxi)*
East Timor	*C*Las	*widespread*	*Diaphorina citri*	*present*
India	*C*Las	*present (Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Delhi, Gujarat, Haryana, Himachal Pradesh, Jammu & Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Mizoram, Nagaland, Orissa, Punjab, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttar Pradesh, West Bengal)*	*Diaphorina citri*	*present (Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Delhi, Gujarat, Haryana, Himachal Pradesh, Jammu & Kashmir, Karnataka, Kerala, Lakshadweep, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Punjab, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttar Pradesh, West Bengal)*
Indonesia	*C*Las	*present (Irian Jaya, Java, Kalimantan, Sulawesi, Sumatra); widespread (Nusa Tenggara)*	*Diaphorina citri*	*present (Java, Maluku, Nusa Tenggara, Sumatra);*
Iran	*C*Las	*restricted distribution*	*Diaphorina citri*	*restricted distribution*
Japan	*C*Las	*present (Ryukyu Archipelago); restricted distribution (Kyushu)*	*Diaphorina citri*	*present, few occurrences (Kyushu); present (Ryukyu Archipelago)*
Laos	*C*Las	*present*	*Diaphorina citri*	*present*
Malaysia	*C*Las	*present (Sarawak, West)*	*Diaphorina citri*	*present (Sabah, West)*
Maldives	*-*	*-*	*Diaphorina citri*	*present*
Myanmar	*C*Las	*present*	*Diaphorina citri*	*present*
Nepal	*C*Las	*widespread*	*Diaphorina citri*	*present*
Oman	*-*	*-*	*Diaphorina citri*	*restricted distribution*
Pakistan	*C*Las	*present*	*Diaphorina citri*	*widespread*
Philippines	*C*Las	*widespread*	*Diaphorina citri*	*present*
Saudi Arabia	*C*Laf	*present*	*Trioza erytreae* *Diaphorina citri*	*restricted distribution* *present*
Sri Lanka	*C*Las	*present*	*Diaphorina citri*	*present*
Taiwan	*C*Las	*present widespread*	*Diaphorina citri*	*restricted distribution*
Thailand	*C*Las	*present*	*Diaphorina citri*	*present*

Country	Bacteria	Status	Vector	Status
United Arab Emirates	-	-	*Diaphorina citri*	*present*
Vietnam	*C*Las	*present restricted distribution*	*Diaphorina citri*	*restricted distribution*
Yemen	*C*Laf	*present restricted distribution*	*Trioza erytreae*	*restricted distribution*
			Diaphorina citri	*present*
Africa				
Angola	-	-	*Trioza erytreae*	*present*
Burundi	*C*Laf	*present*	-	-
Cameroon	*C*Laf	*present*	*Trioza erytreae*	*present*
Camoros	-	-	*Trioza erytreae*	*present*
Congo, Democratic republic of the	-	-	*Trioza erytreae*	*restricted distribution*
Central African Republic	*C*Laf	*present*	-	-
Eritrea	-	-	*Trioza erytreae*	*present*
Ethiopia	*C*Laf	*present*	*Trioza erytreae*	*present*
	*C*Las	*present (few occurrences)*		
Kenya	*C*Laf	*present*	*Trioza erytreae*	*present*
Madagascar	*C*Laf	*present*	*Trioza erytreae*	*present*
Malawi	*C*Laf	*present*	*Trioza erytreae*	*present*
Mauritius	*C*Laf	*present*	*Trioza erytreae*	*present*
	*C*Las	*restricted distribution*	*Diaphorina citri*	*present*
Réunion	*C*Laf	*present*	*Trioza erytreae*	*present*
	*C*Las	*restricted distribution*	*Diaphorina citri*	*present*
Rwanda	*C*Laf	*present*	*Trioza erytreae*	*present*
Saint Helena	*C*Laf	*present (widespread)*	*Trioza erytreae*	*present*
Sao Tome & Principe	-	-	*Trioza erytreae*	*present*
Somalia	*C*Laf	*present*	-	-
South Africa	*C*Laf	*restricted distribution*	*Trioza erytreae*	*widespread*
Sudan	-	-	*Trioza erytreae*	*present*
Swaziland	*C*Laf	*present*	*Trioza erytreae*	*restricted distribution*
Tanzania	*C*Laf	*restricted distribution*	*Trioza erytreae*	*restricted distribution*
			Diaphorina citri	*restricted distribution*
Uganda	-	-	*Trioza erytreae*	*present*
Zambia	-	-	*Trioza erytreae*	*present*
Zimbabwe	*C*Laf	*restricted distribution*	*Trioza erytreae*	*present*

Country	*Bacteria*	*Status*	*Vector*	*Status*
North America				
Mexico	*C*Las	*restricted distribution*	*Diaphorina citri*	*restricted distribution*
USA	*C*Las	*present, few occurrences (California, Georgia, Louisiana, South Carolina, Texas); widespread (Florida)*	*Diaphorina citri*	*present (Florida, Hawai, Texas); few occurrences (Alabama, California, Georgia, Louisiana, Mississippi, South Carolina); restricted distribution (Arizona)*
Central America				
Antigua and Barbuda	-	-	*Diaphorina citri*	*present*
Bahamas	-	-	*Diaphorina citri*	*present*
Barbados	*C*Las	*restricted distribution*	*Diaphorina citri*	*restricted distribution*
Belize	*C*Las	*restricted distribution*	*Diaphorina citri*	*present*
Cayman Islands	-	-	*Diaphorina citri*	*present*
Costa Rica	*C*Las	*restricted distribution*	*Diaphorina citri*	*present*
Cuba	*C*Las	*present (widespread)*	*Diaphorina citri*	*present*
Dominica	*C*Las	*restricted distribution*	*Diaphorina citri*	*present*
Dominican Republic	*C*Las	*restricted distribution*	*Diaphorina citri*	*present*
Guadeloupe	*C*Las	*restricted distribution*	*Diaphorina citri*	*restricted distribution*
Haiti	-	-	*Diaphorina citri*	*present*
Honduras	*C*Las	*present (few occurrences)*	-	-
Jamaica	*C*Las	*present (widespread)*	*Diaphorina citri*	*present*
Martinique	*C*Las	*restricted distribution*	*Diaphorina citri*	*present*
Nicaragua	*C*Las	*present*	-	-
Puerto Rico	*C*Las	*present*	*Diaphorina citri*	*present*
United States Virgin Islands	*C*Las	*present (few occurrences)*	*Diaphorina citri*	*present*

Country	***Bacteria***	***Status***	***Vector***	***Status***
South America				
Brazil	*C*Las *C*Lam	*present (Minas Gerais, Paraná, São Paulo)* *present (Minas Gerais, Paraná, São Paulo)*	*Diaphorina citri*	*present (Amazonas, Bahia, Ceará, Minas Gerais, Pará, Paraná, Pernambuco, Rio de Janeiro, Santa Catarina, São Paulo)*
Columbia	*C*Las	*present (few occurrences)*	*Diaphorina citri*	*widespread*
Paraguay	*C*Las.	*restricted distribution*	*Diaphorina citri*	*restricted distribution*
Uruguay	-	-	*Diaphorina citri*	*few occurrences*
Venezuela	-	-	*Diaphorina citri*	*restricted distribution*
Europe				
Portugal	-	-	*Trioza erytreae*	*restricted distribution*
Spain	-	-	*Trioza erytreae*	*restricted distribution*
Oceania				
American Samoa	-	-	*Diaphorina citri*	*present*
Guam	-	-	*Diaphorina citri*	*present*
Papua New Guinea	*C*Las	*restricted distribution*	*Diaphorina citri*	*restricted distribution*

Leg.: *C*Las: *Candidatus* Liberibacter asiaticus; *C*Laf: *Candidatus* Liberibacter africanus; *C*Lam: *Candidatus* Liberibacter americanus.

4. CAUSING AGENTS AND VECTORS OF HUANGLONGBING

It is well established that HLB is associated with the presence of the gram-negative bacteria genus *Candidatus* Liberibacter (*C*L). Three species are known to cause the symptoms of HLB – *C*L asiaticus (*C*Las), *C*L americanus (*C*Lam) and *C*L africanus (*C*Laf). The Asian and the recently discovered American species, in Brazil, can be transmitted by the psyllid *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) and the African species by the insect *Trioza erytreae* (Hemiptera: Triozidae) (Figure 2) (BOVÉ, 2006). Although HLB was first reported in Brazil and the US 15 years ago, the psyllid vector was reported in SP and Florida as early as 1942 and 1998, respectively (BOVÉ, 2006; TANSEY et al., 2017).

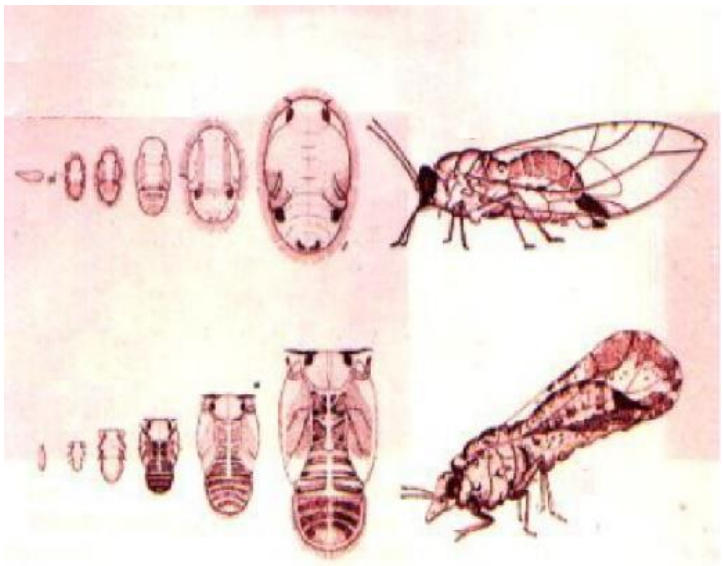

Figure 2. Development stages of Diaphorina citri Kuwayama (Hemiptera: Psyllidae) (upper) and Trioza etrytreae (Del Guercio) (Hemiptera: Triozidae) (lower). (Cheraghian, 2013).

When the symptoms of HLB in orange trees were reported for the first time in Brazil, symptomatic and asymptomatic leaves from sweet orange trees were analyzed for the presence of *C*Las and *C*Laf by polymerase chain reaction (PCR) using two sets of HLB-specific primers for amplification of 16S rDNA and ribosomal protein genes. All samples tested using PCR amplification were

negative, however HLB-affected leaves from the Bordeaux HLB collection were positive. Thus, researchers analyzed the samples again using PCR with universal primers for amplification of bacterial 16S rDNA and found that all symptomatic leaves yielded the same 16S rDNA amplification product. Afterwards, to confirm the existence of bacteria in symptomatic leaves, the 16S rDNA product was cloned, sequenced and compared with those of *C*L*as* and *C*Laf. The homology between the sequences was 93.7% and 93.9%, respectively, while the homology of the two known Liberibacter species was a 97.5% sequence identity. Thus, the bacterium was classified as a new species (TEIXEIRA et al., 2005b).

*C*Lam was the most prevalent bacteria species in Brazil in 2005, which initially affected more than 90% of the infected trees, decreasing to 60% in 2007. During this period, there was an increase in *C*L*as* infection, from 5 to 35% of the infected trees, while a combined infection remained practically the same at 5% (COLETTA-FILHO et al., 2007; GASPAROTO et al., 2012). Among HLB's bacteria, *C*Laf is sensitive to heat and to dry weather and thrives between 20 and 25 ºC, while the other species are heat tolerant and thrive just as well at higher temperatures (CATLING, 1969; CHERAGHIAN, 2013).

5. Symptoms of Huanglongbing and its impact on orange trees

It is uncertain how long a tree can be infected before showing the symptoms of the disease but, when it eventually becomes symptomatic, symptoms manifest on different parts of the tree. Infected trees generally have open growth, stunting, twig dieback and discolored leaves, which appear in contrast to the other healthy or symptomless parts of the tree. The symptomatic leaves can be normal-sized, showing yellow coloration and development of a blotchy-mottle or they can be small, upright and show a variety of chlorotic patterns resembling those induced by zinc or other nutritional deficiencies (Figure 3) (McCLEAN & SCHWARZ, 1970; GRAÇA, 1991; ALBRECHT et al., 2016). The root systems are poorly developed, showing very few fibrous roots, which decay from the rootlets most likely due to starvation (GRAÇA, 1991; BATOOL et al., 2007).

Figure 3. HLB symptomatic orange leaves: symptomatic normal sized leaves with development of blotchy-mottle (on left) (BOVÉ, 2006); and symptomatic small sized leaves (on right) (EMBRAPA, 2011).

Along with the color changes in the leaves, there are some changes in metabolic composition. HLB affects the profile of hydroxycinnamic acids and flavonoids in infected leaves, resulting in lower levels of 6,8-di-C-glucosyl apigenin, apigenin-C-glucosyl-*O*-xyloside, 2”-xylosylvitexin, luteolin rutinoside and apigenin-7-*O*-rutinoside compared to healthy leaves. While healthy leaves contain only trace levels of limonin glucoside, infected leaves contain levels of 300 ± 22 µg/mL (MANTHEY, 2008). Metabolomic analysis using gas chromatography-mass spectrometry (GC-MS) of Valencia sweet orange leaves has been suggested to identify biomarkers for rapid differentiation of HLB infection from zinc deficiency. The combination of L-proline, β-elemene, (-)trans-caryophyllene and α-humulene as HLB biomarkers, is necessary to increase specificity, because the change in concentration of a single compound may not be exclusively attributed to HLB (Cevallos-Cevallos et al., 2011). Tolerance of HLB does not seem to be linked to the accumulation of higher levels of protective metabolites in response to infection, but rather to different concentrations of specific metabolites independent of infection (ALBRECHT et al., 2016).

With respect to the orange fruit, they are reduced in size, lopsided, asymmetric, and contain small, brownish/black aborted seeds which can be seen when the orange is sectioned perpendicularly to the fruit axis (Figure 4). The orange peel turns green with an inversion of colors—when the fruit starts to

change color, from green to yellow/orange, the peduncular end turns orange while the stylar end remains green. In a healthy orange, *C*Las (-), the color change first starts at the stylar end, progressing only later to the peduncular area (Figure 4). HLB causes fruits to drop prematurely, resulting in a 30-100% yield reduction, and, ultimately, premature death of the tree. Tree mortality can occur several months to years after infection (McCLEAN & SCHWARZ, 1970; GRAÇA, 1991; BOVÉ, 2006; BATOOL et al., 2007; BASSANEZI et al., 2011; LIAO & BURNS, 2012).

Figure 4. Typical HLB symptomatic orange: (upper right and upper left) asymmetric fruit containing small, brownish aborted seeds (EMBRAPA, 2011; INIAV, 2015); (lower left) normal change of color in a healthy orange, *C*Las (-) (EMBRAPA, 2011); and (lower right) inversion of colors in a typical HLB symptomatic orange, *C*Las (+) (INIAV, 2015).

Research found in the scientific literature shows that HLB symptomatic fruits from infected trees are smaller in diameter compared to asymptomatic and healthy fruits, which are shown to have similar diameters (Table 2 and Figure 5). The majority of the research also reports that the weights and juice contents of symptomatic oranges are diminished compared to asymptomatic and healthy oranges, which are reported to generally be similar in weight and juice content (Table 2 and Figure 5). Most of the published research analyzed Valencia and Hamlin oranges, and in one study similar tendencies were described in the Valencia Americana, Westin and Pera Rio cultivars. The differences caused by

the disease were the least notable in Valencia Americana cultivar, while the most significant differences were observed in Valencia oranges, followed by Westin oranges (BASSANEZI et al., 2009).

Table 2. Effects on diameter, weight and juice content in fruit affected by Huanglongbing.

Reference	Orange sample		Fruit parameters		
	harvest time	status or conditions	diameter (mm)	weight (g)	juice (g.100 g^{-1})
Valencia orange juice					
Bassanezi et al. (2009)[I]	Blend of different harvests[1]	HLB-AS	73.1[a]	208.1[a]	50.0[a]
		HLB-SY	59.2[b]	118.9[b]	44.6[b]
Liao and Burns (2012)[II]	April 2009	Healthy	73.7[a]	208.5[a]	53.2[a]
		HLB-AS	76.5[a]	214.5[a]	52.9[a]
		HLB-SY	58.4[b]	122.3[b]	46.1[b]
Massenti et al. (2016)[III]	March and May 2013[2]	Healthy	-	183[b]	58.9[a]
		HLB-AS		208[a]	57.8[ab]
		HLB-SY		115[c]	55.5[b]
Hamlin orange juice					
Bassanezi et al. (2009)[I]	Blend of different harvests[3]	HLB-AS	69.1[a]	173.1[a]	42.2[a]
		HLB-SY	60.5[b]	128.6[b]	39.3[b]
Liao and Burns (2012)[II]	December 2007	Healthy	71.5[a]	194.3[a]	52.1[a]
		HLB-AS	68.8[a]	196.6[a]	49.9[a]
		HLB-SY	53.2[b]	109.9[b]	48.8[a]

AS: asymptomatic; SY: symptomatic;

[1]Blend of oranges harvested on Sep. 2004, Jul. and Oct. 2005 and Aug. 2007;

[2]Blend of oranges harvested on Mar. and May 2013;

[3]Blend of oranges harvested on Jul. 2007, Jun. and Jul. 2008.

Values from the same reference with the same letter within columns are not significantly by the following statistical analysis ([I]t test with the probability of error estimated to be lower than 0.000); [II]Duncan's multiple range test $P<0.01$; [III]Tukey test at $P \leq 0.05$).

HLB potentially causes trees to be more susceptible to other pest concerns including citrus longhorned beetle (*Anoplophora chinensis* Forster) attacks. In advanced cases of HLB infection, a combination of citrus longhorned beetles and *Phytophthora* fungi is common (HALBERT & MANJUNATH, 2004; BATOOL et al., 2007).

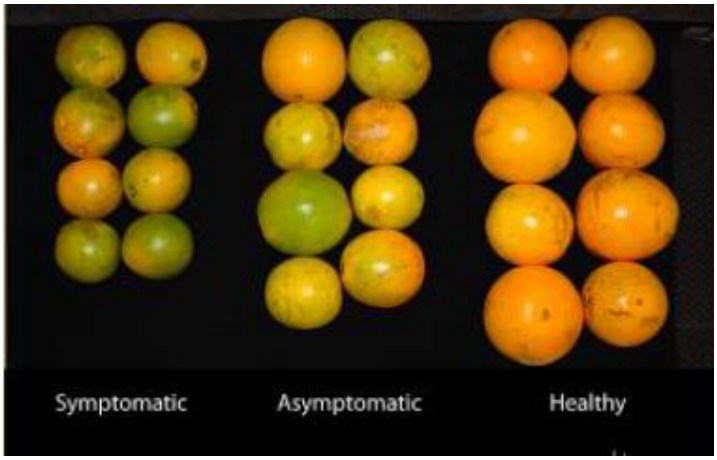

Figure 5. Symptomatic (left) and asymptomatic orange (middle) of HLB disease, *C*Las (+); and healthy oranges (right), CLas (-) (SLIZS et al., 2012).

6. Fresh oranges and orange juice quality affected by *Candidatus* Liberibacter asiaticus

To better understand the influence of HLB on the chemical and physicochemical characteristics of orange juice, it is important to consider the factors which may affect them, such as, cultivar, harvest date, location, degree of maturity and the presence of pulp in the juice. In general, variation due to harvest date is more dramatic compared to variation due to the disease (BASSANEZI et al., 2009; BALDWIN et al., 2010; PLOTTO et al., 2010; BAI et al., 2013; RAITHORE et al., 2015). As the season progresses, the peel color of a healthy orange becomes less green and more orange, juice content declines, sugars and SSC increase and TA, citric acid and total ascorbic acid levels tend to decline (BAI et al., 2013).

6.1 Effects on physicochemical and biochemical characteristics

6.1.1 Peel color

As peel color often determines the attractiveness of an orange to the consumer, the effects of HLB on this important characteristic are of great concern within the citrus industry. Symptomatic oranges, *C*Las (+), are greener or less orange in peel color compared to asymptomatic and healthy oranges. Three studies have covered changes in peel color due to infection. Two of them analyzed Hamlin oranges and reported a less orange colored peel in

symptomatic fruits. All three analyzed Valencia oranges, however only two reported symptomatic fruits having less orange color in their peels, suggesting that the Valencia orange cultivar seems to be the least affected by HLB, regarding changes in peel color (BALDWIN et al., 2010; LIAO & BURNS, 2012; MASSENTI et al., 2016).

6.1.2 Physicochemical characteristics

The physicochemical characteristics of oranges play a vital role in determining the quality of the orange juice produced. There is no general agreement among available results in the scientific literature regarding pH due to status of *C*Las infection. The pH results of asymptomatic orange juice were either higher, lower, or similar compared to healthy orange juice (PLOTTO et al., 2008; PLOTTO et al., 2010; RAITHORE et al., 2015; DALA PAULA et al., 2017b).

TA, SSC and SSC/TA tend to be similar in asymptomatic, *C*Las (+), and healthy orange juice. However, a few studies reported differences in SSC/TA between asymptomatic and healthy Valencia and Hamlin orange juices (BALDWIN et al., 2010; DAGULO et al., 2010; MASSENTI et al., 2016). HLB symptomatic juice usually presents the highest TA, and the lowest SSC and SSC/TA in Valencia, Hamlin (Tables 3 and 4), Westin and Pera Rio orange juices (BASSANEZI et al., 2009). SSC/TA, a parameter commonly used as a fruit maturity index, tends to increase at later harvest dates and is more heavily influenced by harvest time and orange cultivar than HLB infection status (BALDWIN et al., 2010). Among the orange cultivars investigated, evaluation of the effects of HLB predominantly addresses Valencia oranges.

Table 3. Physicochemical characteristics of Valencia orange juice made with healthy fruit and fruit at different stages of HLB infection.

Reference	Orange juice sample		Physicochemical characteristics			
	harvest time	status or conditions	pH	TA (g.100 mL^{-1})	SSC (°Brix)	SSC/TA
Valencia orange juice						
Plotto et al. (2008)[I]	July 2006	Healthy FJ	4.62^{b}	0.64^{a}	12.0^{a}	18.8^{b}
		HLB FJ	4.78^{a}	0.54^{b}	11.3^{a}	21.0^{b}
		Healthy JWP	4.60^{b}	0.63^{a}	11.6^{a}	18.3^{b}
		HLB JWP	4.74^{b}	0.47^{c}	10.1^{b}	21.6^{a}
Bassanezi et al. (2009)[II]	Blend of different harvests[1]	HLB-AS	-	1.22^{a}	9.6^{a}	8.3^{a}
		HLB-SY		1.75^{b}	8.0^{b}	4.8^{b}
Baldwin et al. (2010)[I]	March 2007	Healthy		0.82^{Aa}	10.7^{ABa}	13.2^{Da}
		HLB-AS		0.84^{Aa}	10.3^{Aa}	12.5^{Da}
	April 2007	Healthy		0.68^{Ba}	10.1^{Ba}	15.1^{Ca}
		HLB-AS		0.72^{Ba}	9.7^{Aa}	13.6^{Ca}
	May 2007	Healthy	-	0.57^{Ca}	10.6^{Ba}	18.6^{Ba}
		HLB-AS		0.54^{Ca}	9.6^{Ab}	18.0^{Ba}
	June 2007	Healthy		0.43^{Da}	11.0^{Aa}	25.8^{Aa}
		HLB-AS		0.41^{Da}	10.1^{Ab}	24.8^{Aa}
Dagulo et al. (2010)[III]	April 04, 2008	Healthy				13.7^{a}
		HLB-AS				10.8^{b}
		HLB-SY				5.10^{c}
	April 18, 2008	Healthy				14.8^{a}
		HLB-AS	-	-	-	13.0^{b}
		HLB-SY				5.57^{c}
	May 23, 2008	Healthy				18.2^{b}
		HLB-AS				21.5^{a}
		HLB-SY				9.8^{c}
Plotto et al. (2010)[IV]	April 2008	Healthy	3.78	0.89	14.5	16.2
		HLB-SY	3.68	1.05	14.7	14.1
	June 2008	Healthy	4.37	0.42	12.0	28.7
		HLB-SY	4.27	0.46	13.2	28.4
Liao and Burns, (2012)[V]	April 2009	Healthy		0.85^{b}	11.6^{a}	13.5^{a}
		HLB-AS	-	0.85^{b}	11.2^{a}	13.1^{a}
		HLB-SY		0.91^{a}	9.3^{b}	10.2^{b}
Slisz et al. (2012)[IV]	May 2007	Healthy		0.54	10.6	19.5
		HLB-AS		0.52	9.6	18.5
	June 2007	Healthy	-	0.40	10.8	27.3
		HLB-AS		0.38	9.7	25.7
		HLB-SY		0.69	6.9	10.1
Raithore et al. (2015)[III]	April 2009	Healthy	4.17^{a}	0.62^{b}	12.2^{a}	19.7^{a}
		HLB-SY	3.81^{a}	1.14^{a}	11.6^{a}	10.2^{b}
Massenti et al. (2016)[III]	March + May 2013	Heatlhy	-	0.72^{b}	12.4^{a}	11.0^{a}
		HLB-AS		0.75^{b}	12.2^{a}	10.4^{b}
		HLB-SY		1.22^{a}	8.5^{b}	4.5^{c}
Dala Paula et al. (2017b)[VI]	March 2013	Healthy	4.35^{a}	0.72^{b}	10.5^{a}	14.6^{a}
		HLB-SY	3.86^{b}	0.94^{a}	9.6^{b}	10.1^{b}

Leg.: TA: titratable acidity; SSC: solid soluble content; FJ: filtered juice; JWP: juice with pulp; AS: asymptomatic; SY: symptomatic; [1]Blend of oranges harvested at Sep. 2004, Jul. and Oct 2005, and Aug. 2007; Values from the same reference with the same capital letter within columns do not differ in harvest time and values with the same small letter within columns do not differ in disease status, according to statistical analysis ([I]Fisher's test significant difference test at P = 0.05; [II]*t* test with the probability of error estimated to be lower than 0.000; [III]ANOVA and Tukey's test P ≤ 0.05; [IV]not applicable; [V]Duncan's multiple range test P<0.01; [VI]ANOVA and Tukey's test P ≤ 0.05 for SSC, and P ≤ 0.001 for TA and SSC/TA).

Table 4. Physicochemical characteristics of Hamlin orange juice made with healthy fruit and fruit at different stages of HLB infection.

Reference	Orange juice sample		Physicochemical characteristics			
	harvest time	status or conditions	pH	TA (g.100 mL^{-1})	SSC (°Brix)	SSC/TA
Hamlin orange juice						
Bassanezi et al. (2009)[I]	Fruits of different harvests[1]	HLB-AS HLB-SY	-	0.76^{a} 0.91^{b}	9.6^{a} 8.9^{b}	13.1^{a} 10.7^{b}
Baldwin et al. (2010)[II]	December 2007	Healthy HLB-AS	-	0.49^{Aa} 0.50^{Aa}	7.8^{Ba} 7.6^{Ba}	16.0^{Ba} 15.3^{Ba}
	February 2008	Healthy HLB-SY		0.59^{Aa} 0.50^{Aa}	11.6^{Aa} 10.4^{Ab}	19.8^{Ab} 22.0^{Aa}
Plotto et al. (2010)[III]	February 2008	Healthy HLB-SY	4.19 4.17	0.50 0.52	11.9 11.4	23.8 22.1
Liao and Burns, (2012)[IV]	December 2007	Healthy HLB-AS HLB-SY	-	0.75^{a} 0.80^{a} 0.78^{a}	11.3^{a} 11.5^{a} 9.1^{b}	15.1^{a} 14.3^{ab} 11.7^{b}
Raithore et al. (2015)[V]	January 2009	Healthy HLB-SY	4.22^{a} 4.22^{a}	0.52^{a} 0.52^{a}	11.4^{a} 11.3^{a}	21.7^{a} 21.7^{a}

Leg.: TA: titratable acidity; SSC: solid soluble content; AS: asymptomatic; SY: symptomatic;
[1]Blend of oranges harvested at Jul. 2007, Jun. and Jul. 2008.
Values from the same reference with the same capital letter within columns do not differ in harvest time and values with the same small letter within columns do not differ in disease status, according to statistical analysis ([I]*t* test with the probability of error estimated to be lower than 0.000; [II]Fisher's test significant difference test at P = 0.05; [III]not applicable; [IV]Duncan's multiple range test P<0.01; [V]ANOVA and Tukey's test P ≤ 0.05).

6.1.3 Sugars and organic acids

The results found for glucose in Valencia orange juice due to HLB infection status were not consistent enough to establish an observable trend. Two of three studies reported an increase of glucose in symptomatic juice, *C*Las (+), compared to healthy juice, while one reported a decrease. In two different studies, asymptomatic and healthy orange juice showed similarities in glucose content; however certain harvests (May and June 2007) had higher glucose contents in healthy juice compared to asymptomatic juice. For Hamlin juice, there is one study comparing asymptomatic and healthy juice, from two different harvest times, in which all of the samples showed a decrease of glucose in asymptomatic juice compared to healthy juice (Table 5).

Changes in fructose due to HLB infection status also do not follow a clear pattern. Fructose contents can either increase or decrease in symptomatic orange juice. Between healthy and asymptomatic juices, the content is generally similar. On the other hand, sucrose and total sugar contents decrease in asymptomatic and, more notably, in symptomatic juices of Valencia and

Hamlin oranges (Tables 5 and 6), which would reflect the altered carbohydrate transport in these infected oranges (CHIN et al., 2014). Asymptomatic and healthy orange juice can have sucrose contents approximately 2.5 times higher than that of symptomatic juice (SLISZ et al., 2012).

For individual organic acids, the majority of the studies reported similar citric and ascorbic acid levels in healthy and asymptomatic orange juice, *C*Las (+). However, symptomatic orange juice generally has a higher content of citric acid and a lower content of malic acid compared to healthy juice (Tables 5 and 6). Typically, changes in individual and total sugar contents are more pronounced in Hamlin than in Valencia orange juice (BALDWIN et al., 2010).

6.1.4 Secondary metabolites

Oranges are an important source of secondary metabolites which promote human health, particularly flavonoids, limonoids, hydroxycinnamic acids and polyamines. Many secondary metabolites result from the interaction between the plant and its environment and are induced by biotic and abiotic elicitation. Changes in the levels of certain classes of secondary metabolites are frequently due to stress conditions in plants. In addition to stress conditions, these compounds are influenced by many factors, such as: type of cultivar, cultivating methods, degree of ripeness, and processing and storage conditions (SUDHA & RAVISHANKAR, 2002; RAMAKRISHNA & RAVISHANKAR, 2011; CHIN et al., 2014).

Table 5. Sugars and acids of Valencia orange juice made with healthy fruit and fruit at different stages of HLB infection.

Reference	Orange juice sample		Sugars (g.100 mL^{-1})				organic acids (g.100 mL^{-1})	
	harvest time	status or conditions	glucose	fructose	sucrose	total sugars	citric acid	malic acid
Plotto et al. (2008)[I]	July 2006	Healthy FJ	2.8^{a}	1.9^{a}	4.3^{a}		0.52^{a}	0.13^{a}
		HLB FJ	2.8^{a}	1.9^{a}	4.1^{a}		0.45^{b}	0.10^{b}
		Healthy JWP	2.6^{ab}	1.8^{ab}	4.1^{ab}	-	0.48^{ab}	0.11^{b}
		HLB JWP	2.5^{b}	1.7^{b}	3.7^{b}		0.40^{c}	0.09^{c}
Baldwin et al. (2010)[I]	March 2007	Healthy	1.9^{Aa}	1.9^{Aa}	4.9^{Ba}	8.7^{Ba}		
		HLB-AS	1.9^{Aa}	1.9^{Aa}	4.7^{Aa}	8.6^{Aa}		
	April 2007	Healthy	1.9^{Aa}	2.0^{Aa}	5.2^{ABa}	9.1^{ABb}		
		HLB-AS	1.7^{Aa}	1.8^{Aa}	4.4^{Ab}	8.0^{Ab}		
	May 2007	Healthy	2.0^{Aa}	2.0^{Aa}	5.5^{Aa}	9.5^{ABa}	-	-
		HLB-AS	1.8^{Ab}	1.9^{Aa}	4.8^{Ab}	8.5^{Ab}		
	June 2007	Healthy	2.0^{Aa}	2.0^{Aa}	5.6^{Aa}	9.7^{Aa}		
		HLB-AS	1.8^{Ab}	1.9^{Aa}	4.8^{Ab}	8.4^{Ab}		
Liao and Burns (2012)[II]	April 2009	Healthy				7.1^{a}		
		HLB-AS	-	-	-	6.8^{a}	-	-
		HLB-SY				1.8^{b}		
Slisz et al. (2012)[III]	May 2007	Healthy	1.38	1.70	4.13		0.64	0.26
		HLB-AS	1.30	1.57	3.71		0.57	0.23
	June 2007	Healthy	1.37	1.70	4.64	-	0.47	0.26
		HLB-AS	1.24	1.64	3.90**		0.38	0.22*
		HLB-SY	1.12	1.47	1.54		0.91	0.18
Raithore et al. (2015)[IV]	April 2009	Healthy	2.16^{a}	2.30^{a}	4.95^{a}		0.53^{b}	0.17^{a}
		HLB-SY	2.69^{a}	2.68^{a}	3.39^{b}	-	1.40^{a}	0.12^{b}
Baldwin et al. (2017)[V]	March-April							
	2013	HLB	1.4	1.9	3.9	7.2	0.42	0.13
	2014	HLB	1.9	2.2	3.8	7.9	0.80	0.18
	2015	HLB	1.9	2.2	3.7	7.8	0.67	0.61
Dala Paula et al. (2017b)[VI]	March 2013	Healthy	2.0^{b}	2.3^{b}	5.6^{a}	10.0^{a}	0.84^{b}	0.14^{a}
		HLB-SY	2.3^{a}	2.7^{a}	4.2^{b}	9.0^{b}	1.41^{a}	0.11^{b}

Leg.: FJ: filtered juice; JWP: juice with pulp; AS: asymptomatic; SY: symptomatic.

Values from the same reference with the same capital letter within columns are not significantly by different harvest time and with the same small letter within columns are not significantly by disease status following statistical analysis ([I]Fisher's test significant difference test at P = 0.05; [II] Duncan's multiple range test P<0.01; [III]P-values represent comparisons within harvest *p < 0.05; **p < 0.001; [IV]ANOVA and Tukey's test P ≤ 0.05; [V]not applicable; [VI]ANOVA and Tukey's test P ≤ 0.05 for glucose, total sugars and malic acid, and P ≤ 0.01 for sucrose, fructose and citric acid).

Table 6. Sugars and acids of Hamlin orange juice made with healthy fruit and fruit at different stages of HLB infection.

Reference	Orange juice sample		Sugars (g.100 mL^{-1})				organic acids (g.100 mL^{-1})	
	harvest time	status or conditions	glucose	fructose	sucrose	total sugars	citric acid	malic acid
Baldwin et al. (2010)[I]	December 2007	Healthy	1.5^{Ba}	1.5^{Ba}	3.9^{Ba}	7.0^{Ba}	-	-
		HLB-AS	1.3^{Bb}	1.4^{Ba}	3.2^{Bb}	6.0^{Bb}		
	February 2008	Healthy	2.2^{Aa}	2.2^{Aa}	5.4^{Aa}	9.8^{Aa}		
		HLB-AS	1.8^{Ab}	1.8^{Ab}	4.0^{Ab}	7.6^{Ab}		
Raithore et al. (2015)[II]	January 2009	Healthy	2.9^{a}	3.0^{a}	5.4^{a}	-	0.53^{a}	0.16^{a}
		HLB-SY	2.7^{a}	2.7^{a}	4.7^{a}		0.55^{a}	0.17^{a}

Leg.: AS: asymptomatic; SY: symptomatic.
Values from the same reference with the same capital letter within columns are not significantly by different harvest time and with the same small letter within columns are not significantly by disease status following statistical analysis
([I]Fisher's test significant difference test at P = 0.05;
[II]ANOVA and Tukey's test P ≤ 0.05 for glucose, total sugars and malic acid, and P ≤ 0.01 for sucrose, fructose and citric acid).

Generally, higher concentrations of phenolic compounds are found in sprouts and seedlings compared to mature plants, consistent with the notion that plant phenolics provide a degree of protection against predation (DREWNOWSKI & GOMEZ-CARNERO, 2000). Similarly, there is an increase of phenolic compound levels in orange juice and leaves from trees infected with *C*Las (DAGULO et al., 2010; HIJAZ et al., 2013; KIEFL et al., 2017). Flavonoid contents, particularly narirutin and hesperidin, are higher in the peel, pulp and juice of HLB symptomatic fruit (MASSENTI et al., 2016; KIEFL et al., 2017) compared to the respective healthy fruit parts. The pulp of HLB symptomatic Valencia oranges from two different harvest times (March and May 2013) showed an increase of 147.9% and 16.9%, in narirutin, respectively, and an increase of 85.5% and 94.1% in hesperidin, respectively, compared to the corresponding healthy fruit pulp (MASSENTI et al., 2016). Juice from symptomatic Valencia oranges harvested in March 2013, contained higher amounts of tangeretin (> 4x), nobiletin (> 2x), heptamethoxyflavone (> 1.5x), diosmin (> 2x), didymin (> 1.5x), 6,8-di-C-glucosyl apigenin (> 1.5x), nomilin (> 20x), limonin (> 7.5x) and limonin glucoside (> 1.5x) compared to healthy juice (DALA PAULA et al., 2017b). The polymethoxyflavone, tangeretin, is also high in symptomatic Valencia and Hamlin juice (KIEFL et al., 2017).

Juice made with asymptomatic, *C*Las (+), and healthy, *C*Las (-), Hamlin oranges harvested in February and April 2008, respectively, had similar limonin, nomilinic acid glucoside, nomilin glucoside, narirutin, limonin glucoside, narirutin-4'-glucoside, feruloyl-putrescine, 6,8-di-C-glucosyl apigenin, alkaloid and hydroxycinnamic acid contents. Healthy and asymptomatic Valencia oranges differed in nomilin glucoside and nomilin levels in their juice (BALDWIN et al., 2010).

Juices made with asymptomatic and, especially, symptomatic oranges, *C*Las (+), contain high levels of nomilin and limonin. Both, nomilin and limonin are known to provide bitterness in citrus fruit and its juice, however their levels in HLB orange juice are usually below the threshold for bitter perception (DEA et al., 2012) and, in fact, only symptomatic oranges have their taste compromised (BALDWIN et al., 2010; PLOTTO et al., 2010; SLISZ et al., 2012; CHIN et al., 2014; RAITHORE et al., 2015; DALA PAULA et al., 2017b). This

suggests that there may be other compounds involved with the bitter taste in *C*Las (+) symptomatic orange juice.

6.1.5 Amino acids and bioactive amines

The accumulation of proline, arginine and the branched chain amino acids is expected in plants subjected to conditions which induce stress, such as drought, high salinity and acidity, high incidence of light, high concentration of heavy metals in the soil, changes in temperature, as well as in response to biotic stress, such as plant diseases (RAI, 2002; SHARMA & DIETZ, 2006; SLISZ et al., 2012; MALIK et al., 2013). However, the amino acids: alanine, arginine, isoleucine, leucine, proline, threonine, and valine are found in lower concentrations in symptomatic orange juice, *C*Las (+). In symptomatic Valencia and Hamlin orange juices, the concentrations of asparagine and phenylalanine are over 2 times higher and histidine content is also increased (SLISZ et al., 2012; CHIN et al., 2014). A suggested explanation for this trend is that *C*Las may have inhibited the tree's defense mechanism which, in turn, reduced the action of proline dehydrogenase, an enzyme responsible for the activation of the biosynthetic pathways of proline from ornithine and glutamate. Thus, the levels of this amino acid could not increase (SLISZ et al., 2012).

The changes in amino acid contents due to infection status in orange trees allowed the use of their levels as biomarkers to identify early detection of asymptomatic trees. The differences of the local harvest and cultivars did not compromise the use of metabolite composition in the discernment of HLB infection status in the oranges (CHIN et al., 2014).

Hamlin and Valencia symptomatic oranges, *C*Las (+), show high contents of synephrine, an aromatic amine, however asymptomatic and healthy juices have similar contents (SLIZS et al., 2012; CHIN et al., 2014). In plants, putrescine is a necessary diamine precursor of polyamines (spermidine and spermine) synthesis and its increase is usually associated with environmental stress in plants (COELHO et al., 2005; GLÓRIA, 2006; SHARMA & DIETZ, 2006); however, putrescine content is not affected in symptomatic juice (CHIN et al., 2014). On the other hand, feruloyl putrescine, a conjugate of putrescine and ferulic acid, is found at high concentrations in symptomatic Hamlin juice

compared to asymptomatic and healthy juice. The same trend does not seem to be observed in Valencia oranges (BALDWIN et al., 2010).

6.2 Effects on sensory characteristics

The aroma of orange juice is due to the complex combination of various flavor and odor components. Alcohols, aldehydes, esters, ketones, hydrocarbons, sugars and secondary metabolites, such as phenolic compounds and bitter limonoids have been investigated in orange juice affected by *C*Las (PLOTTO et al., 2008; 2010; 2017; BALDWIN et al., 2010; 2012a; DAGULO et al., 2010; MASSENTI et al., 2016; DALA PAULA et al., 2017b). Some taste descriptors have been used to describe the effects of HLB infected oranges since the 1970s, for example, bitter and salty (McCLEAN & SCHWARZ, 1970; MOLL & VAN VUUREN, 1977; BALDWIN et al., 2010; PLOTTO et al., 2010; RAITHORE et al., 2015).

Years later, after HLB had been reported in both South and North America, some researchers began to take a deeper look into the sensory effects of orange juice caused by HLB. Recently, with the evolution of sensory analysis as an increasingly important component of food science and the increased interest on the topic, *C*Las (+) orange juice has been associated with several negative effects regarding taste (astringency, tingling, harshness, bitterness, metallic-taste, low sweetness, saltiness/umami, musty, sourness/fermented, pungent/peppery, low citrusy taste) (Tables 7 and 8), usually due to an imbalance in the chemical composition (BALDWIN et al, 2010; 2012b; PLOTTO et al., 2010; RAITHORE et al., 2015; DALA PAULA et al., 2017b; KIEFL et al., 2017).

Table 7. Sensorial descriptors ascribed to Huanglongbing in Valencia orange juice

Sensorial descriptor*	Harvest time	Juice specifications	Reference
Acidic	Jul. 2006, Apr. 2008	frozen juice with pulp and filtered[I], hand-squeezed juice [II]	1; 4
Astringent	Jun. 2008; Mar. 2013	commercially processed juice[III]	2; 5
Bitter/slight bitter	Jun. 2008; Mar. 2013	commercially processed juice	2; 5
Bland	Jun. 2008	commercially processed juice	2
Burning	Mar. 2013	commercially processed juice	5
Fermented	Jul. 2006	frozen juice with pulp and filtered	1
Grapefruit-like flavor	Apr. 2008, Jun. 2008; Mar. 2013	commercially processed juice	2; 5
Green flavor	Mar. 2013	commercially processed juice	5
HLB-bitter	Monthly basis during the season 2014 and 2015	hand-squeezed juice	4
Less fruity non-citrus flavor**	Mar; 2013	commercially processed juice	5
Less orange flavor**	Mar. 2013	commercially processed juice	5
Less sweet**	Apr. 2008, Mar. 2013	commercially processed juice	2; 5
Metallic	Jun. 2008; Apr. 2009	commercially processed juice	3
Off flavor	Apr. 2008	commercially processed juice	2
Overripe	Jul. 2006	frozen juice with pulp and filtered	1
Peel oil	Apr. 2008, Jun. 2008; Mar. 2013	commercially processed juice	2; 5
Salty/umami	Apr. 2009; Mar. 2013	commercially processed juice	3; 5
Sharp	Apr. 2008, Jun. 2008	commercially processed juice	2
Sour	Apr. 2008, 2009; Mar 2013	commercially processed juice	2; 3; 5
Stale	Mar. 2013	commercially processed juice	5
Sweeter**	Apr. 2008	commercially processed juice	2
Tangy	Apr. 2008	commercially processed juice	2
Tingly	Apr. 2009	commercially processed juice	3
Typical HLB flavor	Mar. 2013	commercially processed juice	5
Unidentifiable different flavor	Jun. 2008	commercially processed juice	2
Weak in taste	Jul. 2006	frozen juice with pulp and filtered	1

*The list of sensorial descriptors includes commentaries realized by the panel during sensory evaluations and attributes significantly higher in asymptomatic or symptomatic orange juice, *C*Las (+), compared to healthy juice (control);
**in comparison with control juice - healthy orange juice, *C*Las (-);
***According to the authors, HLB-bitter refers to a long-lasting metallic, astringent and harsh taste.
[I]frozen juice thawed overnight served with the pulp and without pulp. Juice was filtered then flash pasteurized at 71 °C for 10 s and immediately cooled then served;
[II]oranges were hand juiced and lightly pasteurized using at 71 °C for 15 s, and frozen at - 20 °C;
[III]fruit were extracted using a commercial JBT 391 single head extractor with premium juice extractor settings, and pasteurized under simulated commercial conditions (1.2 L/m, 8 to 10 s hold time, 83 to 90 °C).
Leg.: [1]PLOTTO et al., 2008; [2]PLOTTO et al., 2010; [3]RAITHORE et al., 2015; [4]KIEFL et al., 2017; [5]DALA PAULA et al., 2017b.

Table 8. Sensorial descriptors ascribed to Huanglongbing in Hamlin orange juice

Sensorial descriptor*	Harvest time	Juice specifications	Reference
Astringent	Feb. 2008;	commercially processed juice	1
Bitter	Dec. 2007; Feb. 2008; Jan. 2009	hand-squeezed juice, commercially processed juice	1; 2
Cooked	Jan. 2009	commercially processed juice	2
Earthy	Feb. 2008;	commercially processed juice	1
Fatty	Feb. 2008	commercially processed juice	1
Fermented	Feb. 2008;	commercially processed juice	1
Grapefruit-like	Dec. 2007; Feb. 2008; Jan. 2009	hand-squeezed juice, commercially processed juice	1; 2
HLB bitter***	Monthly basis during the season 2014 and 2015	hand-squeezed juice	3
Less freshness	Feb. 2008	commercially processed juice	1
Less orange flavor**	Feb. 2008	commercially processed juice	1
Less sweet**	Feb. 2008;	commercially processed juice	1
Metallic	Feb. 2008;	commercially processed juice	1
Musty	Feb. 2008;	commercially processed juice	1
Overripe	Jan. 2009	commercially processed juice	2
Peel oil/citrus oil	Dec. 2007; Feb. 2008; Jan. 2009	hand-squeezed juice, commercially processed juice	1; 2
Peppery	Feb. 2008;	commercially processed juice	1
Pungent	Feb. 2008	commercially processed juice	1,
Salty/umami	Feb. 2008	commercially processed juice	1
Sharp	Dec. 2007;	hand-squeezed juice	1
Sour	Dec. 2007; Feb. 2008; Jan. 2009	hand-squeezed juice, commercially processed juice	1; 2
Sour milk	Dec. 2007;	hand-squeezed juice	1
Sulfury	Jan. 2009	commercially processed juice	2
Tingly	Feb; 2008	commercially processed juice	1

*The list of sensorial descriptors includes commentaries realized by the panel during sensory evaluations and attributes significantly higher in asymptomatic or symptomatic orange juice, *C*Las (+), compared to healthy juice (control);
**in comparison with control juice - healthy orange juice, *C*Las (-);
***According to the authors, HLB-bitter refers to a long-lasting metallic, astringent and harsh taste.
[I]frozen juice thawed overnight served with the pulp and without pulp. Juice was filtered then flash pasteurized at 71 °C for 10 s and immediately cooled then served;
[II]oranges were hand juiced and lightly pasteurized using at 71 °C for 15 s, and frozen at - 20 °C;
[III]fruit were extracted using a commercial JBT 391 single head extractor with premium juice extractor settings, and pasteurized under simulated commercial conditions (1.2 L/m, 8 to 10 s hold time, 83 to 90 °C).
Leg.: [1]PLOTTO et al., 2010; [2]RAITHORE et al., 2015; [3]KIEFL et al., 2017;

According to the chemical and physicochemical changes observed in orange juice as the season progresses, the effects of HLB on sensory characteristics are more pronounced in symptomatic juice made from oranges harvested earlier in the season (McCLEAN & SCHWARZ, 1970; RAITHORE et

al., 2015). In addition, there is a large variation in the effect of *C*Las on the quality of orange juice due to cultivar, maturity, and individual tree. Comparing Hamlin and Valencia, the off-flavor resulting from the disease is generally more noticeable in Hamlin oranges. Juice made with these symptomatic, *C*Las (+) oranges generally has the most off-flavors, commonly described as “bitter”, “sour” and “sour/fermented”. On the other hand, for certain harvests, symptomatic Valencia orange juice is no different from its healthy juice. When they are different, the HLB juice is described as “bitter” or “off-flavor” (PLOTTO et al., 2010).

The HLB off-flavor is so pronounced that processing healthy fruits with infected ones can affect the sensory quality of the orange juice, negatively impacting the citrus industry (BASSANEZI et al., 2009). Juice from *C*Las (+) symptomatic fruit can be blended with juice from *C*Las (-) fruit, up to 25% symptomatic juice, without being perceived as off-flavored. Considering the increased spread of HLB in the US, this may be considered valuable information for the citrus industry and can help maintain commercial acceptability of orange juice in regions where HLB is prevalent (RAITHORE et al., 2015).

HLB-infected juice with lower sugar contents, an altered volatile profile and sometimes higher acids, tastes unpleasant. In addition to these components, the orange fruit also contains some secondary metabolites including different classes of flavonoids (flavanone, flavone, polymethoxyflavone, hydroxycinnamic acid derivatives, coumarins, and anthocyanins) and triterpenoids which influence taste. Limonin and nomilin exist in glycosides forms being tasteless, and aglycone forms. The aglycone of the triterpenoids limonin and nomilin are well known to be bitter and/or metallic in taste. Interestingly, the levels of bitter limonoids were either at or below reported thresholds (the concentration at which a compound can be detected); yet, panelists were able to detect a bitter and metallic flavor in symptomatic *C*Las (+) orange juice. Further research showed that the threshold for the detection of bitterness in orange juice was lowered if the two bitter limonoids (limonin and nomilin) were present together, indicating synergy between them (HASEGAWA, 1983; DEA et al., 2013).

There is a strong correlation between limonoid and flavonoid concentrations and the off-flavor and quality of the oranges (KIEFL et al., 2017).

It is known that commercial polymethoxyflavone (PMF) preparations from citrus peel possess a bitter taste. Nobiletin and tangeretin were reported as the two main bitter PMFs present in citrus peel, with tangeretin bitterness determined to be 2-3 times higher than nobiletin by sensory analysis and 3-4 times higher using a receptor-based method in vitro (BATENBURG et al., 2016). Recently, hesperidin was also suggested to be a key compound of HLB induced orange juice off-flavor, indirectly acting as a taste modulator enhancing unacceptably harsh, metallic and bitter characteristics (KIEFL et al., 2017). Additionaly, in hesperidin can contribute to the formation of sediments which result in undesirable cloudiness in orange juice (GATTUSO et al., 2007).

Unlike other tastes, the detection thresholds for bitterness are generally extremely low. For example, the bitter quinine was detected at 25 µmol/L. Furthermore, the bitter after-taste can also be more prolonged than others (DREWNOWSKI & GOMEZ-CARNEROS, 2000). Bitterness is appreciated in some kinds of food and beverages, such as bitter chocolate and coffee however, it greatly reduces the acceptability of orange juice (PLOTTO et al., 2008; 2010).

The polyphenol contained in foods and beverages are classified into many classes, from small molecules, such as hydroxycinnamic acids, to big polymers, such as lignin. This vast group of compounds may be responsible for the bitterness and astringency of many foods and beverages. Astringency, a typical mouthfeel, is perceived as a long-lasting trigeminal sensation in the oral cavity which may be due to a reaction between dietary polyphenols and proteins in the mouth and saliva and can be classified into sub qualities such as velvety, grainy, drying or puckering (DREWNOWSKI & GOMEZ-CARNERO, 2000; HUFNAGEL & HOFMANN, 2008;). In general, phenolics of lower molecular weight are bitter, while higher molecular weight polymers tend to be more astringent (DREWNOWSKI & GOMEZ-CARNEROS, 2000). However, this assumption is not consistently applicable. For example, the phenolic compounds of black tea were separated into three fractions according to the different molecular weights and then described by a sensory panel. The fraction containing low molecular weight compounds was described as the most astringent and represented the typical taste profile of the black tea (SCHARBERT et al., 2004).

6.3 Effect on the levels and profile of volatile compounds

Ten odor-active compounds were chemically identified in pasteurized orange juice and their odors were described utilizing multidimensional gas chromatography coupled with olfactometry and mass spectrometry. Grassy and plastic-like odors were attributed to α-pinene; fruity and sweet odors to ethyl butanoate; grassy and green odors to hexanal; citric, mint and sweet odors to heptanal, methyl hexanoate and D-limonene; fresh, lemony, green, fatty and bug odors to octanal; flower and lemon odors to linalool; and mint, green, fruity and citrus odors to α-terpineol and citral. Other than these odor descriptions, other unidentified compounds were described as mushroom, spicy, wood, cheese, citric and caramel odors (MASTELLO et al., 2015). Some of these descriptors were very similar to those obtained from the taste dilution fractionation test of phenolic compounds extracted by fast centrifugal partition chromatography in healthy and symptomatic *C*Las (+) Valencia orange juice (DALA PAULA et al., 2017a; 2017b), as well as the observations recorded by the panel during the sensory evaluation of differences between healthy and asymptomatic Hamlin orange juice (PLOTTO et al., 2010). However, unlike Mastello et al. (2015), in the study of Valencia orange juice, the authors only evaluated non-volatile compounds.

Regarding other chemical and physicochemical attributes, the harvest date is a more influential factor in changing the volatile contents of orange juice compared to infection status (BALDWIN et al., 2010). However, HLB infection significantly affects the levels of odor active compounds in orange peel oil (KIEFL et al., 2017) and orange juice. Symptomatic, *C*las (+), Valencia juice made with oranges harvested from December to May in 2007/2008 showed higher contents of 26 volatile compounds and lower contents of the following 9 compounds: ethyl butanoate, ethyl hexanoate, 1-Octen-3-ol, β-selinene, ethyl-3-hydroxyhexanoate, valencene, α-selinene and β-Ionone) compared to healthy juice (DAGULO et al., 2010).

Among the 22 volatile compounds identified in juice made with healthy and asymptomatic, *C*Las (+), Hamlin oranges harvested in different months in 2007 and 2008, only two volatiles showed significant differences due to infection

status. The samples had higher contents of ethyl hexanoate and lower contents of sabinene compared to control juice. Homemade Valencia juice made with asymptomatic oranges, *C*Las (+), harvested in different months of 2007 showed significant differences in 9 compounds. The samples had lower contents of octanal, decanal, trans-2-hexenol, valencene and ethyl butanoate and higher contents of 2-methylpropanol, cis-3-hexenol, sabinene and ethyl hexanoate compared to control juice. Commercial Valencia juice made with asymptomatic oranges harvested in various months of 2008 showed significant differences in two compounds, the samples had higher contents of ethanol and lower contents of ethyl acetate compared to the control (BALDWIN et al., 2010). These results suggest different levels of volatiles due to different harvest times, types of processes used to prepare the orange juice (BALDWIN et al., 2012a) and HLB infection status. It is important to emphasize that, generally, asymptomatic orange juice is similar to healthy orange juice.

Orange juice made with HLB symptomatic fruit, *C*Las (+), has higher levels of α- and β-sinensal (37 and 59%, respectively), linalool (20%), and numerous limonene and linalool degradation compounds compared to healthy orange juice. On the other hand, healthy orange juice has higher levels of octanal (22%), decanal (6%), undecanal (6%), 6-methyloctanal (23%), ethyl butanoate (163%), ethyl hexanoate (169%), ethyl octanoate (57%), nootkatone (128%), valencenes (51%), (Z)-4decenal (38%) and (E,E)-2,4-decadienal (19%) compared to HLB orange juice. The difference in ethyl-butanoate concentration was more noticiable in healthy than in HLB orange juice. This ethyl ester imparts fruity and sweet odors in healthy orange juice (KIEFL et al., 2017).

6.4 Huanglongbing control and mitigation of its symptoms

To this date, there is no cure for HLB and the prevention of the spread of the disease relies primarily on controlling psyllid populations (STANSLY et al., 2010; MARTINI et al., 2016). Currently, preventing *C*Las from infecting healthy citrus trees is much easier than trying to eradicate or control it. The control of HLB is still difficult, especially if the bacteria are widespread and their vectors are well established. The most effective control strategy has been to remove the trees infected with HLB in an area and then replant with HLB-free trees

(ABDULLAH et al., 2009). Other common management strategies include chemical insect control and nutritional spray applications (ALBRECHT et al., 2012).

Florida growers have been using foliar nutritional spray products that often contain micro-nutrients and compounds that supposedly activate systemic acquired resistance to help boost tree health and defense response (MASUOKA et al., 2011; BALDWIN et al., 2012b). The benefits of this approach to disease management in the field are questionable because the inoculums remain after application. Unfortunately, this method of managing HLB potentially contributed to the propagation of the disease in Florida because many Floridian farms stopped eliminating their affected trees. Unless the vectors are thoroughly controlled, the spread of HLB to other orchard trees and neighboring farms is inevitable (TIMMER et al., 2011; GOTTWALD et al., 2012).

In an evaluation of nutritional spray treatments, Hamlin oranges from trees that received the treatment had the same off-flavor as oranges from trees that did not receive the treatment, whereas Valencia oranges were notably sweeter. The nutritional treatments did not consistently result in less pathogen DNA for either cultivar (BALDWIN et al., 2012b). The implementation of combined nutrient programs and insecticide treatments has been studied and the results suggest that the beneficial effect of increased orange juice quality may have been cumulative, only manifesting later on during the third year (BALDWIN et al., 2017; PLOTTO et al., 2017).

7. Final considerations

HLB affects the physicochemical characteristics of orange juice. Symptomatic juice tends to have high TA, low SSC and SSC/TA, while asymptomatic juice tends to be similar to healthy juice. In general, HLB causes a decrease in sucrose, total sugar and malic acid contents while ascorbic acid does not seem to be significantly affected by the disease. On the other hand, levels of citric acid, bitter limonoids (limonin and nomilin), hydroxycinnamic acids, flavonoids, particularly tangeritin, nobiletin, narirutin, hesperidin, diosmin and didymin are higher in HLB symptomatic juice compared to healthy juice. Thus, there is a strong correlation between the limonoid and flavonoid

concentrations and the off-flavor and quality of the oranges. The amino acid contents, alanine, arginine, asparagine, histidine, isoleucine, leucine, phenylalanine, proline, threonine and valine are altered by HLB. Additionally, symptomatic Hamlin orange juice has high synephrine and feruloyl putrescine levels.

Regarding the typical HLB-off flavor in orange juice, the loss of sweetness can generally be explained by lower sucrose levels, total sugar levels and SSC, along with higher citric acid levels. The sensory descriptors of sourness, umami and tingling were correlated with some volatile compounds, and with tangeretin and nobiletin. The sourness can be partially explained by higher TA and citric acid content generally found in HLB orange juice. Elevated levels of limonin and nomilin, which occur in juice made with oranges harvested from symptomatic and/or asymptomatic HLB infected trees, are partially responsible for the typical HLB-bitterness. These two liminoids have a synergistic effect which decreases their perception and identification thresholds in orange juice. Furthermore, there is evidence indicating that other compounds, possibly hydroxycinnamic acids, are involved with the typical HLB-bitterness. Additionally, the lowest SSC, SSC/TA and sugar contents typically perceived in symptomatic orange juice, *C*Las (+) can reinforce the perception of bitterness.

There are relatively few published papers evaluating the effects of HLB on orange juice's chemical, physicochemical and, especially its sensorial qualities and most of the research available was performed using Valencia oranges, followed by Hamlin samples. The evaluation of the effects of HLB is complex due to various factors that can obscure them. Thus, subsequent research is required to better understand the effects of HLB on orange juice and to identify solutions for its negative sensory attributes.

CAPÍTULO III - ACTIVE TASTE COMPOUNDS IN JUICE MADE FROM ORANGES SYMPTOMATIC OF HUANGLONGBING (HLB) GREENING DISEASE

ABSTRACT

Citrus greening disease, also known as Huanglongbing (HLB), compromises the quality of citrus fruit and juice, causing increased bitterness and metallic taste, astringency and a burning mouthfeel. The chemical basis responsible for these changes remains largely unknown other than the roles of the bitter limonoids, limonin and nomilin, and of flavonoids that may cause astringency. A combination of chemical and sensory analyses was used to identify bitter components in orange juice made from oranges symptomatic for HLB, and comparisons were made with juice made with healthy fruit. The results showed that there were statistical differences in pH, total acidity (TA), soluble solids content (SSC), SSC/TA, total sugars, organic acids, secondary metabolites and sensory characteristics between healthy and HBL-affected orange juices. Nonvolatile juice compounds were fractionated using fast centrifugal partition chromatography and semipreparative HPLC. Some fractions were described as bitter, but did not contain limonoids, polymethoxylated flavones (PMF) or hesperidin, and instead they were overwhelmingly composed of hydroxycinnamates, indicating that these compounds might also be involved with this sensory attribute.

KEYWORDS: Huanglongbing. Bitterness. Hydroxycinnamic acids. Limonoids. Orange juice.

Artigo submetido a LWT – Food Science and Technology

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1. INTRODUCTION

Worldwide citrus production has been adversely affected by citrus greening disease or Huanglongbing (HLB), associated with the presence of the gram-negative bacteria *Candidatus* Liberibacter asiaticus (*C*las) transmitted by the Asian Citrus psyllid *Diaphorina citri* (BOVÉ, 2006). The HLB was first reported in 1919 in Southern China, and has now spread throughout more than 40 countries in Africa, Asia and the Americas (South and North) (UF/IFAS Extension, 2013). The first case of HLB in the Western Hemisphere was reported in the State of São Paulo, Brazil, in March 2004 (TEIXEIRA et al., 2005a), and a year later, in August 2005, HLB was confirmed in South Florida, USA (BOVÉ, 2006). Now, HLB is present in all of the Florida citrus-growing counties, and the production of oranges for processing fell from 140 million boxes in 2005-2006 to less than 70 million boxes in 2016-2017 (UF/IFAS Extension, 2013; USDA NASS, 2016). Texas and Arizona also have HLB which is affecting their production as well (USDA/FAS, 2017).

In addition to causing the deterioration and death of citrus trees, HLB has a negative impact on fruit quality and results in off-flavored orange juice. Since 90% of the oranges grown in Florida are juiced, the quality of the final product is vital for competition in the global market. Juice produced from HLB-affected fruit has been characterized as having negative attributes including sour, bitter, salty, metallic, astringent, tingling, with bitter, astringent and burning aftertaste (PLOTTO et al., 2010; 2017), purportedly resulting from changes in the chemical composition of the disease-affected fruit (BASSANEZI et al., 2009; BALDWIN et al, 2010; DAGULO et al., 2010; RAITHORE et al., 2015; KIEFL et al., 2017).

In stress situations as caused by diseases such as HLB, plants respond by accelerated synthesis of certain secondary metabolites. Several authors observed higher bitter limonoids, sometimes higher acids, lower sugar contents, and generally higher flavonoids in HLB-affected fruit compared to healthy fruit (BALDWIN et al., 2010; DAGULO et al., 2010; PLOTTO et al., 2010; MASSENTI et al., 2016). It has been shown that the negative taste effect is caused by interactions of these various chemical classes rather than one single component. The roles of reduced sugar and increased limonoids in bitter taste

are well understood (HOROWITZ & GENTILI, 1963; DREWNOWSKI & GOMEZ-CARNEROS, 2000; DEA et al., 2013; BATENBURG et al., 2016). Dea and co-authors (2013) showed how adding sucrose to orange juice spiked with limonin and nomilin resulted in decreased bitterness induced by these compounds. Similarly, spiking limonin at 10 $mg.L^{-1}$ to Valencia juice with high soluble solids content had little effect on bitterness contrary to spiking the same amount of limonin to a low sugar Hamlin juice (KIELF et al., 2017). Adding to the complexity is the synergism found between limonin and nomilin which decreased the detection thresholds when combined together in orange juice (DEA et al., 2013).

Until recently, focus on orange juice bitterness has been nearly exclusively on limonoids, so it is not well documented whether other secondary metabolites such as flavanone neohesperidosides (naringin, neohesperidin, poncirin and neoeriocitrin) or polymethoxylated flavones - PMF (nobiletin and tangeretin), also play any role or not. Batenburg et al. (2016) showed that tangeretin and nobiletin were the main bitter components in citrus peel-derived PMF preparations, with tangeritin 2-3 times more bitter than nobiletin. However, in juice, these compounds occur at concentrations far below their detection thresholds, making it unlikely that they directly contribute to orange juice bitterness (KIEFL et al., 2017; PLOTTO et al., 2017). Kiefl et al. (2017) attempted to demonstrate bitterness in orange juice by spiking with either limonin, PMFs, poncirin and hesperidin, alone or in combination. They were able to show an increase in bitterness from the mixture of all flavonoids plus limonin, but it was not clear which specific flavonoids would contribute to any of these complex taste descriptors.

Multiple words describe taste and flavor of orange juice from HLB-affected fruit and suggest that changes in taste due to HLB are results of complex alterations in chemical composition. Therefore, the objectives of this study were to quantify the differences between healthy and HLB-affected orange juice, and to identify sensory-active fractions in HLB-affected orange juice extracts possessing bitter, astringent or harsh properties. This work will contribute to potential identification of compounds responsible for the bitterness (other than limonin, nomilin, tangeretin, and nobiletin) typical of HLB

symptomatic fruit and juice to potentially detect the presence of compounds that mitigate off-flavors in HLB-affected orange juice.

2. MATERIAL AND METHODS

2.1 Juice samples

2.1.1 Sample preparation

'Valencia' oranges (*Citrus sinensis* (L.) Osbeck) were harvested from a commercial grove in March 2013, from multiple healthy and HLB-affected trees. The fruit were then separated into healthy controls and HLB-symptomatic fruit (small green and lopsided, testing positive for *C*Las by qPCR), and juice was extracted using a JBT Food Tech extractor system and pasteurized (BALDWIN et al., 2012b) resulting in healthy control juice (COJ) and HLB juice (HLBOJ).

2.1.2 DNA extraction and qPCR detection of CLas from juice

DNA was extracted from 500 µL of orange juice using a modified CTAB method (ZHAO et al., 2015; BALDWIN et al., 2017). Briefly, DNA quality (260/280 and 260/230 ratio) and quantity were assessed by spectrophotometry (Nano Drop, Thermo Scientific, Waltham, MA). *C*Las detection was accomplished by qPCR. Specific primers targeting *C*Las 16S rRNA gene (Li primers) (LI et al., 2006) or *C*Las hyv1 (LJ primers) (MORGAN et al., 2012) were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA). The qPCR parameters were as follows: 95 °C for 10 minutes, followed by 40 cycles at 95 °C for 15 seconds, and 60 °C for 1 minute, with fluorescence signal capture at each stage of 60 °C. For SYBR® Green Real-Time PCR (with LJ primers), the default Melt Curve (disassociation) stage is continued after the 40 cycles of PCR.

2.2 Chemical analysis of COJ and HLBOJ

2.2.1 Titratable acidity and soluble solids

For quality determination, solid soluble content (SSC) and titratable acidity (TA) were determined prior to individual sugar and acid analyses. SSC

was determined by refractive index measured with a digital ATAGO PR-101 refractometer (Atago Co, Tokyo, Japan) and TA was calculated by titration of 10 mL of juice with 0.1 mol.L^{-1} NaOH to a pH 8.1 endpoint using an autotitrator (Metler Toledo DL50, Columbus, OH, USA) (BALDWIN et al., 2017).

2.2.2 Total sugar, sucrose, glucose and fructose

Individual sugars were analyzed with a high performance liquid chromatography (HPLC) system using a Perkin-Elmer Series 200 autosampler and pump (Perkin-Elmer, Waltham, MA). Sample preparation was according to Baldwin et al. (2012a), where juice samples were extracted with 80% (v/v) aqueous ethanol, boiled for 15 min, cooled, and run through several levels of filtration. The separation column was a Sugar-Pak™ I (10 µm, 6.5 x 300 mm) (Waters, Milford, MA) operated at 90 °C in a CH-30 column heater and a TC-50 controller (FIAtron, Milwaukee, WI). Run conditions were an isocratic system of 0.001 mol.L^{-1} calcium ethylenediaminetetraacetic acid mobile phase at a flow rate of 0.3 mL.min^{-1}. Detection of peaks was done with an Agilent 1100 series refractive index detector (Agilent Technologies, Santa Clara, CA). Quantification was based on the external standard method (EZChrom Elite software, Version 3.3.2. SP2, Santa Clara, CA) using standards for sucrose, glucose and fructose. All results are expressed as g.100 mL^{-1} of juice (BALDWIN et al., 2012a).

2.2.3 Citric acid, malic acid and ascorbic acid analyses

Organic acids were analyzed by HPLC using the same extracts that were prepared for sugar analysis. Chromatographic separation was accomplished with an AltechOA1000 Prevail organic acid column (9 µm, 6.5 x 300 mm) (Grave Davison Discovery Sciences, Deerfield, IL). Samples were introduced to the HPLC system by injecting 60 µL at a flow rate of 0.2 mL.min^{-1} at 35 °C and a mobile phase of 0.005 mol.L^{-1} H_2SO_4. The analytes of interest (citric and malic acids) were detected with a Spectra System UV 6000 LP photo diode array detector at 215 and 245 nm (for ascorbic acid) (Thermo Fisher Scientific, Waltham, MA). Quantification was based on the calibration curves for citric and malic acids, expressed as g.100 mL^{-1} of juice (BALDWIN et al., 2017).

2.2.4 Secondary metabolites analyses

Concentrations of limonoids and flavonoids in orange juice were determined by HPLC-mass spectrometry (MS) according to Baldwin et al. (2017). Juice samples were extracted with methanol using a succession of shaking, heating, cooling and centrifugation (BALDWIN et al., 2017). The supernatants were combined and concentrated using a rotary evaporator model RE111 (Buchi, Switzerland). The concentrated extract was then passed through a 0.45 µm PTFE filter for HPLC-MS analysis. A Waters 2695 Alliance HPLC (Waters, Medford, MA) connected in parallel with a Waters 996 Photodiode Array (PDA) detector and a Waters/Micromass ZQ single quadrupole mass spectrometer equipped with an electrospray ionization source was used. Compound separations were achieved with a Waters Atlantis dC18 column (5 µm, 2.1 x 100 mm). Solvent gradient, HPLC and MS parameters were as reported previously (BALDWIN et al., 2017). MassLynx software ver.4.1 (Micromass, Division of Waters Corp., Beverly, MA) was used for data handling. Quantification was based on calibration curves of authentic standards of each flavonoid and limonoid compound, expressed as g.100 mL^{-1} of juice.

2.3 Fractionation of phenolic compounds from orange juice

2.3.1 Preparation of phenolic compound extracts of COJ and HLBOJ

Five liters of COJ and HLBOJ were centrifuged at 27000 x *g* for 30 min at 5 °C. The supernatant was added to 600 g of Sepabead resin SP-207 (Supelco, Bellefonte, PA, USA) and the mixture was shaken 1 h at 170 rpm. The liquid was filtered and the resin was stirred with 2 L deionized water at 140 rpm for 20 min, then the water decanted. The procedure was repeated with 1 L deionized water to remove unbound sugars. Compounds adsorbed to the resin were removed by washing and shaking the resin three times with 2 L ethanol at 50 °C for 60 min on a shaker at 140 rpm, twice more with 1.5 L ethanol for 30 min, and finally with 1.5 L acetone/water (8:2 v/v) for 30 min. The organic solvents used to extract the phenolic compounds were evaporated using a rotary evaporator at 35-40 °C. The final volume was adjusted to 45 mL using

water/ethanol (1:1 v/v). The concentrated extract was lyophilized for 24 hours, and the dried samples (~2 g) stored in a desiccator.

2.3.2 Fractionation of phenolic compound extracts by Fast Centrifugal Partition Chromatography (FCPC)

Semi-preparative scale fractionation of the 'Valencia' juice compounds was achieved using a fast centrifugal partition chromatography (FCPC) unit (Kromaton, Angers, France) equipped with an A1000 rotor. The adjoining HPLC system consisted of a Waters Delta 600 pump and controller (Waters, Milford, MA), a sample injector (Rheodyne, Cotati, CA) with a 100 mL loop, and a Waters 996 photodiode array detector. Fractionation was achieved using a 2 L biphasic solvent system comprised of ethyl acetate/acetonitrile/0.5% aqueous formic acid (1/1/2, v/v/v). The FCPC was operated in ascending mode where stationary and mobile phases were the organic and aqueous phases, respectively. The stationary phase was first introduced into the system with rotors remaining stationary. Mobile phase was then pumped through the stationary phase with the rotor spinning at 950 rpm at the flow rate of 4 $mL.min^{-1}$. Samples were prepared by dissolving the dried phenolic fraction (~ 2 g) in equal volumes (50 mL) of the organic and aqueous phases prior to sample injection. Fractions were then collected at 1 min intervals, and every fifth fraction was analyzed by HPLC to help determine which fractions should be combined. Peak elution was monitored at 330 nm. A total of five fractions, A-E, were obtained.

2.3.3 Chemical characterization of phenolic compounds by HPLC– MS

The FCPC fractions were analyzed by the same HPLC-MS system used for limonoids and flavonoids. Compound separations were achieved with a Waters XBridge C8 column (5 µm, 4.6 × 150 mm). Elution conditions included gradients of aqueous 0.5% formic acid/acetonitrile, initially 90/10 (v/v), and changed with linear gradients to 80/20, 75/25, 60/40, 30/70, 30/70, 90/10 and 90/10 (v/v) at 10, 15, 23, 40, 45, 53, and 60 min, respectively. The same chemical characterization was used for subsequent sub-fractions (BALDWIN et al., 2017).

2.3.4 Sub-fractionation of fractions A and B by HPLC

The first two FCPC fractions, **A** and **B**, were further fractionated using semipreparative HPLC, Varian ProStar, model 210 pumps and a Prostar 335 photodiode array detector at 330 nm. Separations were achieved with an Atlantis^{TM} dC18 OBD^{TM} column (5 µm, 19 x 100 mm, Waters, USA), using linear gradients of 0.5 % v/v formic acid (solvent 1) and acetonitrile (solvent 2) as shown in Table 1 for fractions **A** (Gradient 1): healthy (COJ) and HLB (HLBOJ) and **B**: HLB (HLBOJ). For better separation, the gradient was slightly modified for analysis of fraction **B**: healthy (COJ) (Gradient 2, Table 1). The eluted compounds were collected in 1 min intervals and analyzed by analytical HPLC-MS, as described above. Column fractions with similar chemical compositions were combined. After complete solvent removal, each dried sample was stored in a vacuum desiccator until sensory analysis.

Table 1. Mobile phases gradients (1 and 2) used to separate fractions A and B: from healthy and huanglongbing (HLB)-affected Valencia orange juice into sub fractions.

Time of analysis	**Gradient 1***		**Gradient 2****	
(min)	**% Solvent 1**	**% Solvent 2**	**% Solvent 1**	**% Solvent 2**
00:00	90	10	90	10
05:00	85	15	90	10
10:00	75	25	-	-
28:00	50	50	75	25
35:00	-	-	70	30
40:00	30	70	60	40
45:00	-	-	30	70
48:00	30	70	-	-
50:00	-	-	30	70
55:00	90	10	90	10
65:00	90	10	90	10

*Gradient 1 was used to separate fraction A: healthy and HLB and fraction B: HLB into sub-fractions; **gradient 2 was used to separate fraction B: healthy into sub-fractions. Solvent 1: 0.5% v/v formic acid; solvent 2: acetonitrile. Flow rate was set at 5 mL.min^{-1}

2.4 Sensory evaluation

2.4.1 Comparative sensory analysis of COJ and HLBOJ

Twelve panelists were specifically trained (12 one-hour sessions) for orange juice descriptive analysis, with a core of seven panelists having evaluated orange juice samples for over 5 years. Eighteen descriptors and reference standards were developed including seven descriptors for aromatics, five for taste, three for mouth feel and three for aftertaste (Table 2).

Only the 'HLB flavor' descriptor was rated according to each panelist's perception, based on their experience of tasting juice affected with HLB. Descriptors were rated using a 16-point intensity scale where 0 = none, 1 = low, 7-8 = medium and 15 = high, and data were recorded using Compusense® *five*. Samples were prepared as in Plotto et al. (2017) and were served in duplicate. All taste panels took place in isolated booths equipped with computers, and under positive air pressure and red lighting.

2.4.2 Descriptions of flavor attributes of fractions obtained from COJ and HLBOJ

Based on HPLC-MS analyses, five FCPC fractions (A-E) were obtained from COJ and HLBOJ. Drinking water, 4 mL, was added to each fraction and homogenized in a vortex (Genie 2, Model No G560, Scientific Industries, USA). The insoluble fractions were heated to 40 ± 2 °C in a hot water bath and then completely homogenized using a vortex. This represented a stock solution, which was tasted by two or three experienced panelists. Serial dilutions (1:1) were performed with these fractions and subsequent sub-fractions, until the panelists could not perceive any taste. Each team member tasted an average volume of 0.5 mL of each dilution. Panelists cleansed palates between samples with drinking water and consumed salted cracker as needed to dispel bitter aftertaste. Individual observations were recorded followed by discussion of taste impressions. The same procedure was used to taste sub-fractions from A and B fractions. Sensory descriptors that are repeated at least two times among the three panelists are retained.

Table 2. Descriptors and reference standards with suggested intensity for orange juice sensory descriptive panel, using a 16-point intensity scale*

Sensory modality	Descriptor (suggested intensity)	Reference standard
Aromatics (Flavor)	Orange (7)	Orange juice, 100 % Florida, Gourmet Pasteurized (Natalie's Orchid Island Juice Company, Fort Pierce, FL).
	Grapefruit (15)	Grapefruit juice, 100 % Florida, Gourmet Pasteurized (Natalie's Orchid Island Juice Company Fort Pierce, FL).
	Fruity-non-citrus (12)	A mixture of passion fruit (Welch's, Westfield, NY), mango (Frito-Lay, Inc., Dallas, TX) and pineapple (Dole Food Company Inc., Westlake Village, CA) juices and guava (Sunshine bottling Company, Doral, FL) and peach (Santiago Felippelli Conway, Miami, FL) nectars and water
	Orange peel (7)	Zests from Hamlin oranges (washed and sanitized before zesting) cut in ~50 mm^2 pieces (1.4 ± 0.3 g),
	Green (10)	A mixture of (*Z*)-3-hexenal (2 $\mu g.mL^{-1}$, Sigma-Aldrich) and (*Z*)-3-hexenol (7 $\mu g.mL^{-1}$, Sigma-Aldrich) in solution at 0.09 % ethanol
	Stale (10)	0.005 %v/v in water of N&A Old Flavor Type, Stale (Givaudan Flavors Corp., Cincinnati, OH)
	HLB flavor	Any off-flavor related to HLB disease
Taste	Sweet (7)	8 % sucrose (pure sugar, Publix, Lakeland, FL) in water
	Sour (7)	0.2 % citric acid (≥ 99.5%, Sigma-Aldrich) in water
	Umami (7)	0.08 % Monosodium glutamate (Ac'cent®, B&G Foods Inc., Parsippany, NJ)
	Bitter (7)	11.5 $\mu g.mL^{-1}$ of quinine monohydrochloride dihydrate (90 % Sigma-Aldrich) in water
	Metallic (10)	Canned orange juice (Ruby Kist®, 100 % Orange juice from concentrate (Clement Pappas & Co., Inc., Seabrook, NJ)
Mouthfeel	Tingly (15)	Carbonated water, ClubSoda (Publix, Lakeland, FL)
	Astringent (15)	Premium English Breakfast Black tea (Publix, Lakeland, FL)
	Burn (7)	Zests from Hamlin oranges cut in ~50 mm^2 pieces (1.4 ±0.3 g), washed and sanitized before zesting.
Aftertaste	Bitter (7)	11.5 $\mu g.mL^{-1}$ of quinine monohydrochloride dihydrate (90 % Sigma-Aldrich) in water
	Astringent (15)	Premium English Breakfast black tea (Publix, Lakeland, FL)
	Burning (7)	Zests from Hamlin oranges cut in ~50 mm^2 pieces (1.4 ±0.3 g), washed and sanitized before zesting.

*16-point intensity scale (1 = low, 7-8 = medium and 15 = high).

To determine the impacts of FCPC fractions **A**-**E** on orange juice flavor, they were added to healthy orange juice at 120 $mg.L^{-1}$, an amount estimated to be slightly higher than normally present in orange juice, and evaluated by the 12-member trained panel. Samples were presented as pairs comprising of the reference and a coded sample. The coded samples were the spiked samples and included an unspiked control. Panelists rated sweetness, sourness, bitterness, astringency and aftertaste of the coded sample on a -50 to +50 linear

scale in relation to the reference (negative being less, positive being more, and “zero” as no difference from unspiked control).

2.5 Statistical analyses

All analyses were performed with two (sensory), three (limonoids and flavonoids) or four (sugars and acids) replications. Statistical analyses were performed as one-way ANOVA and comparison of means were undertaken by F, Student *t* ($P \leq 0.01$) and Tukey tests ($P \leq 0.05$, 0.01 and 0.001) (Minitab® 16.2.3 Statistical Software).

3. RESULTS

The sensory evaluation of both Valencia juices, COJ and HLBOJ, showed significant differences in all analyzed attributes (Figure 1). The positive descriptors, “orange flavor”, “fruity-non-citrus” and “sweetness” were rated higher for COJ compared to HLBOJ, while other descriptors representing negative attributes such as “grapefruit flavor”, “orange peel”, “green”, “stale”, “typical HLB flavor”, “sourness”, “bitterness”, “metallic”, “tingling”, “astringent” and “burning”, with aftertastes of bitterness, astringency and burning, were most frequently applied to HLBOJ samples.

The two juice types, COJ and HLBOJ were also different in that HLBOJ had statistically higher TA and reduced pH, SSC and SSC/TA. (Figure 2). Analysis of individual sugars revealed lower sucrose but higher glucose and fructose levels in HLBOJ compared to COJ, with total sugars being significantly lower. HLBOJ exhibited higher citric acid and ascorbic acid (not statistically significant) and lower malic acid levels compared to COJ.

Levels of all secondary metabolites analyzed were higher in HLBOJ than in COJ, except sinensetin (Figure 3), which was around 75% higher in COJ. Nomilin, limonin, tangeretin, nobiletin and diosmin presented the greatest difference in levels between HLBOJ and COJ corresponding to 2051, 780, 417, 231 and 229%, respectively, higher in the first.

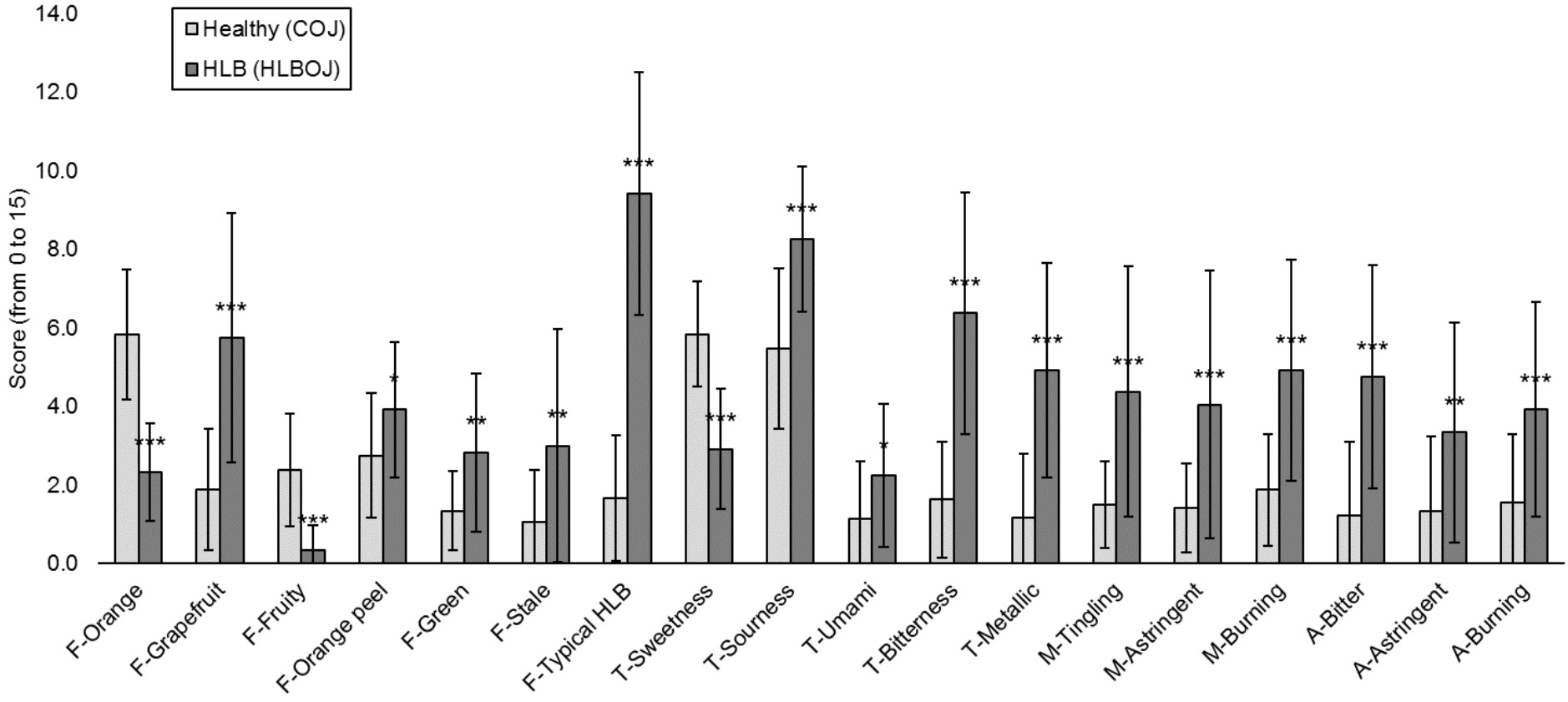

Figure 1 Sensory scores (average ± standard deviation for 12 trained panelists) for Valencia orange juice from healthy (COJ) or HLB-affected (HLBOJ) fruit. Descriptors preceded with the letters "F-", "T-", "M-" and "A-" indicate "flavor", "taste", "mouthfeel" and "aftertaste", respectively. *, ** and *** above each pair of bars indicate significant difference between COJ and HLBOJ by ANOVA ($P \leq 0.01$) and Tukey test at $P \leq 0.05$, 0.01 and 0.001, respectively.

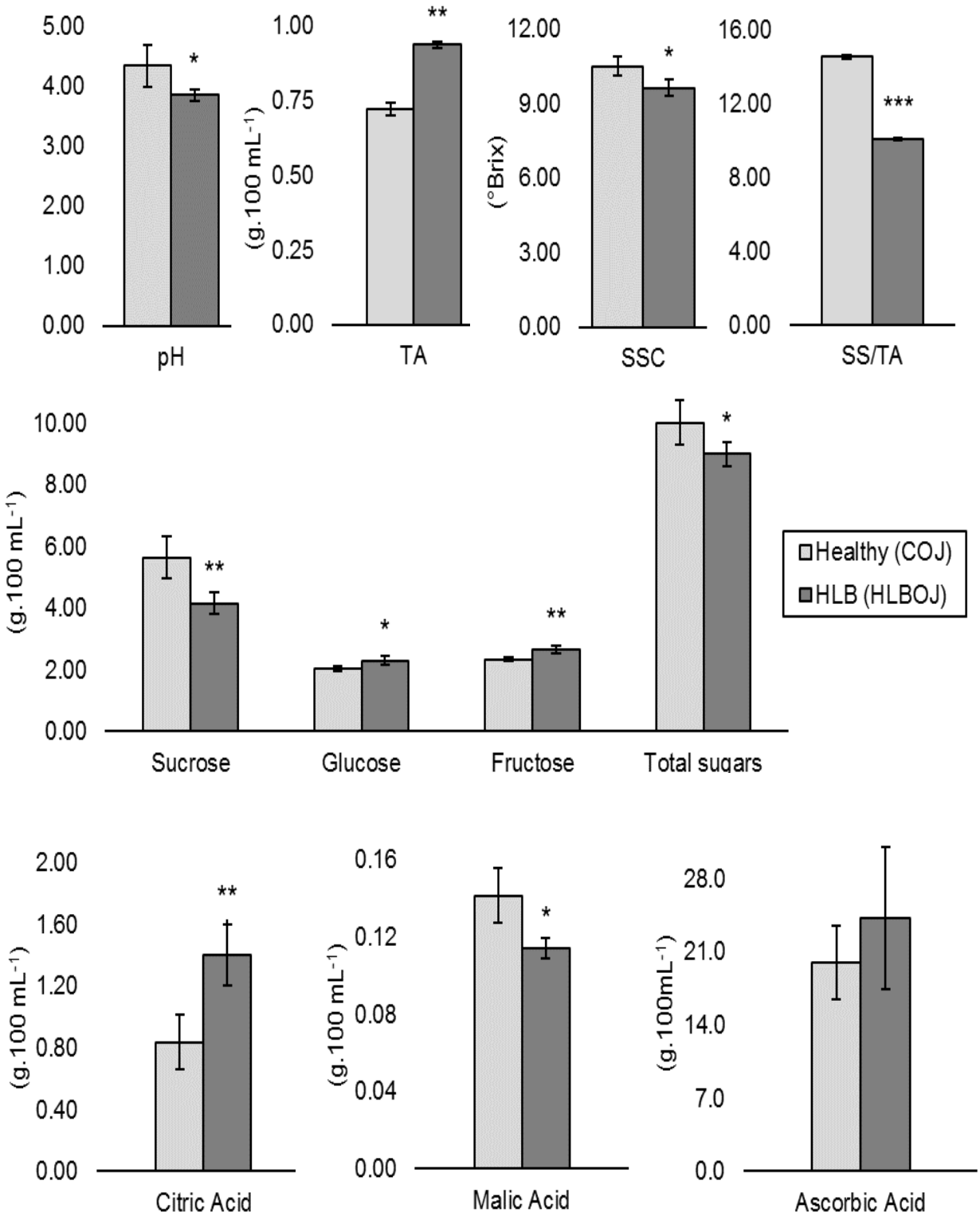

Figure 2. Quality attributes (average ± standard deviation for four replicates) in Valencia orange juice from healthy (COJ) or HLB-affected (HLBOJ) fruit. TA = titratable acidity expressed in citric acid equivalent; SSC = soluble solids content. Bars followed by *, ** and *** are significantly different by ANOVA ($P \leq 0.01$) and Tukey test at $P \leq 0.05$, 0.01 and 0.001, respectively.

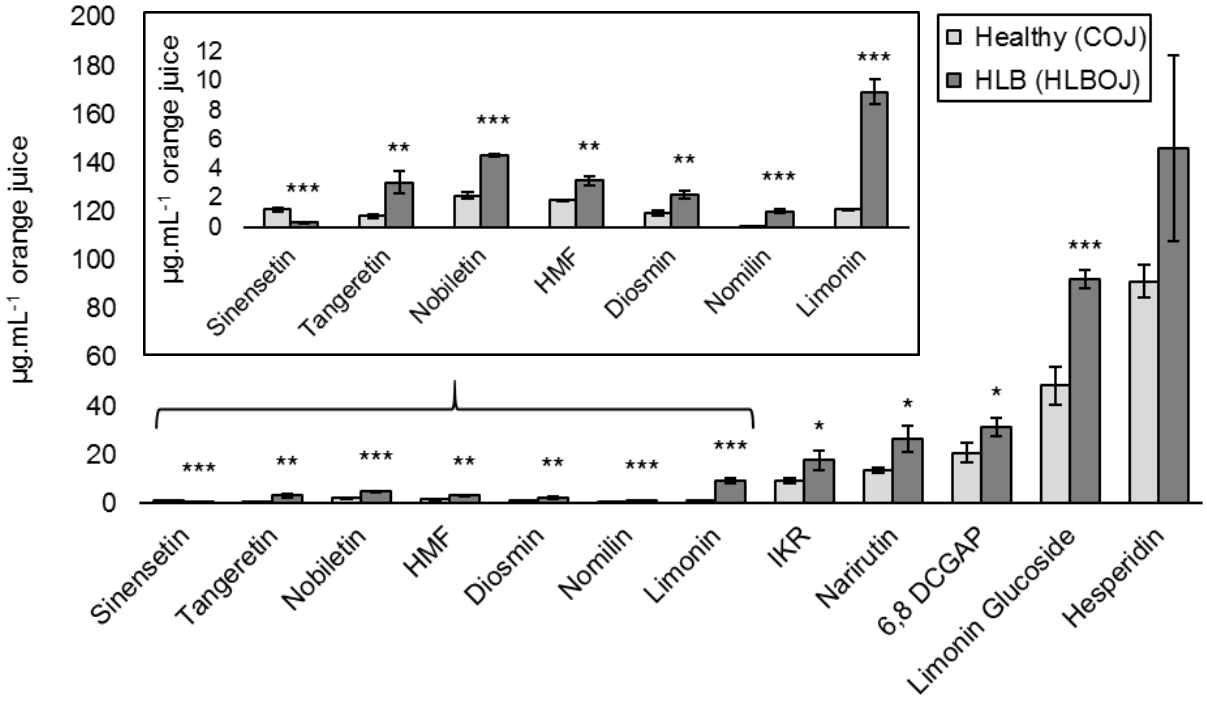

Figure 3. Secondary metabolites (average ± standard deviation for three replicates) in Valencia orange juice from healthy (COJ) or HLB-affected (HLBOJ) fruit. Bars followed by *, ** and *** are significantly different by ANOVA ($P \leq 0.01$) and Tukey test at $P \leq 0.05$, 0.01 and 0.001, respectively. Compounds present at concentrations lower than 10 µg.mL^{-1} are presented in the insert. HMF: heptamethoxyflavone; IKR: isosakuranetin-7-O-rutinoside or didymin; 6,8 DCGAP: 6,8-di-*C*-glucosyl apigenin.

Initial fractionation of the nonvolatile compounds in COJ and HLBOJ by FCPC produced five distinct fractions (**A**-**E**). Tasting by two trained sensory panelists revealed that the first two fractions (**A** and **B**) were the most bitter and astringent with HLB off-flavor (data not shown). This sensory analysis was performed by diluting with water the HLB fractions **A** and **B** to initial concentrations of 836 mg.L^{-1} and 496 mg.L^{-1}, respectively. Samples were repeatedly tasted after successive 1:1 dilutions. Fractions **A** and **B** had detectable residual taste after 12 and 14 dilutions, respectively, representing final concentrations of 0.40 mg.L^{-1} and 0.06 mg.L^{-1}. Fractions **A**-**B** were subsequently added to healthy orange juice at 120 mg.L^{-1}. The spiked orange juice was then presented to a trained panel who evaluated taste activities of **A** and **B** in comparison with an unspiked control juice. The panelists detected a 16% increase in bitterness for the orange juice spiked with **A** and a 10% increase in astringency for the orange juice spiked with **B** (data not shown). The major chemical components of **A** included PMFs (sinensetin, tangeretin, heptamethoxyflavone and nobiletin), two bitter limonoid aglycones (limonin and

nomilin) and numerous hydroxycinnamates. Fraction **B** contained no PMFs but it did contain didymin as a minor component, and additional hydroxycinnamic acids different from those in **A**.

Fractions **A** and **B** were further chromatographed by preparative HPLC to better separate and selectively detect compounds or groups of compounds that were responsible for the bitter, astringent and harsh tastes, typical of HLB juice. Each resulting sub-fraction was sensorially evaluated in serial dilution tests as done previously with fractions **A**-**E**, until the taste characteristics were imperceptible. The lowest concentration tested for each sub-fraction of **A** is presented in Figure 4 and Table 3 for COJ and HLBOJ, with the lower level of the last dilution tasted representing a stronger flavor. Results of analyses of the sub-fractions of **B** are listed in Table 4 and are discussed later.

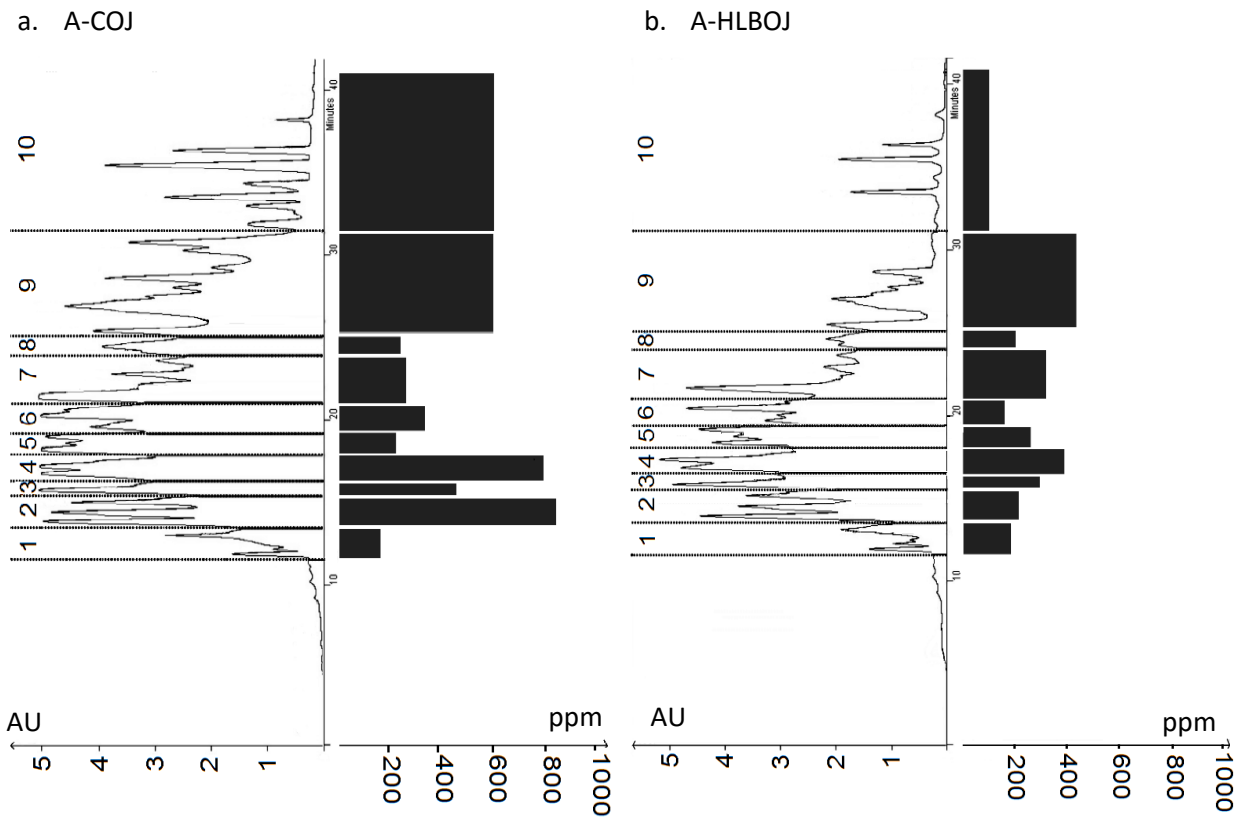

Figure 4. HPLC chromatograms of healthy (COJ) (a) or huanglongbing (HLB)-affected (HLBOJ) (b) Valencia orange juice from fraction A showing 10 sub-fractions with their respective lowest concentration at which taste was perceived.

Table 3. Sensory descriptors of each sub-fraction (SF) obtained from A-COJ and A-HLBOJ from healthy and huanglongbing (HLB)-affected Valencia orange juice, respectively.

SF	Tentative identification of major compounds	Fraction A-COJ Sensory descriptors*	SS ($\mu g.mL^{-1}$)	FD ($\mu g.mL^{-1}$)	Fraction A-HLBOJ Sensory descriptors	SS ($\mu g.mL^{-1}$)	FD ($\mu g.mL^{-1}$)
1	[Ferulic acid + glucaric acid] [p-Coumaric acid + glucaric acid] [Sinapinic acid + glucose] [Sinapinic acid + ferulic acid]	Caramel/honey, coffee	1275	159	Astringent, coffee, herbal, smoky	1455	182
2	[p-Coumaric acid + glucaric acid] [Ferulic acid + glucaric acid] [Sinapinic acid + ferulic acid]	Sour, acidic	3315	829	Intensely sour, intensely bitter, astringent, harsh, herbal	3390	212
3	[Ferulic acid + galactaric acid] [Sinapinic acid + ferulic acid] [Sinapinic acid + glucuronic acid] [Dimer of ferulic acid + citric acid] [Ferulic acid + sinapinic acid + glucose] [Ferulic acid + glucose]	Sour, astringent, caramel, herbal, smoky	3568	446	Intensely bitter, astringent, herbal, smoky	2370	296
4	[Ferulic acid + glucaric acid] [Sinapinic acid + glucaric acid] [Dimer of ferulic acid + glucaric acid]	Intensely astringent, sour, herbal, floral	6253	782	Intensely bitter, sour, astringent, herbal	6193	387
5	Ferulic acid [Dimer of ferulic acid + glucaric acid]	Intensely bitter, astringent, sour, herbal, burn, harsh	3493	218	Bitter, astringent, sour, herbal	2070	259
6	[Sinapinic acid + ferulic acid]	Bitter, astringent, burning, metallic, herbal, floral (sweet), harsh	5266	329	Intensely bitter, astringent, burn, pungent, harsh, herbal	5075	159
7	Unknown (high mwt HCAs) [p-Coumaric acid + glucaric acid] [Ferulic acid + glucaric acid]	bitter, astringent, herbal	2099	262	Intensely bitter, sour, astringent, herbal	2548	319
8	[Sinapinic acid + ferulic acid] [Ferulic acid + glucaric acid] [Dimer of ferulic acid + glucaric acid]	Astringent, herbal, yerba mate**	1910	239	Astringent, herbal	1608	201
9	[Dimer of ferulic acid + glucaric acid] [Sinapinic acid + unknown]	Intensely bitter, astringent	4719	590	Intensely bitter, intensely harsh, pungent, herbal	3553	444
10	HCA; SIN; QHME; NOB; TMS; HMF; TAN; limonin; nomilin	Bitter, astringent, vegetable	4701	588	Intensely bitter, astringent, intense vegetable	3200	100

SS: stock solution; FD: final dilution; *: Sensory descriptors that are repeated at least two times among the 3 panelists are presented here; **: yerba mate is a bitter, astringent South American tea (Ilex paraguariensis St. Hilaire) that contains caffeine, phenolic compounds and saponins. HCA: hydroxycinnamate acids; SIN: sinensetin; QHME: quercetagetin hexamethyl ether; NOB: nobiletin; TMS: tetramethoxyflavone; HMF: heptamethoxyflavone; TAN: tangeretin.

Sub-fractions from A-HLBOJ had generally stronger taste activity than those from A-COJ as indicated by the lower concentrations at which taste was perceived (Figure 4). Concentrations of the final dilutions (FD) tasted in sub-fractions 2, 3, 4, 6, 9 and 10 of A-COJ were 74, 34, 51, 52, 25 and 83% higher than the respective A-HLBOJ sub-fractions, indicating higher taste thresholds (Table 3). Concentrations of the final dilutions (FD) tasted in A-HLBOJ sub-fractions 1, 5, 7 and 8 were in the same range or slightly higher (13-18%) than A-COJ sub-fractions. In fact, descriptors of sub-fractions 7 and 8 are very similar to each other.

Overall, A-HLBOJ sub-fractions received more negative descriptors compared to A-COJ sub-fractions. Of the five A-COJ sub-fractions classified as bitter, two were described as intensely bitter, whereas out of the eight sub-fractions from A-HLBOJ classified as bitter in taste, seven were described as intensely bitter. Furthermore, in sub-fraction 3, 6, 9, and 10, initial concentrations tasted in stock solution (SS) were slightly lower in A-HLBOJ than in A-COJ and still presented intensely bitter descriptors. Eight sub-fractions from A-COJ and nine sub-fractions from A-HLBOJ were described as having an astringent mouthfeel and three sub-fractions from both samples were classified as harsh, especially sub-fraction 9 from A-HLBOJ, which was described as intensely harsh in spite of having lower initial concentration.

Sub-fractions 1, 3, 4 and 6 from A-COJ were characterized by positive descriptors, such as caramel, honey and floral tastes, whereas none of the sub-fractions from A-HLBOJ were described as such. Conversely, sub-fractions 6 and 9 from A-HLBOJ were described as pungent, whereas none of the sub-fractions from A-COJ were. All sub-fractions from A-HLBOJ were described as having herbal or vegetable-like tastes, with sub-fraction 10 described as having an intense vegetable-like taste. Among sub-fractions from A-COJ, sub-fractions 1, 2 and 9 did not receive vegetable-like or herbal taste descriptors.

Sub-fractions for B-COJ and B-HLBOJ 1-10 are not quite comparable to those of Fraction A, as the gradient had to be slightly modified to obtain better separation of B-COJ. Nevertheless there is plenty of overlap between the B-COJ and B-HLBOJ fractions (Table 4).

Table 4. Sensory descriptors and concentration of each sub-fraction obtained from B-COJ and B-HLBOJ from healthy and huanglongbing (HLB)-affected Valencia orange juice, respectively

SF	**Fraction B-COJ** Sensory descriptors*	SS	FD	SF	**Fraction B-HLBOJ** Sensory descriptors	SS	FD
		($\mu g.mL^{-1}$)				($\mu g.mL^{-1}$)	
1	Honey, astringent	1413	177	**1'**	Sour, astringent, herbal	2150	269
2	Astringent, umami, pungent, burning	3120	390	**2'**	Bitter, astringent, acidic, tingly, pungent, irritating	2008	251
3	bitter, astringent, harsh, herbal	4752	594	**3'**	Intensely bitter, astringent, sour, tingly	5693	712
4	Sour, astringent	3070	384	**4'**	Intensely bitter; astringent, citric, yerba mate**	3184	816
5	bitter, astringent, pungent	2874	359	**5'**	Intensely bitter, astringent	2338	292
6	bitter, astringent, herbal	2752.5	344	**6'**	Intensely bitter, astringent, herbal	2535	634
7	bitter, sour, astringent, pungent, herbal	6981	428	**7'**	Intensely bitter, astringent, sour, herbal, irritating	5338	667
8	bitter, astringent	1348	337	**8'**	Intensely bitter, astringent, irritating, coffee	1590	398
9	Intensely bitter, astringent, harsh, herbal	6849	438	**9'**	Intensely bitter, astringent, irritating, coffee	5570	348
10	Citrus, fruity, astringent	3803	238	**10'**	Intensely bitter, astringent, yerba mate, vegetable, fruity	3183	398

Sub-fractions for Fraction B-COJ and B-HLBOJ do not exactly correspond in their retention times because a slightly different fractionation gradient was used to optimize separation in B-HLBOJ. *: The sensory descriptors are result of the final discussion between the panelists; SS: stock solution; FD: final dilution; **: yerba mate is a bitter, astringent South American tea (*Ilex paraguariensis* St. Hilaire) that contains caffeine, phenolic compounds and saponins.

All B-COJ and B-HLBOJ sub-fractions were described as astringent. There were no PMFs nor limonoids in B-COJ and B-HLBOJ sub-fractions; however, nine sub-fractions of B-HLBOJ were described as bitter, with eight being described as intensely bitter, and six B-COJ sub-fractions as bitter, with sub-fraction 9 described as intensely bitter. Among B-HLBOJ sub-fractions, sub-fractions 8' and 9' had coffee taste, sub-fractions 4' and 10' had yerba mate taste, while none of the sub-fraction from B-COJ had these descriptors. Both sub-fractions 10' from B-COJ and B-HLBOJ received the positive descriptor “fruity”, while only sub-fraction 1' from B-COJ received the positive descriptor “honey”.

Initial analyses of sub-fractions from A-COJ and A-HLBOJ by UV and mass spectrometry suggest most compounds in these fractions are hydroxycinnamates with various levels of polymerization and glycosylation (Table 3). Only sub-fraction 10 was composed of known compounds sinensetin, nobiletin, tangeritin, limonin and nomilin. The elution times of the majority of hydroxycinnamates in FCPC fraction **A**, and hence in the sub-fractions of A-COJ and A-HLBOJ, were typically later (15-25 min) compared to the elution times of the hydroxycinnamates in FCPC fraction **B** (4-11 min) (data not shown). This implies that the hydroxycinnamates in the **A** sub-fractions (Table 3) are more lipophilic than in the **B** sub-fractions. Consistent with this are the frequent observations of 14 amu neutral losses from the molecular ions, suggesting the presence of hydroxycinnamate methyl esters, generally absent in **B** sub-fractions. Additionally, MS spectra of a number of the later-eluting HCAs in FCPC **A** show neutral losses of ferulic and sinapinic acid subunits of the HCAs, suggesting the occurrence of diferulic acid (neutral losses of 194 amu and 193 *m/z* fragment ions) and disinapinic acid (neutral mass losses of 224 amu and 223 *m/z* fragment ions) chemical species which are also further conjugated to aldaric acids (evidenced by 209 and 191 *m/z* fragment ions) among these compounds. Such compounds have been previously described in sweet orange (RISCH et al., 1987; RISCH et al., 1988; HIJAZ et al., 2013;).

4. DISCUSSION

Differences between COJ and HLBOJ in both taste and compositional analysis confirmed previous studies (BALDWIN et al., 2010; DAGULO et al., 2010; PLOTTO et al. 2010; MASSENTI et al, 2016; KIELF et al, 2017), in which higher levels of many secondary metabolites in juice made from oranges symptomatic for HLB were observed. Fruit that are symptomatic of HLB tend to be smaller, and therefore more peel components enter in the juice stream, explaining the higher level of secondary metabolites. In general, the decrease in sweetness in HLBOJ can be explained by lower sucrose, total sugars and soluble solids content, together with higher citric acid. In another study, researchers correlated the sensory descriptors sourness, umami and tingling with some volatiles compounds, as well as tangeretin and nobiletin (PLOTTO et

al., 2017). Both flavones were present at higher levels in HLBOJ compared to COJ in this study. The higher score in the sensory evaluation pertaining to sourness can be partially explained by the higher titratable acidity and higher citric acid content found in HLBOJ. However, sourness was also detected in several sub-fractions of both A-COJ and A-HLBOJ extracts (Tables 3 and 4), which suggest that some other compounds besides organic acids could be responsible for the perception of sourness in the orange juice fractions. But also, organic acids could have remained in the fractions and not be detected by the HPLC system used in this study.

The increased bitterness score pertaining to HLBOJ can be partially correlated with higher limonin, nomilin, nobiletin and tangeretin levels. The limonin level in HLBOJ at 9.26 ± 0.87 $\mu g.mL^{-1}$, is above its reported recognition threshold in orange juice (4.7 $\mu g.mL^{-1}$, DEA et al., 2013), while nomilin level at 1.08 ± 0.14 $\mu g.mL^{-1}$, is below its reported recognition threshold (2.6 $\mu g.mL^{-1}$, DEA et al., 2013). In contrast, limonin and nomilin concentrations in COJ were 1.19 ± 0.03 $\mu g.mL^{-1}$ and 0.05 ± 0.01 $\mu g.mL^{-1}$, respectively (Figure 3), both concentrations are below the detection thresholds (DEA et al., 2013). Higher levels of limonin and nomilin in orange juice made with HLB symptomatic oranges in comparison with juice made with non-symptomatic oranges have been reported (BALDWIN et al., 2010; DAGULO et al., 2010).

HLBOJ contained higher levels of tangeretin and nobiletin than COJ, however, the average levels were 3.06 ± 0.73 and 4.90 ± 0.08 $\mu g.mL^{-1}$, respectively, which were below thresholds for bitterness reported by Batenburg et al. (2016). That particular study shows that the threshold levels of these PMFs are above 20 $\mu g.mL^{-1}$, and they are more abundant in citrus peel. Hesperidin levels in HLBOJ were higher compared to COJ, which may have contributed to the higher bitter score in the sensory evaluation as suggested by Kiefl et al. (2017). However, in the fractionation of both samples, detectable levels of these compounds were only in A-COJ and A-HLBOJ sub-fractions 10, meanwhile eight other A-HLBOJ and five other A-COJ sub-fractions were described as bitter, while lacking these compounds. In B-COJ and B-HLBOJ sub-fractions there were no limonoids or PMFs, however eight B-HLBOJ sub-fractions and five B-COJ sub-fractions were described as intensely bitter. This result suggests that there are other compounds, other than limonin, nomilin,

tangeretin, nobiletin and hesperidin, involved with the bitter perception in HLBOJ. These bitter-inducing compounds may likely be due to the readily detectable hydroxycinnamates, or to other yet undetected unknowns.

5. CONCLUSION

The difference between HLBOJ and COJ was verified and partially explained by chemical analysis, but the fractionation of HLBOJ and COJ showed that bitterness and sourness are imparted by other compounds besides the bitter limonoids (nomilin and limonin), bitter flavonoids (tangeretin, nobiletin and hesperidin) and sour organic acids. The sensory descriptions of the HLBOJ and COJ fractions implicate hydroxycinnamic acids or other compounds which could not be identified by UV and Mass Spectrometry detectors. More studies are warranted to determine the role of these compounds obtained from HLB-affected orange juice, to clarify their taste activity, chemical identity and contribution to HLB-induced off-taste.

CONCLUSÕES INTEGRADAS

A colheita de laranjas Valência em diferentes épocas durante uma mesma safra influenciou nas características físico-químicas, nos teores de compostos voláteis e metabólitos secundários do fruto. Sendo as laranjas Valência colhidas no meio da estação as preferidas para o processamento por apresentarem níveis ideais de SS, AT e *ratio*, além de reduzido conteúdo de compostos que contribuem para o sabor amargo. Os resultados desse estudo demonstram que a época de colheita é uma variável importante ao se estudar os efeitos provocados pelo HLB no suco de laranja.

O suco de laranjas sintomáticas para o HLB apresenta menores teores de açúcares totais, SS e ratio; maior acidez, elevado conteúdo de flavonoides, limonoides e significativas diferenças sensoriais, dentre elas, os acentuados sabores amargo e azedo. Foi demonstrado que outros compostos, além de limonina, nomilina, tangeritina, nobiletina e hesperidina, podem contribuir para o sabor amargo, e outros, que não os ácidos orgânicos, estão envolvidos com o sabor azedo em suco de laranjas acometidas pelo HLB. As análises químicas das frações de compostos não voláteis extraídos do suco de laranja, descritas como intensamente amargas e azedas, sugerem o envolvimento de ácidos hidroxicinâmicos ou outros compostos ainda não identificados. Além de agrupar sistematicamente os estudos sobre os efeitos do HLB nas características físico-químicas e sensorias de suco de laranja, o inédito levantamento bibliográfico permitiu identificar o predomínio de pesquisas envolvendo laranjas Valência e Hamilin em oposição às outras cultivares. Apesar do destaque na produção das duas cultivares no Brasil e nos Estados Unidos, o estudo das demais contribuirá para a busca de novos híbridos ou cultivares de laranja resistentes ao HLB.

REFERÊNCIAS BIBLIOGRÁFICAS

ABDULLAH, T.L.; SHOKROLLAH, H.; SIJAM, K.; ABDULLAH, S.N.A. Control of Huanglongbing (HLB) disease with reference to its occurrence in Malaysia. *African Journal of Biotechnology*, v. 8, n. 17, p. 4007-4015, 2009.

ACKERLEY, J.; CORREDIG, M.; WICKER, L. Clarification of citrus juice is influenced by specific activity of thermolabile pectinmethylesterase and inactive PME-pectin complexes. *Journal of Food Science*, v. 67, p. 2529-2533, 2002

ADAMI, A.C.O. Risco e retorno de investimento em citros no Brasil. Piracicaba: Escola Superior de Agricultura “Luiz de Queiroz” da Universidade de São Paulo. 150 p., 2010. (Tese de doutorado em Economia Aplicada).

ADAMS, R.P. Identification of essential oil components by gas chromatography/mass spectrometry. 4ª ed. *Allured Publishing*, Carol Stream, IL. p. 804, 2007.

ALBRECHT, U.; McCOLLUM, G.; BOWMAN, K.D. Influence of rootstock variety on Huanglongbing disease development in field-grown sweet orange (*Citrus sinensis* [L.] Osbeck) trees. *Scientia Horticulturae*, v. 138, p. 210-220, 2012.

ALBRECHT, U.; FIEHN, O.; BOWMAN, K.D. Metabolic variations in different citrus rootstock cultivars associated with different responses to huanglongbing. *Plant Physiology and Biochemistry*, v. 107, p. 33-44, 2016.

AYERS, J.; TOMES, M. The effect of two uniform ripening genes on chlorophyll and carotenoid contents of tomato fruit. *Proceedings of the Amerian Society of Horticultural Science*, v. 88, p. 550, 1966.

BAI, J.; BALDWIN, E.A.; PLOTTO, A.; MANTHEY, J.A.; MCCOLLUM, T.G.; IREY, M.; LUZIO; G. Influence of harvest time on quality of ‘Valencia’ oranges and juice. *Proceedings of the Amerian Society of Horticultural Science*, v. 122, p. 308-315, 2009.

BAI, J.; BALDWIN, E.A.; PLOTTO, A.; CAMERON, R.; FORD, B.L.; LUZIO, G.; MANTHEY, J.; NARCISO, J.; DEA, S. A comparison of processed and fresh squeezed ‘Hamlin’ orange juice flavor quality. *Proceedings of the Amerian Society of Horticultural Science*, v. 123, p. 199-206, 2010.

BAI, J.; BALDWIN, E.A.; PLOTTO, A. Stability of headspace volatiles in a Fallglo tangerine juice–NaCl matrix system at room temperature. *Proceedings of the Amerian Society of Horticultural Science*, v. 124, p. 207-212, 2011.

BAI, J.; BALDWIN, E.; McCOLLUM, G.; MANTHEY, J.; PLOTTO, A.; PAULA, B.M.D.; GLORIA, M.B.A.; WINDMER, W.; LUZIO, G.; CAMERON, R.; NARCISO, J. Influence of harvest time on quality of 'Valencia" oranges and juice, second season. *Proceedings of the Amerian Society of Horticultural Science*, v. 126, p. 232-238, 2013.

BALDWIN, E.A.; NISPEROS-CARRIEDO, M.O.; BAKER, R.; SCOTT, J.W. Quantitative analysis of flavor parameters in six Florida tomato cultivars (*Lycopersicon esculentum* Mill). *Journal of Agriculture and Food Chemistry,* v. 39, p. 1135-1140, 1991.

BALDWIN, E.A. Citrus fruit, p. 107–149. In: SEYMOUR, G.B.; TAYLOR, J.E.; TUCKER, G.A. (eds.). Biochemistry of fruit ripening. *Chapman and Hall*, New York. 1993.

BALDWIN, E.; PLOTTO, A.; MANTHEY, J.; MCCOLLUM, G.; BAI, J.; IREY, M.; CAMERON, R.; LUZIO, G. Effect of Liberibacter infection (Huanglongbing disease) of citrus on orange fruit physiology and fruit/fruit juice quality: chemical and physical analyses. *Journal of Agriculture and Food Chemistry,* v. 58, p. 1247-1262, 2010.

BALDWIN, E.; BAI, J.; PLOTTO, A.; CAMERON, R.; LUZIO, G.; NARCISO, J.; MANTHEY, J.; WIDMER, W.; FORD, B.L. Effect of extraction method on quality of orange juice: Hand-squeezed, commercial-fresh squeezed and processed. *Journal of the Science of Food and Agriculture*v, v. 92, p. 2029-2042, 2012a.

BALDWIN, E.; BAI, J.; PLOTTO, A.; MANTHEY, J.; NARCISO, J.; DEA, S.; IREY, M. Effect of nutritional spray regimes on orange juice flavor quality and juice Liberibacter (*C*Las) DNA detection. *Proceedings of the Florida State Horticultural Society*, v. 125, p. 239-242, 2012b.

BALDWIN, E.; BAI, J.; PLOTTO, A.; MANTHEY, J.; RAITHORE, S.; DETERRE, S.; ZHAO, W.; do NASCIMENTO NUNES, C.; STANSLY, P.A., TANSEY, J.A. Effect of vector control and foliar nutrition on quality of orange juice

affected by Huanglongbing (HLB): Chemical analysis. *HortScience*. *In press*, 2017.

BASSANEZI, R.B.; MONTESINO, L.H.; STUCHI, E.S. Effects of huanglongbing on fruit quality of sweet orange cultivars in Brazil. *European Journal of Plant Pathology*, v. 125, n. 4, p. 565-572, 2009.

BASSANEZI, R.B.; MONTESINO, L.H.; GASPAROTO, M.C.G.; BERGAMIN FILHO, A.; AMORIM, L. Yield loss caused by huanglongbing in diferente sweet orange cultivars in São Paulo, Brazil. *European Journal of Plant Pathology,* v. 130, p. 577-586, 2011.

BATENBURG, A.M.; JOODE, T.de; GOUKA, R.J. Characterization and modulation of the bitterness of polymethoxyflavones using sensory and receptor-based methods. *Journal of Agricultural and Food Chemistry*, v. 64, p. 2616-2626., 2016.

BATTOL, A.; IFTIKHAR, Y.; MUGHAL, S.M.; KHAN, M.M.; JASKANI, M.J.; ABBAS, M.; KHAN, L.A. Citrus greening disease – a major cause of citrus decline in the world – a review. *Horticultural Science* (Prague), v. 34, n. 4, p. 159-166, 2007.

BENAVENTE-GARCÍA, O.; CASTILLO, J. Uptade on uses and properties of citrus flavonoids: new findings in anticâncer, cardiovascular, and anti-inflammatory activity. Jounal of Agricultural and Food Chemistry, v. 56, p. 6185-6205, 2008.

BOVÉ, J.M. Huanglongbing: a destructive, newly-emerging, century-old disease of citrus*. Journal of Plant Pathology*, v. 88(1), p. 7-37, 2006.

BRUEMMER, J.H. Terminal oxidase activity during ripening of Hamlin orange. *Phytochemistry,* v. 28, p. 2901-2902, 1989.

CABI. Citrus huanglongbing (greening) disease (citrus greening). In: Invasive Species Compedium. Wallingford, UK: CAB International. Disponível em: <http://www.cabi.org/isc/datasheet/16567>. Acesso em 06.22.2017.

CAMERON, R.G.; BAKER, R.A.; GROHMANN, K. Multiple forms of pectinmethylesterase from citrus peel and their effects on juice cloud stability. *Journal of Food Science*, v. 63, p. 253-256, 1998.

CAPOOR, S.P. Decline of citrus trees in India*. Bulletin of the National Institute of Science, India*, v. 24, p. 48-64, 1963.

CAPUTO, M.M. Avaliação de doze cultivares de laranja doce de maturação precoce na região sudoeste do Estado de São Paulo. Escola Superior de Agricultura “Luiz de Queiroz” da USP,84 p. 2012 (Tese de doutorado em Ciências, área de concentração: Fitotecnia).

CATLING, H.D. The bionomics of the South African citrus psylla, *Trioza erytreae* (Del Guercio) (Homoptera: Psyllidae). II. The Influence of parasites and notes on the species involved. *Journal of Entomology Society South Africa*, v. 32, p. 191-208, 1969.

CDA (Coordenadoria de Defesa Agropecuária). Portaria CDA – 21, de 15/12/2011. Disponível em: < http://www.defesa.agricultura.sp.gov.br/ legislacoes/portaria-cda-21-de-15-12-2011,940.html>. Acesso em: 01 de nov. 2012.

CETESB (Companhia de Tecnologia e Saneamento Ambiental). Cítricos, 45 p., 2005. Disponível em: <http://www.cetesb.sp.gov.br>. Acesso em: 31 nov. 2012.

CEVALLOS-CEVALLOS, J.M.; GARCIA-TORRES, R.; ETXEBERRIA, E.; REYES-DE-CORCUERA, J.I. GC-MS analysis of headspace and liquid extracts for metabolomic differenctiation of citrus huanglongbing and zinc deficiency in leaves of ‘Valencia’ sweet orange from commercial groves. *Phytochemical. Analysis*, v. 22, p. 236-246, 2011.

CHEN, C. Models for seasonal changes in °Brix and ratio of citrus fruit juice. *Proceedings of the Florida State Horticultural Society*, v. 103, p. 251-255, 1990.

CHEN, H.; MCCOLLUM, G.; BALDWIN, E.; BAI, J. Impacts of Huanglongbing symptom severity on fruit detachment force and mechanical properties of sweet oranges (*Citrus sinensis*). *HortScience*, v. 51, n. 4, p. 356-361, 2016.

CHERAGHIAN, A. A guide for diagnosis & detection of quarantine pests – African citrus psyllid *Trioza etrytreae* (Del Guercio) Hemiptera: Triozidae. *Bureau of Plant Pest Surveillance and Pest Risk Analysis*, p. 13, 2013.

CHITARRA, M.I.F.; CHITARRA, A.B. Pós-colheita de frutas e hortaliças: fisiologia e manuseio. 2ª ed. Lavras, Editora: *UFLA*, 785 p., 2005.

CHIN, E.L.; MISHCHUK, D.O.; BREKSA, A.P.; SLUPSKY, C.M. Metabolite signature of *Candidatus* Liberibacter asiaticus infection in two citrus

varieties. *Journal of Agricultural and Food Chemistry*, v. 62, p. 6585-6591, 2014.

CITRUSBR, Consumo de suco de laranja. 2016a, Disponível em: <http://www.citrusbr.com/mercadoexterno/?me=08>. Acesso em 16 mai. 2017.

CITRUSBR, Exportações totais de suco de laranja em toneladas de FCOJ – MUNDO. 2016b. Disponível em: <http://www.citrusbr.com/mercadoexterno/ ? me=01>. Acesso em: 17 mai. 2017.

CITRUSBR, CitrusBR divulga dados de fechamento da safra 2017/2018 – Notícia, 2017. Disponível em: <http://www.citrusbr.com/destaques/?id=312425>. Acesso em 19 mai. 2017.

CITRUSBR, Laranja e suco, produtos e subprodutos. Data de publicação não infomada. Disponível em: <http://www.citrusbr.com/laranjaesuco/?ins=19>. Acesso em: 16 mai. 2017.

COELHO, A.F.S.; GOMES, E.P.; SOUSA, A.deP.; GLÓRIA, M.B.A. Effect of irrigation level on yield and bioactive amine content of American lettuce. *Journal of the Science of Food and Agriculture*, v. 85, p. 1026-1032, 2005.

COLLETA-FILHO, H.D.; TARGON, M.L.P.N.; TAKITA, M.A.; DE NEGRI, J.D.; POMPEU, J.J.; MACHADO, M.A. First report of the causal agent of huanglongbing ("*Candidatus* Liberibacter asiaticus") in Brazil. *Plant Disease*, v. 88, p. 1382, 2004.

COLETTA-FILHO, H.D.; CARLOS, E.F.; LOTTO, L.L.; LUCIANE, F.C.; ALVES, K.C.S; PEREIRA, M.A.R.; MACHADO, M.A. Prevalence of *Candidatus* Liberibacter spp. in HLB-diseased citrus plants in São Paulo State, Brazil. In Conference *Proceedings of the Seventeenth International Organization of Citrus Virologists*, Oct. 2007, p. 110-115, 2007.

CONAB (Companhia Nacional de Abastecimento). Acompanhamento da safra brasileira – laranja, safra 2011/2012. Terceiro levantamento – SP, estimativa da safra no triângulo mineiro – MG. 12 p., 2011.

CONAB (Companhia Nacional de Abastecimento). Conjutura mensal da produção de laranjas – período 01 a 28/02/2017, *MAPA*, 7 p., 2017. Disponível em

COSTA, C.C. Cenários futuros da citricultura brasileira determinados pelo HLB (Greening) – Boletim de pesquisa e desenvolvimento. *EMBRAPA*, 34, 23 p., 2011.

GRAÇA, J.V.da. Citrus Greening Disease. *Annual Reviews of Phytopathol*ogy, v. 29, p. 109-136, 1991.

GRAÇA, J.V.da. Biology, history and world status of huanglongbing. *Hermosillo, Sonora. México*, p. 1-7, 2008.

DAGULO, L.; DANYLUK, M.D.; SPANN, T.M.; VALIM, M.F. GOODRICH-SCHNEIDER, R.; SIMS, C.; ROUSEFF, R. Chemical characterization of orange juice from trees infected with citrus greening (Huanglongbing). *Journal of Food Science,* v. *75, n. 2*, p. 199-207, 2010.

DALA PAULA, B.M.; RAITHORE, S; MANTHEY, J.A.; PLOTTO, A.; BAI, J.; GLÓRIA, M.B.A.; BALDWIN, E. A deeper look into the causes of off-flavor in orange juice affected by Huanglongbing (HLB). Journal Citrus Pathology, v. 4, n. 1, p.8, 2017a. (Apresentação de Trabalho/Conferência ou palestra). Disponível em: <http://escholarship.org/uc/item/2cr0f2kc>.

DALA PAULA, B.M.; RAITHORE, S.; MANTHEY, J.A.; BALDWIN, E.; BAI. J.; GLÓRIA, M.B.A.; PLOTTO, A. Identification of active taste compounds in juice made fom oranges symptomatic of Huanglongbing – greening disease. *LWT – Food Science and Technology*, in press, 2017b.

DEA, S.; PLOTTO, A..; MANTHEY, J.A.; RAITHORE, S.; IREY, M.; BALDWIN, E. Interactions and thresholds of limonin and nomilin in bitterness perception in orange juice and other matrices. *Journal of Sensory Studies*, v. 28, p. 311-323, 2013.

DREWNOWSKI, A.; GOMES, C.C. Bitter taste, phytonutrients, and the consumer: a review. *The American Journal of Clinical Nutrition,* v. 72, p. 1424-1435, 2000.

DUQUE, A.L.R.F.; MONTEIRO, M.; ADORNO, M.A.T.; SAKAMOTO, I.K.; SIVIERI, K. An exploratory study on the influence of orange juice on gut microbiota using a dynamic colonic model. *Food Research International,* v. 84, p. 160-169, 2016.

ECHEVERRIA, E.; VALICH, J. Carbohydrate and enzyme distribution in protoplasts from valencia orange juice sacs. *Phytochemistry*, v. 27, p. 73–76, 1988.

EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária). Guia de identificação do Huanglongbing (HLB, ex-greening) dos citros. *EMBRAPA*, 34 p., 2011.

EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária). Variabilidade genética e melhoramento dos citros. Trabalho apresentado no Simpósio de recursos genéticos e melhoramento de plantas para o Nordeste do Brasil, realizado pela EMBRAPA Semi-Árido, p.1–23, 1998.

EMBRAPA. BASTOS, D.C.; FERREIRA, E.A.; PASSOS, O.S.; DE SÁ, J.F.; ATAÍDE, E.M.; GALGARO, M. Cultivares copa e porta-enxertos para a citricultura brasileira, Informe agropecuário, v. 35, n. 281, p. 36-45, 2014.

EMBRAPA. Mandioca e Fruticultura, p. 1-5, 2012. Disponível em: <http://www.cnpmf.embrapa.br/index.php?p=pesquisa-culturas_pesquisadas-citros.php&menu=2#producao>. Acesso em: 22 de out. 2012.

EPPO, 2017. PQR database. Paris, France: European and Mediterranean Plant Protection Organization. http://www.eppo.org.

FIGUEIRA, R.; NOGUEIRA, A.M.P.; VENTURINI FILHO, W.G.; DUCATTI, C.; QUEIROZ, E.C.; PEREIRA, A.G.S. Análise físico-química e legalidade em bebidas de laranjas*. Alimentação e Nutrição*, v. 21, n. 2, p. 267-272, 2010.

FLORIDA DEPARTMENT OF AGRICULTURE AND CONSUMER SERVICES. Florida citrus statistics 2014-2015, 115 p., 2016.

FLORIDA DEPARTMENT OF CITRUS – Economic and Market Research Department. Citrus reference book, 94 p, 2016. Disponível em: < https://fdocgrower.app.box.com/shared/dt42f2c7kk>. Acesso em: 17 de mai. 2017.

FRANKE, S.I.R.; PRA, D.; ERDTMANN, B.; HENRIQUES, J.A.P.; DA SILVA, J. Influence of orange juice over the genotoxicity induced alkylating agents: an in vivo analysis. *Mutagenesis*, v. 20, p. 279-283, 2005.

FRASER, L.R.; SINGH, D.; CAPOOR, S.P.; NARIANI, T.K.. Greening vírus, the likely cause of citrus dieback in India. *FAO Plant Protection Bulletin,* v. 14, p. 127-130, 1966.

FRASER, L.R.; SINGH, D. Citrus dieback in India – the contribution of greening vírus. *Procedings of 4th Conference IOCV, Riverside*, p. 141-144, 1968.

FUNDECITRUS – FUNDO DE DEFESA DA CITRICULTURA. Inventário de árvores do cinturão citrícola de São Paulo e Triângulo/Sudoeste Mineiro: retrato dos pomares em março de 2017, Araraquara, SP, 95 p., 2017

GALAVERNA, G.; DI SILVESTRO, G.; CASSANO, A.; SFORZA, S.; DOSSENA, A.; DRIOLI, E.; MARCHELLI, R. A new integrated membrane process for the production of concentrated blood orange juice. Effect on bioactive compounds and antioxidant activity. *Food Chemistry*, v. 106, p. 1021-1030, 2008.

GAMA, J.J.T.; SYLOS, C.M. Effect of thermal pasteurization and concentration on carotenoid composition of Brazilian Valencia orange juice. *Food Chemistry*, v. 100, p. 1686-1690, 2007.

GASPAROTO, M.C.G.; COLETTA-FILHO, H.D.; BASSANEZI, R.B.; LOPES, S.A.; LOURENÇO, S.A.; AMORIM. S.A. Influence of temperature on infection and stablishment of '*Candidatus* Liberibacter americanus' and '*Candidatus* Liberibacter asiaticus' in citrus plants. *Plant Pathology,* v. 61, p. 658-664, 2012.

GATTUSO, G.; BARRECA, D.; GARGIULLI, C.; LEUZZI, U. CARISTI, C. Flavonoid composition of citrus juices. *Molecules,* v.12, p. 1641–1673, 2007.

GIL-IZQUIERDO, A.; GIL, M.I.; FERRERES, F. Effect of processing techniques at industrial scale on orange juice antioxidant and beneficial health compounds, *Journal of Agriculture and Food Chemistry,* v. 50, n. 18, p. 5107-5114, 2002.

GLÓRIA, M.B.A. Bioactive amines. In: HUI, H.; NOLLET, L.L. Handbook of food. Science, Technology and Engineering. *Marcel Dekker*, v. 4, p. 1-38, 2006.

GOTTWALD, T.R.; GRAHAM, J.H.; IREY, M.S.; McCOLLUM, T.G.; WOOD, B.W. Inconsequential effect of nutritional treatments on huanglongbing control, fruit quality, bacterial titer and disease progress. *Crop Protection*, v. 36, p. 73-82, 2012.

GUTHRIE, N.; HASEGAWA, S.; MANNERS, G.; VANDENBERG, T. Inhibition of human breast cancer cells by citrus limonoids, p. 164–174. In: BERHOW, M.; HASEGAWA, S.; MANNERS, G. (eds.). Citrus limonoids: Functional chemicals in agriculture and foods. *ACS Symposium Series*, 758 p., 2000.

HALBERT, S.E.; MANJUNATH, K.L. Asia citrus psyllids (Sternorrhycha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. *Florida Entomologist*, v. 87, p. 330-353.

HALBERT, S.E. The discovery of huanglongbing in Florida. *Proc. 2nd Int. Citrus Canker & Huanglongbing Res*. Workshop, p. 50, 2005.

HARDING, P.; WINSTON, J.; FISHER, D. Seasonal changes in Florida oranges. v. 753, *USDA Technical Bulletim*, p. 1–89, 1940.

HASEGAWA, S.P.V. Reduction of nomilin bitterness in citrus juices and juice serum with Arthrobacter globiformis cells immobilized in acrylamide gel. *Journal of Agriculture and Food Chemistry***,** v. *31*, p. 178-180, 1983.

HIJAZ F.M.; MANTHEYM J.A.; FOLIMONOVA, S.Y.; DAVIS, C.L.; JONES, S.E.; REYES-DE-CORCUERA, J.I. Na HPLC-MS Characterization of the changes in sweet orange leaf metabolite profile following infection by the bacterial pathogen *Candidatus* Liberibacter asiaticus. *Plos One*, v. 8, n. 11, p. 1-11, 2013.

HOROWITZ, R.M.; GENTILI, B. Flavonoids of citrus – VI The structure of neohesperidose. Tetrahedron, v. 19, p. 773-782, 1963.

HUFNAGEL, J.C.; HOFMANN, T. Orosensory-directed identification of astringent mouthfeel and bitter-tasting compounds in red wine. *Journal of Agriculture and Food Chemistry*, v. 56, p. 1376-1386, 2008.

HUTTON, R.J.; LANDSBERG, J.J. Temperature sums experienced before harvest partially determine the post-maturation juicing quality of oranges grown in the Murrumbidgee Irrigation Areas (MIA) of New South Wales. *Journal of the Science of. Food and Agriculture,* v. 80, p. 275-283, 2000.

IBGE. Indicadores IBGE - Estatística da produção agrícola, 77 p., 2017. Disponível em: <http://www.ibge.gov.br/home/estatistica/indicadores/agropecuaria/lspa/>. Acesso em: 15 de mai. 2017.

IBGE. Indicadores IBGE – Estatística da produção agrícola. 80 p., 2013.

IBGE/SIDRA, Sistema do IBGE de Registro Agrícola. Produção agrícola municipal PAM-2015. Disponível em: <https://sidra.ibge.gov.br/tabela/1618>. Acesso em: 11 de mai. de 2017.

IBGE/SIDRA, Sistema do IBGE de Registro Agrícola. Produção agrícola municipal PAM-2016-2017, 2017. Disponível em: < https://sidra.ibge.gov.br/tabela/1618# resultado>. Acesso em: 11 de jun. 2017.

INIAV (INSTITUTO NACIONAL DE INVESTIGAÇÃO AGRÁRIA E VETERINÁRIA), *Candidatus* Liberibacter sp. e o citrus greening. Boletim Técnico. 2p. *SAFSV–BT*.02/2015.

JBT. Linha de processamento de citros. 8 p, 2015. Disponível em: < http://www.jbtfoodtech.com/~/media/JBT%20FoodTech/Images/Modules/Citrus%20Processing/PDF/409_PT%20Citrus-Processing%20Line_LR.ashx>. Acesso em: 25 de jul. 2017.

JOHNSON, J.R.; BRADDOCK, R.J.; CHEN, C.S. Flavor losses in orange juice during ultrafiltration and subsequent evaporation. *Journal of Food Science,* v. 61, p. 540-543, 1996.

KIEFL, J.; KOHLENBERG, B.; HARTMANN, A.; OBST, K.; PAETZ, S.; KRAMMER, G.E.; TRAUTZSCH, S. Investigation on key molecules of Huanglongbing (HLB)-induced orange juice off-flavor. *Journal of Agricultural and Food Chemistry*, Publicated date (web) March 2017 (DOI: 10.1021/acs.jafc.7b00892).

KONDJOYAN, N.; BERDAGUÉ, J.L. A compilation of relative retention indices for the analysis of aromatic compounds. INRA de THEIX, Saint Genes Champanelle, France. p. 234, 1996.

KROON, P.A.; WILLIAMSON, G. Hydroxycinnamates in plants and food: current and future perspectives. *Journal of the Science of Food and Agriculture,* v. 79, p. 355-361, 1999.

LEE, H; AEDIN, C. A review of the health care potential of bioactive compounds. *Journal of the Science of Food and Agriculture,* v. 86, p. 1805-1813, 2006.

LI, W.; HARTUNG, J.S.; LEVY, L. Quantitative real-time PCR for detection and identification of Candidatus Liberibacter species associated with citrus huanglongbing. *Journal of Microbiological Methods*, v. 66, p. 104-115, 2006.

LIAO, H.K.; BURNS, J. Gene expression in *Citrus sinensis* fruit tissues harvested from huanglongbing-infected trees: comparison with girdled fruit. *Journal of Experimental Botany*, v. 63, n. 8, p. 3307–3319, 2012.

LIMA, A.C., Insetos do Brasil, Homopteros. Serie didática 4 Escola Nacional de Agronomia, v. 3, 327 p., 1942

LLAUGER, R.; LUÍS, M.; COLLAZO, C.; PEÑA, L.; GONZÁLEZ, C.; BATISTA, L.; TEIXEIRA, D.C.; MARTINS, E.; PERDOMO, A.; CASÍN, J.C.; PERÉZ, J.L.; CUETO, J.R.; BOVÉ, J.M. Current situation of citrus huanglongbing in Cuba. *International Research Conference on Huanglongbing* - Orlando, Florida, p. 97-100, 2008.

MACHADO, T.V. Avaliação sensorial e físico-química do suco de laranja proveniente das etapas do processamento do suco concentrado e congelado. Araraquara: Faculdade de Ciências Farmacêuticas da Universidade Estadual Paulista “Júlio de Mesquita Filho”. 117 p., 2010. (Dissertação de mestrado em Alimentos e Nutrição).

MAIER, V.; HASEGAWA, S.; BENNETT, R.; ECHOLS, L. Limonin and limonoids: Chemistry, biochemistry, and juice bitterness, p. 3–24. In: NAGY, S.; ATTAWAY, J.A. (eds.). Citrus nutrition and quality. *American Chemistry Society*, Washington, DC, 1980.

MALIK, N.S.A.; PEREZ, J.L.; KUNTA, M. Changes in free amino acid levels in sour Orange leaves in response to cold stress and during recovery from cold stress. *Journal of Food, Agriculture & Environment*, v. 11, n. 1, p. 1086-1088, 2013.

MANACH, C.; SCALBERT, A.; MORAND, C.; RÉMÉSY, C.; JIMÉNEZ, L. Polyphenols: food sources and bioavailability. The American Journal of Clinical Nutrition, v. 79, p. 727-747, 2004.

MANNERS, G.D.; BREKSA, A.P.; SCHOCH, T.K.; HIDALGO, M.B. Analysis of bitter limonoids in citrus juices by atmospheric pressure chemical ionization and electrospray ionization liquid chromatography–mass spectrometry. *Journal of Agriculture and Food Chemistry,* v. 51, p. 3709-3714, 2003.

MANTHEY, J.A.; GROHMANN, K. Phenols in citrus peel by products. Concentrations of hydroxycinnamates and polymethoxylated flavones in citrus peel molasses. *Journal of Agriculture and Food Chemistry*, v. 49, p. 3268-3273, 2001.

MANTHEY, J.A.; Differences in secondary metabolites in leaves from orange (*Citrus sinensis* L.) trees affected with greening disease (huanglongbing disease) (HLB). *Proceedings* of the *Florida State Horticultural Society*, v. 121, p. 285-288, 2008.

MAPA (Ministério da Agricultura e Abastecimento). Instrução normativa nº 01 de 07 de janeiro de 2000. Aprova o regulamento técnico geral para fixação dos padrões de identidade e qualidade para polpa e suco de frutas. Disponível em: <http://www.ivegetal.com.br/p4-doc.htm>. Acesso em: 07 de set. 2012.

MAPA (Ministério da Agricultura, Pecuária e Abastecimento). Instrução Normativa nº 10, de 18 de março de 2005. Disponível em: <http://www.agricultura.gov.br/vegetal/registrosautorizacoes/registro/registro-nacional-cultivares/legislacao-rnc>. Acesso em: 01 de nov. 2012.

MAPA (Ministério da Agricultura, Pecuária e Abastecimento). Instrução Normativa nº 32, de 29 de setembro de 2006. Disponível em: <http://www.agricultura.gov.br/vegetal/registrosautorizacoes/registro/registro-nacional-cultivares/legislacao-rnc>. Acesso em: 01 de nov. 2012.

MAPA (Ministério da Agricultura, Pecuária e Abastecimento). Instrução Normativa nº 53, de 16 de outubro de 2008. Disponível em: <http://www.agricultura.gov.br/vegetal/registrosautorizacoes/registro/registro-nacional-cultivares/legislacao-rnc>. Acesso em: 01 de nov. 2012.

MARKESTRAT. Consumo de sucos em geral. 2016. Disponível em: <http://www.citrusbr.com/download/3-Consumo%20de%20Sucos%20em%20Geral.pdf>. Acesso em: 17 de jun. 2017.

MASSENTI, R.; LO BIANCO, R.; SANDHU, A.K.; GU, L.; SIMS, C. Huanglongbing modifies quality components and flavonoid content of 'Valencia' oranges. *Journal of the Science of Food and Agriculture*, v. 96, p. 73-78, 2016.

MARTINI, X.; PELZ-STELINSKI, K.S.; STELINSKI, L. Factors affecting the overwintering abundance of the Asian citrus psyllid (Hemiptera: Liviidae) in Florida citrus (Sapindales: Rutaceae) orchards. *Florida Entomological Society*, v. 99, n. 2, p. 178-186, 2016

MASTELLO, R.B.; CAPOBIANGO, M.; CHIN, S.T.; MONTEIRO, M.; MARRIOTT, P.J. Identification of odour-active compounds of pasteureized Orange juice using multidimensional gas chromatography techniques. *Food Research International*, v. 75, p. 281-288, 2015.

MASUOKA, Y.; PUSTIKA, A.; SUBANDIYAH, S.; OKADA, A.; HANUNDIN, E.; PURWANTO, B.; OKUDA, M.; OKADA, Y.; SAITO, A.; HOLFORD, P.; BEATTIE, A.; IWANAMI, T. Lower concentrations of microelements in leaves of citrus infected with '*Candidatus* Liberibacter asiaticus' *Japan Agricultural Research Quarterly*, v. 45, p. 269-275, 2011.

MATOS, L.; HILF, M.E.; CAMEJO, J. First reporto f '*Candidatus* Liberibacter asiaticus' associated with citrus huanglongbing in the Dominican Republic. *American Phytopathological Society Journal*, v. 93, n. 6, p. 668, 2009.

MATTHEWS, R.F. Frozen concentrated orange juice from Florida oranges. University of Florida, Fact Sheet FS. p. 1-4, 1994.

MATTOLI, L.; CANGI, F.; MAIDECCHI, A.; GHIARA, C.; TUBARO, M.; TRALDI, P. A rapid liquid chromatography electrospray ionization mass spectrometry^n method for evaluation of synephrine in *Citrus aurantium* L. samples*. Journal of Agricultural and Food Chemistry*, v. 53, p. 9860-9866, 2005.

MCCLEAN, A.P.D.; SCHWARZ, R.E. Greening or blotchy-mottle disease of citrus. *Phytophylactica*. v. 2, p. 177-194, 1970.

MILLER, E.G.; FANOUS, R.; RIVERA-HIDALGO, F.; BINNIE, W.H.; HASEGAWA, S. The effect of citrus limonoids on hamster buccal pouch carcinogenesis. *Carcinogenesis* v. 10, p. 1535-1537, 1989.

MIRANDA, S.H.G.; ADAMI, A.C.O.; BASSANEZI, R.B. Economic impacts of Huanglongbing disease in São Paulo state. Epidemiology, Fundecitrus (Fundo de Defesa da Citricultura), p. 1-20, 2012. Disponível em: < http://iaae.confex.com/iaae/iaae28/webprogram/Paper17434.html>. Acesso em: 31 de nov. 2012.

MOLL, J.N.; VAN VUUREN, S.P. Greening disease in Africa. *Proceedings of the International Society of Citriculture*, v. 3, p. 903-912, 1977.

MORGAN J.K.; ZHOU, L.; LI, W.; SHATTERS, R.G.; KEREMANE, M.; DUAN, Y.P. Improved real-time PCR detection of 'Candidatus Liberibacter asiaticus' from citrus and psyllid hosts by targeting the intragenic tandem-repeats of it's prophage genes. *Molecular and cellular probes*, v. 26, n. 2, p. 90-98, 2012.

NAPPO (North American Plant Protection Organization`s). Phytosanitary alert system: detection of huanglongbing (*Candidatus* Liberibacter asiaticus) in the municipality of Tecoman, Colima, Mexico. NAPPO, 2010. Disponível em: <http:// www.pestalert.org/oprDetail.cfm?oprID=427>. Acesso em: 21 de jun. 2017.

NEPA-UNICAMP (Núcleo de Estudos e Pesquisas em Alimentação – Universidade Estadual de Campinas). Tabela de Composição Química de Alimentos, versão II, 2ª Ed. São Paulo, 113 p., 2006.

NEVES, M.F.; TROMBIN, V.G. Análise de uma década na cadeia de laranja. Markestrat Value Generation. São Paulo. 36 p., 2011. Disponível em: <http://www.markestrat.org/up_arqs/pub_20111216161046_uma_decada_da_citricultura_markestrat.pdf>. Acesso em: 19 de set. 2012.

NEVES, M.F.; TROMBIN, V.G.; MILAN, P.; LOPES, F.F.; CRESSONI, R.; KALAKI, R. O retrato da citricultura brasileira. Markestrat, Centro de pesquisa e projetos em marketing e estratégia, 138 p., 2009. Disponível em: <http://www.citrusbr.com/download/Retrato_Citricultura_Brasileira_MarcosFava.pdf>. Acesso em: 07 de set. 2012.

NISPEROS-CARRIEDO, M.O.; SHAW, P.E. Comparison of volatile flavor components in fresh and processed orange juices. *Journal of Agriculture and Food Chemistry,* v. 38, p. 1048-1052, 1990.

NOGATA, Y.; SAKAMOTO, K.; SHIRATSUCHI, H.; ISHII, T.; YANO, M.; OHTA, H. Flavonoid composition of fruit tissues of citrus species. Bioscience, Biotechnology, & Biochemistry, v. 70, n. 1, p. 178-192, 2006.

PENSO, G. Index Plantarum Medicinalium Totius Mundi Eorumque Synonymorum, 2ª ed.; O. E. M. F. Int.: Milano, p. 286-288, 1997.

PLOTTO, A.; MARGARIA, C.A.; GOODNER, K.L.; GOODRICH, R.; BALDWIN, E.A. Odour and flavour thresholds for key aroma components in an orange juice matrix: Terpenes and aldehydes. *Flavour Fragrance Journal.* v. 19, p. 491-498, 2004.

PLOTTO, A.; MARGARÍA, C.A.; GOODNER, K.L.; BALDWIN, E.A. Odour and flavour thresholds for key aroma components in an orange juice matrix: Esters and miscellaneous compounds. *Flavour Fragrance Journal,* v. 23, p. 398-406, 2008.

PLOTTO, A.; BALDWIN, E.; McCOLLUM, G.; MANTHEY, J., NARCISO, J.; lREY, M. Effect of Liberibacter infection (huanglongbing or “greening disease) of citrus on orange juice flavor quality by sensory evaluation. *Journal of Food Science*, v. 75, n. 4, p. 220–230, 2010.

PLOTTO, A.; BALDWIN, E.; BAI, J.; MANTHEY, J.; RAITHORE, S.; DETERRE. S.; ZHAO, W.; do NASCIMENTO NUNES, C.; STANSLY, P.A.; TANSEY, J.A. Effect of vector control and foliar nutrition on quality of orange juice affected by Huanglongbing (HLB): Sensory evaluation. *HortScience. In press*, 2017.

RAI, V.K. Role of amino acids in plant responses to stress. *Biologia Plantarum*, v. 45, n. 4, p. 481-487, 2002.

RAITHORE, S.; DEA, S.; PLOTTO, A.; BAI, J.; MANTHEY, J.; NARCISO, J.; IREY, M.; BALDWIN, E. Effect of blending Huanglongbing (HLB) disease affected orange juice with juice from healthy orange on flavor quality. *Food Science and Technology*, v. 62, p. 868–874, 2015.

RAMAKRISHNA, A.; RAVISHANKAR, G.A. Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling & Behavior*, v. 6, n. 11, p. 1720-1731, 2011.

RISCH, B.; HERRMANN, K.; WRAY, V.; GROTJAHN, L. 2'-(*E*)-*O*-*p*-coumaroylgalactaric acid and 2'-(*E*)-*O*-feruoylgalactaric acid in citrus. *Phytochemistry*, v. 26, n. 2, p. 509-510, 1987.

RISCH, B.; HERRMANN, K.; WRAY, V. (*E*)-*O*-*p*-coumaroyl-, (*E*)-*O*-feruoyl-derivatives of glucaric acid in citrus. *Phytochemistry*, v. 27, n. 10, p. 3327-3329, 1988.

RITENOUR, M.A. ORANGE. IN: K. GROSS, C.Y. WANG AND M. SALTVEIT (eds.). Agricultural Handbook, No. 66. USDA, Beltsville, MD. 2004. Disponível em: <http://www.ba.ars.usda.gov/hb66/100orange.pdf>. Acesso: 15 Jun. 2013.

RODRIGUES, R.S.; FERRI, V.C. Tecnologia de Frutas: polpas e sucos, 2ª Edição revisada e ampliada, Editora Universitária da UFPEL, 83 p, 2012.

ROUSE, A.H.; ATKINS, C.D.; MOORE, E.L. The occurrence and evaluation of pectin in component parts of Valencia oranges during maturation. *Proceedings of the Florida State Horticulture Society*. v. 75, p. 307-311, 1962.

ROUSEFF, R. Flavonoids and citrus quality, p. 83–108. In: NAGY, S.; ATTAWAY, J.A. (eds.). Citrus nutrition and quality. *American Chemical Society*, Washington, DC., 1980.

RUIZ, G.J.P.; TOZZE JÚNIOR, H.J.; SGUAREZI, C.N.; USBERTI, R.; MARTELLO, V.P.; TOMAZELA, M.S. Ações de defesa sanitária vegetal no estado de São Paulo contra o Huanglongbing. *Citrus Research & Technology*, v. 31, n. 2, p. 155-162, 2010.

RYGG, G.L.; GETTY, M.R. Seasonal changes in Arizona and California grapefruit. *USDA Technical Bulletim.* 1130 p, 1955.

SANTIAGO-SILVA, P.; LABANCA, R.A.; GLORIA, M.B.A. Functional potential of tropical fruits with respect to free bioactive amines. *Food Research. International*, v. 44, p. 1264-1268, 2011.

SCHARBERT, S.; HOLZMANN, N.; HOFMANN, T. Indentification of the astringent taste compouds in black tea infusions by combining instrumental analysis and human bioresponse. Journal of Agricultural and Food Chemistry, v. 52, p. 3498-3508, 2004.

SCOTT, W.C.; VELDHUIS, M.K. Rapid estimation of recoverable oil in citrus juices by bromate titration. *Journal of the Association of Official Analytical Chemists*, v. 49, p. 628-633, 1966.

SHARMA, S.S.; DIETZ, K.J. The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *Journal of Experimental Botany*, v. 57, n. 4, p. 711-726, 2006.

SHAW, P.E. Fruit II, p. 305–327. In: MAARSE, H. (ed.). Volatile compounds in foods and beverages. Marcel Dekker, New York, 1991.

SINCLAIR, W.B.; JOLLIFFE, V.A. Changes in pectic constituents of Valencia oranges during growth and development. *Botanical Gazette,* v. 119, p. 217-223, 1958.

SINCLAIR, W.B.; JOLLIFFE, V.A. Pectic substances of Valencia oranges at different stages of maturity. *Journal of Food Science,* v. 26, p. 125-130, 1961.

SLISZ, A.M.; BREKSA, A.P.; MISHCHUK, D.O.; MCCOLLUM, G. Metabolomic analysis of citrus infection by '*Candidatus* Liberibacter' reveals insight into pathogenicity. *Journal of Proteome*, v. 11, p. 4233-4230, 2012.

SOULE, J.; GRIERSON, W.; BLAIR, J. Quality tests for citrus fruits. *Florida Agricultural. Ext*. Circ. 315 p., 1967.

SPREEN, T.H.; ZANSLER, M.L. Citrus production projections and consumption scenarios 2016-2017 through 2025-2026. *Economic Market Research Department – Florida Department of Citrus*, p. 41, 2015.

STANSLY, P.A.; AREVALO, H.A.; ZEKRI, M. Area-wide psyllid sprays in Southwest Florida: an update on the cooperative program aimed at controlling the HLB vector. *Citrus Industry,* v. 91, p. 6-8, 2010.

SUDHA, G.; RAVISHANKAR, G.A. Involvement and interaction of various signaling compounds on the plant metabolic events during defense

response, resistance to stress factors, formation of secondary metabolites and their molecular aspects. *Plant Cell, Tissue and Organ Culture*, v. 71, p. 181-212, 2002.

SWINGLE, W.T.; REECE, P.C. In The Botany of Citrus and Its Wild RelatiVes; REUTHER, W.; WEBBER, H.; BACHELOR, L. Eds.; *The Citrus Industry: University of California*, v. 1, p. 190-430, 1967.

TANSEY, A.J.; VANACLOCHA, P.; MONZO, C.; JONES, M.; STANSLY, P. Costs and benefits of insecticide and foliar nutrient applications to huanglongbing-infected citrus trees. *Pest Management Science*, v. 73, p. 904-916, 2017.

TASSONI, A.; GERMANA, M.A.; BAGNI, N. Free and conjugated polyamine content in *Citrus sinensis* Osbeck, cultivar Brasiliano N.L. 92, a navel orange, at different maturation stages. *Food Chemistry,* v. 87, p. 537-541, 2004.

TEIXEIRA, D.C.; AYRES, A.J.; KITAJIMA, E.W.; TANAKA, F.A.O.; DANET, J.L.; JAGOUEIX-EVEILLARD, S.; SAILLARD, C.; BOVÉ, J.V. First report of a Huanglongbing-like disease of citrus in Sao Paulo State, Brazil and association of a new liberibacter species, "*Candidatus* Liberibacter americanus", with the disease. *Plant Disease*, v. 89, p. 107, 2005a.

TEIXEIRA, D.C.; DANE, J.L.; EVEILLARD, S.; MARTINS, E.C.; JESUS JUNIOR, W.C.de.; YAMAMOTO, P.T.; LOPES, S.A.; BASSANEZI, R.B.; AYRES, A.J.; SAILLARD, S.; BOVÉ, J.M. Citrus huanglongbing in São Paulo State, Brazil: PCR detection of the "*Candidatus*" Liberibacter species associated with the disease. *Molecular Cellular Probes*, v. 19, p. 173-179, 2005b.

TEIXEIRA, D.C.; SAILLARD, C.; COUTURE, C.; MARTINS, E.C.; WULFF, N.A.; EVEILLARD-JAGOUEIX, S.; YAMAMOTO, P.T.; AYRES, A.J.; BOVÉ, J.M. Distribution and quantification of *Candidatus* Liberibacter americanus, agent of huanglongbing disease of citrus in São Paulo State, Brasil, in leaves of an affected sweet orange tree as determined by PCR. *Molecular and Cellular Probes.* v. 22, p. 139-150, 2008.

TIMMER, L.W.; BOVÉ, J.M.; AYRES, A.J.; BASSANEZI, R.B.; BELASQUE JÚNIOR, J.; CHAMBERLAIN, H.K.; DAWSON, W.O.; DEWDNEY, M.M.;

GRAHAM, J.H.; IREY, M. HLB: it's not too late – yet, Citrus Industry, v. 92, n. 1, p. 6-7, 2011.

TOLEDO, M.; CASTILLO, R. Grandes empresas e o uso corporativo do território: o caso do circuito espacial produtivo da laranja. *Geosul*, Florianópolis, v. 23, n. 46, p. 79-93, 2008.

TRIBESS, T.B. Estudo da cinética de inativação térmica da pectinesterase em suco de laranja natural minimamente processado. Escola Politécnica da Universidade de São Paulo. 117 p., 2003 (Dissertação de mestrado em Engenharia).

TRIPOLI, E.; GUARDIA, M.L.; GIAMMANCO, S.; MAJO, D.D.; GIAMMANCO, M. Citrus flavonoids: Molecular structure, biological activity and nutritional properties: A review. *Food Chemistry,* v. 104, p. 466-479, 2007.

UF/IFAS Extension. Greening History. 2013. Disponível em : <http://www.crec.ifas.ufl.edu/extension/greening/history.shtml>. Acesso em 16 jun. 2017.

USDA/FAS, Foreign Agricultural Service. Citrus: World Markets and Trade, 2017. Disponível em: <http://usda.mannlib.cornell.edu/usda/current/citruswm/citruswm -01-25-2017.pdf>. Acesso em: 20 de abr. 2017.

USDA NASS. Citrus Forecast. USDA, National Agricultural Statistics Service 2016. Disponível em: <https://www.nass.usda.gov/Statistics_by_State/Florida/Publications/Citrus/cit/2015-16/cit0516.pdf>. Acesso em: 13 de mar. 2017.

VAN DER MERWE, A.J.; ANDERSEN, F.G. Chromium and manganese toxicity. It is important in Transvaal citrus greening? *Farming in South Africa*, v.12, p. 439-440, 1937.

VERSTEEG, C.; ROMBOUTS, F.M.; SPAANSEN, C.H.; PILNIK, W. Thermostability and orange juice cloud destabilizing properties of multiple pectinesterases from orange. *Journal of Food Science,* v. 45, p. 969-971, 1980.

VIEIRA, S.M. Metodologia analítica para aminas bioativas como índice da autenticidade e da proporção de suco em refrigerante de laranja. Belo Horizonte: Faculdade de Farmácia da UFMG. 121 p., 2006. (Tese de doutorado em Ciência de Alimentos).

VIEIRA, S.M.; GLÓRIA, M.B.A. Profile and levels of bioactive amines in orange juice and orange soft drink. *Food Chemistry*, v. 100, p. 895-903, 2007.

VIEIRA, S.M.; SILVA, T.M.; GLÓRIA, M.B.A. Influence of processing on the levels of amines and proline on the physico-chemical characteristics of concentrated orange juice. *Food Chemistry*, v. 119, p. 7-11, 2010.

ZHAO, X.Y. Citrus yellow shoot (huanglongbing) in China: A review. *Proceedings of the International Society of Citriculture,* v. 1, p. 466-469, 1981.

ZHAO, W.; BALDWIN, E.A.; BAI, J.; PLOTTO, A.; IREY, M.S. Method for assessing juice/cider quality and/or safety. US patent application: 2015/0093755 A1, 2015.

(176 referências)

PRODUÇÃO CIENTÍFICA DURANTE O DOUTORADO

Artigos completos publicados em periódicos

1. BAI,J.; BALDWIN, E.; MCCOLLUM, G.; MANTHEY, J.; PLOTTO, A.; DALA PAULA, B.M.; GLORIA, M.B.A; WIDMER, W.; LUZIO, G.; CARMERON, R.; NARCISO, J. Influence of harvest time on quality of 'Valencia' oranges and juice, second season. Proceeding of the Florida State Horticultural Society, v. 126, p. 232-238, 2013.

Artigos a serem submetidos para publicação

1. DALA-PAULA, B.M.; PLOTTO, A.; GLÓRIA, M.B.A. Effect of Huanglongbing (greening disease) on orange juice quality, a review.

2. DALA-PAULA, B.M.; RAITHORE, S.; MANTHEY, J.A.; BALDWIN, E.A.; BAI, J.; ZHAO, W.; GLÓRIA, M.B.A.; PLOTTO, A. Active-taste compounds in juice made from oranges symptomatic of Huanglongbing (HLB) greening disease.

Resumos publicados em anais de congressos

1. DALA PAULA, B.M.; RAITHORE, S; MANTHEY, J.A.; PLOTTO, A.; BAI, J.; GLÓRIA, M.B.A.; BALDWIN, E. A deeper look into the causes of off-flavor in orange juice affected by Huanglongbing (HLB). Journal Citrus Pathology, v. 4, n. 1, p.8, 2017. (Apresentação de Trabalho/Conferência ou palestra). Disponível em: <http://escholarship.org/uc/item/2cr0f2kc>.

Resumos submetidos para congressos

1. DALA PAULA, B.M.; RAITHORE, S.; MANTHEY, J.; PLOTTO, A.; BAI, J.; GLÓRIA, M.B.A.; BALDWIN, E.A. Compounds other than limonoid aglycones impart bitterness to orange juice affected by Huanglongbing citrus greening disease (12th Pangborn Sensory Science Symposium – http://www.pangbornsymposium.com/)

2. DALA PAULA, B.M.; MACHADO, G.M.; BALDWIN, E.; MANTHEY, J.A.; PLOTTO, A.; GLÓRIA, M.B.A. Effect of Huanglonbing disease (HLB) and different nutritional foliar treatments in amino acid and bioactive amine contents in Hamlin orange juice (12º SLACA – A Ciência de Alimentos e seu impacto no mundo em transformação, Campinas, UNICAMP, 2017).