**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

**Use of non-destructive instrumental techniques to evaluate the
effects of gamma irradiation, quality and sensory attributes in
orange juice**

Marilia Bizzani

Thesis presented to obtain the degree of Doctor in
Science: Area: Food Science and Technology

**Piracicaba
2021**

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

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EPÍGRAPH

"A persistência é o menor caminho do êxito".

Charles Chaplin

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RESUMO

Uso de técnicas instrumentais não destrutivas para avaliar os efeitos da irradiação gama, qualidade e atributos sensoriais em suco de laranja

O setor citrícola é um dos mais tradicionais do agronegócio brasileiro e responde por 34% da produção de laranja em todo mundo. Entretanto, juntamente com a competitividade do mercado atual, a produção de laranja vem sendo muito impactada pelas variações climáticas. Neste contexto, nota-se a importância de ferramentas que analisem o suco de laranja em grandes escalas e de forma rápida em cenários de grande produtividade, bem como garantir o controle de qualidade em tempos de baixa oferta do produto. Relacionada à qualidade do suco de laranja, encontram-se alguns fatores como uso de métodos de conservação e também a presença de atributos sensoriais. Entre os métodos de conservação de alimentos, a irradiação pode ser uma alternativa na conservação de sucos de frutas, porém medir os efeitos da irradiação é um desafio. Referente aos atributos sensoriais, o método de análise descritiva quantitativa (ADQ) é capaz de proporcionar a descrição sensorial completa de um produto, entretanto requer um longo período de treinamento de provadores. Neste contexto, análises instrumentais como ressonância magnética nuclear no domínio do tempo (RMN-DT) e espectroscopia de infravermelho médio (MIR) e próximo (NIR) vêm sendo exploradas em frutos intactos e sucos demonstrando correlação com parâmetros de qualidade associados à percepção sensorial e controle de processos. Entretanto, as tecnologias limpas, rápidas e não destrutivas pouco foram relacionadas ao processo de irradiação e atributos sensoriais. Portanto, o presente estudo discute no primeiro capítulo: o uso de métodos instrumentais não destrutivos como RMN-DT, MIR e NIR para analisar suco de laranja irradiado. Nos demais capítulos os estudos foram direcionados para avaliação da qualidade de suco de laranja pelo método de ADQ e sua relação com os métodos instrumentais (RMN-DT, MIR e NIR). Os resultados evidenciaram desempenho na classificação de amostras não-irradiadas e irradiadas utilizando os dados espectrais de MIR e RMN-DT. O estudo de relação entre métodos instrumentais não destrutivos e ADQ mostrou melhor desempenho nos modelos de classificação para os descritores amarelo sabor natural, frescor do aroma, aroma e sabor cozido com habilidades preditivas válidas entre 70 e 87% utilizando os dados espectrais do MIR. Por outro lado, os modelos de classificação baseados em dados de RMN-DT alcançaram 79% de acerto na classificação da cor amarela e 72% na classificação corporal do suco de laranja. Desta forma, o extenso estudo dos métodos analíticos de NIR, MIR e RMN-DT apresentaram grandes avanços no campo de estudo da qualidade de sucos de frutas e análises rápidas e limpas acopladas a técnicas estatísticas multivariadas para análise de alimentos.

Palavras-chave: Citros, Conservação por irradiação, Análise sensorial, RMN-DT, MIR, NIR, Quimiometria

ABSTRACT

Use of non-destructive instrumental techniques to evaluate effects of gamma irradiation, quality and sensory attributes in orange juice

The citrus sector is one of the most traditional in Brazilian agribusiness and accounts for 34% of orange production worldwide. However, together with the competitiveness of the current market, orange production has been greatly impacted by climatic variations. In this context, we notice the importance of tools that analyze orange juice on a large scale and quickly in scenarios of high productivity, as well as ensuring quality control in times of low product supply. Related to the quality of orange juice, there are some factors such as the use of conservation methods and the presence of sensory attributes. Among food preservation methods, irradiation can be an alternative in the conservation of fruit juices, but measuring the effects of irradiation is a challenge. Regarding the sensory attributes, the method of quantitative descriptive analysis (ADQ) is capable of providing a complete sensory description of a product, however it requires a long period of training of tasters. In this context, instrumental analyzes such as time domain nuclear magnetic resonance (TD-NMR) and medium-infrared (MIR) and near (NIR) spectroscopy have been explored in intact fruits and juices demonstrating a correlation with quality parameters associated with sensory perception and control of processes. However, clean, fast and non-destructive technologies in combination with irradiation processes and sensory attributes were weakly explored in the literature. Therefore, the present study discusses in the first chapter: the use of non-destructive instrumental methods such as TD-NMR, MIR and NIR to analyze irradiated orange juice. In the following chapters, the studies were directed to assess the quality of orange juice using the ADQ method and its relationship with instrumental methods (TD-NMR, MIR and NIR). The results demonstrated a good performance in the classification of non-irradiated and irradiated samples using the spectral data of MIR and TD-NMR. The study of the relationship between non-destructive instrumental methods and ADQ showed better performance in the classification models for the descriptors yellow, natural flavor, aroma freshness, aroma and flavor cooked with predictive skills valid between 70% and 87% using the MIR spectral data. On the other hand, the classification models based on TD-NMR data reached 79% of correctness in the classification of yellow color and 72% in the body classification of orange juice. Thus, the extensive study of the analytical methods of NMR-DT, MIR and NIR showed great advances in the field of study of the quality of fruit juices and quick and clean analyzes coupled with multivariate statistical techniques for food analysis.

Keywords: Citrus, Irradiation conservation, Sensory analysis, TD-NMR, MIR, NIR, Chemometrics

1. INTRODUCTION

The citrus sector is one of the most traditional in Brazilian agribusiness and accounts for 34% of orange production worldwide. According to the United States Department of Agriculture (USDA, 2020), Brazil still has a 76% share of the world trade in orange juice, becoming the most important supplier of this product.

However, this important sector has been undergoing several structural transformations. In an increasingly competitive landscape, the presence of substitutes for orange juice like nectars and still drinks is increasing, presenting a new reality to orchards and processing plants. Along with the competitiveness of the current market, orange production has been greatly impacted by climatic variations. According to a report by Fundecitrus, the current harvest is expected to decline 30.8% when compared to the 2017/2018 harvest and as a consequence, the orange juice stocks of the 2018/2019 harvest are expected to remain low again, as only 146.7 thousand tons of juice, which is the second lowest amount ever recorded since 1988/89.

Even in a scenario of major oscillation where it is possible for the processing industries to reduce shipments of the commodity throughout the season, Brazilian orange juice will still remain high in 2019/2020, due to the fact that other suppliers such as Florida, are controlling greening, which is a long-term problem ("HortiFruti Brasil," 2018). Therefore, in this context of competitiveness in the current market and oscillations in citrus productivity, we notice the importance of tools that analyze orange juice on a large scale and quickly in high productivity scenarios, as well as ensure quality control in times of low supply of the product.

Related to the quality of orange juice, there are some factors such as the use of conservation methods to maintain its shelf life (Wahia et al., 2020), and also the presence of desirable sensory attributes such as color, aroma, flavor and texture (Kim, Lee, Kwak, & Kang, 2013; Kim, Jang, & Lee, 2018).

Food preservation methods include conventional and unconventional methods (Nowacka et al., 2019; Ricciardi et al., 2019; Ștefănoiu, Tănase, Miteluț, & Popa, 2016). For unconventional methods, ionizing radiation can be an alternative in the conservation of fruit juices (Keyser, Muller, Cilliers, Nel, & Gouws, 2008; Singh & Pal, 2009). The irradiation process involves the intentional exposure of food to a defined dose of irradiation (Jan, Bashir, & Maurya, 2020). This process used the ionizing rays include X-rays, Gamma rays and energized electrons. The type of rays

to be used depends upon the food properties and desired objectives of the irradiation. Gamma irradiation due the high penetration power and satisfactory results, has been preferred over X-rays and electron beam irradiation (Bashir & Aggarwal, 2016; Bhatia, Wall, Kerth, & Pillai, 2018; Song et al., 2012). The treatment with ionizing radiation aims to increase the food preservation time, and may involve, among other mechanisms, the inactivation of microorganisms and disinfestation (Iemma et al., 1999).

For fruit juices, ionizing radiation can be a promising treatment in combination with the refrigeration process (Spoto, 1988). However, measuring the effects of irradiation is a challenge, since the quality analyzes of fruit juices are carried out by sampling, before or after, the irradiation. The loss in ascorbic acid content was observed in the evaluation of the use of irradiation in orange juice (Arteaga et al., 1988), as well as significant variations in pH, soluble solids and total acidity were found with a dose of 2.0kGy (Munhoz-Burgos, 1985).

Sensory attributes, another factor responsible for the quality of orange juice, are decisive for its prominence among other juices (Aptekmann & Cesar, 2010; Khandpur & Gogate, 2015; Tiwari, O' Donnell, Muthukumarappan, & Cullen, 2009; Vervoort et al., 2011). These sensory attributes depend on their physical-chemical composition. The color of orange juice, for example, is mainly due to the presence of pigments and carotenoids present in a complex mixture of more than 115 natural substances (Lee & Coates, 2003; Meléndez-Martínez et al., 2005). The flavor and aroma of orange juice, on the other hand, are related to the presence of key compounds such as limonene, mircene, α -pinene, decanal, octanal, ethyl butanoate, linalool and also with the composition of glycosidic flavonones (Moshonas & Shaw, 1997; Rojas et al., 2007). Also, attributes such as total soluble solids (TSS), total titratable acidity (ATT), ratio (SST / ATT) and pH are parameters that are highly linked to the taste perceived in orange juice (Flores, Colnago, Ferreira, & Spoto, 2016).

Sensory analysis of specific descriptors with trained tasters is a very efficient method, capable of detecting and discriminating qualitative and quantitative sensory components, or even providing a complete sensory description of a product (Meilgard, Vance Civille, & Thomas Carr, 1990; Vilanova, Genisheva, Masa, & Oliveira, 2010). Quantitative descriptive analysis (QDA) is used as a tool in quality analysis including orange juice (Kim, Kim, & Lee, 2016). In this study, for the

differentiation of 18 commercial samples, sensory attributes were defined, such as orange pulp, orange peel, cooked orange, burnt, citrus, medicine aroma and sweet flavor. In particular for the b^* values obtained in determination color of the product, the sensory characteristics showed a high correlation, in which samples with greater yellowing may have a stronger correlation with unwanted sensory characteristics (Kim et al., 2016).

Techniques such as gas chromatography, olfactometry or mass spectrometry, associated with mathematical regressions, have been linked to sensory descriptors in orange juice and other foods such as great quality oils and wines (Andreu-Sevilla, Mena, Martí, García Viguera, & Carbonell-Barrachina, 2013; Baxter, Easton, Schneebeil, & Whitfield, 2005; Vilanova et al., 2010). Although these analytical techniques are efficient, the high cost of equipment acquisition and operation, as well the need for expert analysts, makes usability in industrial applications difficult. Also, because they are destructive analysis, sample preparation and analysis time, associated with small injected volumes, generate difficulties in the analysis routine of many samples. In addition to measures of aromatic compounds, it is essential to carry out measures related to taste and process / harvest control, such as SST, ATT and ratio, which are invasive analysis and do not allow the evaluation of large volumes due to the operational time of each analysis.

Current food quality control methods are destructive, time-consuming and require specialized analysts. The methods already used in the detection of irradiated foods, such as thermoluminescence, electrophoresis of isolated cells in microgels, analysis of volatile species and electronic paramagnetic resonance spectroscopy, are expensive, time consuming and do not allow the analysis of large volumes of samples. In the same way, the sensory method of QDA, despite being efficient for a complete sensory description of a product, requires a long period of training of tasters, which makes it difficult to perform in processing companies. In this context, instrumental analysis such as time domain nuclear magnetic resonance (TD-NMR) and infrared spectroscopy have been explored in intact fruits and juices showing a correlation with quality parameters associated with sensory perception and process control (Cayuela, 2008; Cayuela & Weiland, 2010; Zude et al., 2008).

Spectroscopy in the near infrared region ($12500\text{-}4000\text{ cm}^{-1}$) and medium ($4000\text{-}400\text{ cm}^{-1}$) are studied for non-destructive analysis of fruits and juices (Oliveira, de Castilhos, Renard, & Bureau, 2014; Reid, Woodcock, O'Donnell, Kelly, & Downey,

2005; Tavakolian et al., 2013; Vélez Rivera et al., 2014). These techniques are based on the interaction of energy with relative movements of atoms in a molecule, that is, its vibrations and rotations (Oliveira et al., 2014). The advantage of this analysis is to enable the identification of specific compounds, due to the absorption of energy in known bands, called fingerprints. In these regions, significant changes in the distribution and absorption of the bands may indicate differences in the structure and chemical composition of the samples, making it possible to perform mathematical calibrations to quantify and classify compounds of interest such as organic acids and sugars (Bellon-Maurel & McBratney, 2011; Magwaza, Landahl, et al., 2014).

TD-NMR analysis observes the transition of nuclei with non-zero spins, such as Hydrogen and Carbon, exposed to the influence of a magnetic field and radio frequency (rf) (Colnago et al., 2014). Its application has been motivated by the low cost of the equipment with permanent magnet technology, measurement of whole and packaged samples, preservation of sample integrity, as well as simple and quick analysis procedures (Pereira, Carvalho, Cabeça, & Colnago, 2013; P. M. Santos, Pereira-Filho, & Colnago, 2016). TD-NMR analysis allows to study the changes that occur in the internal environment of the samples via relaxometry techniques. These techniques are based on the disturbance of the thermal equilibrium state of nuclei, for example, of hydrogen, via radiofrequency pulses. In this way, the time constant to return to the thermal equilibrium state is known longitudinal relaxation time (T_1). The transverse relaxation time (T_2) is the time constant related to the loss of coherence of the spins (Colnago et al., 2014; Ribeiro et al., 2010). T_2 measurement using Carr-Purcell-Meiboom-Gil (CPMG) rf pulse sequence has already been studied to classify intact oranges by TSS difference (Flores et al., 2016) and plums also due to total solid soluble (TSS) difference (Pereira, Carvalho, et al., 2013) both showing good results and indicating the high potential of the analysis of intact fruits.

At the same time, with the advancement of technology in food analysis, more data and information are produced, which makes it essential to apply chemometric tools or data science. This combination allows several applications in food analysis, including the extraction of important information from the spectra by principal component analysis (PCA) (Andrade, Medeiros Coelho, & Uarrota, 2020), development of predictive and classification models, by the method of partial least squares regression (PLS) (Croce et al., 2020) and method of discriminant analysis by

partial least squares (PLS-DA), respectively. Thus, new applications of non-destructive methods combined with data science, may represent alternatives for juice processing companies to measure the impact of fruit quality at the end and thus adopt corrective measures to improve juice production processes throughout the year.

Therefore, the study on the effects of irradiation on orange juice via non-destructive techniques by TD-NMR, MIR and NIR that allow the analysis of the chemical molecular profile of the product inside the packaging and even before and after the process of irradiation may help to measure the effects and doses of irradiation that affect or not the orange juice significantly. In addition, most of the studies that evaluated the relationship between instrumental analysis and sensory analysis used instrumental methods such as GC-MS, MIR and NIR, or only demonstrated the potential of TD-NMR to classify whole fruits by TSS. Therefore, the general objective of this thesis was to evaluate the use of non-destructive techniques of TD-NMR, MIR and NIR combined with data science for analysis of irradiated orange juice and also to relate the same non-destructive methods with sensory attributes of orange juice. The thesis was developed in 4 chapters:

Chapter 1. Study of gamma irradiation in orange juice using MIR, NIR, and TD-NMR spectroscopies coupled with machine learning.

Chapter 2. Sensory panel training by quantitative descriptive analysis to evaluate original orange juice from the Brazilian Citrus belt.

Chapter 3. Application of NIR and MIR to the sensory evaluation of orange juice.

Chapter 4. Orange juice sensory evaluation with TD-NMR combined with multivariate analysis data processing.

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2. STUDY OF GAMMA IRRADIATION ON ORANGE JUICE USING INFRARED AND TIME DOMAIN NUCLEAR MAGNETIC RESONANCE SPECTROSCOPIES COUPLED WITH MACHINE LEARNING

Abstract

Time domain nuclear magnetic resonance (TD-NMR), medium and near infrared spectroscopy (MIR and NIR) were used as non-invasive methods to investigate the possibility to rapidly detect orange juice submitted to a gamma irradiation at 0; 2; 4 and 6kGy. All instrumental data were subjected to a principal component analysis (PCA) and machine learning by support vector machine (SVM) to develop classification models of non-irradiated and irradiated samples and also distinguish samples according to the irradiation dose. PCA showed the best separation of non irradiated and irradiated samples using physicochemical, MIR and TD-NMR data. For the classification, MIR and TD-NMR SVM models showed an accuracy of 89.89 and 79.79% respectively, both for irradiated and non-irradiated samples and higher than the physicochemical model. The different irradiation doses measures by TD-NMR, NIR and MIR were overlapped each other in PCA analysis. The results obtained demonstrate the potential for rapid and easy detection of irradiated foods using non-destructive methods by MIR and TD-NMR combined with chemometric tools.

Keywords: Irradiation, orange juice, MIR and NIR spectroscopy, TD-NMR, chemometrics.

2.1. Introduction

Orange juice is one of the most consumed and globally accepted sensory fruit juices (Katariya, Arya, & Pandit, 2020). In addition to its sensory qualities, orange juice contains vitamins (C and B), minerals, carotenoids, flavonoids and antioxidants (Mears & Shenton, 1973). Due to this rich composition, the consumption of orange juice has been associated with the prevention of several diseases such as kidney disorders, high cholesterol, blood pressure and stroke (Zhao et al., 2017). In the processing of orange juice, the application of thermal treatment is necessary to control the activity of deteriorating microorganisms and to inactivate enzymes. In this context, pasteurization is the thermal treatment conventionally used (Brugos, Gut, & Tadini, 2018).

However, even most commercial juices are pasteurized (temperature $\geq 80^{\circ}\text{C}$; waiting time $> 30\text{ s}$), the application of thermal preservation methods in juices can change the flavor, the content of nutrients and vitamins of the product (Petruzzi et al., 2017; Zinoviadou et al., 2015). Pasteurization by means of heat exchangers can still

contribute to changes in color, texture and, consequently, in the acceptance of the final product (Van Den Broeck, Ludikhuyze, Weemaes, Van Loey, & Hendrickx, 1999). For this reason, many studies have been developed to verify the possibility of using alternative treatments (Arjmandi et al., 2017). In this context, for many years, gamma irradiation have been tested for sterilization and conservation of several food matrices such: fresh vegetables (Rubio et al., 2001), apple (Wang & Chao, 2003) (Constantinovici, Oancea, & Zaharescu, 2009), grapefruit (Patil, Vanamala, & Hallman, 2004), papaya (D'innocenzo & Lajolo, 2001), chickpea starch (Khalid Bashir & Aggarwal, 2017), brown rice (Kumar et al., 2017), wheat flour and starch (Khalid Bashir & Aggarwal, 2017), buckwheat and oat starch (Dar et al., 2018), cowpea and potato starch (Verma, Jan, & Bashir, 2019). Gama irradiation was also applied in the conservation of orange juice observing its effects and changes in the final product (Groppo, 2012; Iemma et al., 1999; Verruma-Bernardi & Spoto, 2003).

The first principle, when talking about food irradiation is that it must be non-thermal (Khalid Bashir, Swer, Rani, Jan, & Jan, 2021). Defined by the Codex Alimentarius Commission there are three types of radiation used commercially for food irradiation, which include: X-rays, accelerated electrons and gamma rays (Bashir & Aggarwal, 2016b; Bashir, Swer, Prakash, & Aggarwal, 2017; Bhat, Sridhar, & Tomita-Yokotani, 2007). Furthermore, irradiation is classified according to the absorbed dose, where: Radappertization (30–40 kGy), Radurization (0.75–2.5 kGy) and Radicidation (2.5–10 kGy) (Khalid Bashir et al., 2021). The process consists of a physical treatment where the food is exposed to a pre-established dosage of radiation. This process has an effect similar to that of other processing techniques, such as pasteurization, canning, blanching, with effects lower than the quality of the product and nutritional value (Khalid Bashir et al., 2021). Among the three types of irradiation, gamma irradiation has the advantage of high penetration power (Bashir & Aggarwal, 2016b, 2019).

The irradiation process exposes food to a source of radiation, which is sufficient to create free radicals, positive and negative charges molecules. Thus, the molecules when absorbing ionizing energy become reactive forming ions or free radicals that react to form stable radiolitic products (C.D'Oca & Bartolotta, 2018). In the case of food irradiation, these radiolitic products formed are generally the same as those formed when the food is cooked or treated with infrared radiation (C.D'Oca & Bartolotta, 2018). The main effect of radiation on food is due to an extremely small

breakdown of chemical bonds, but with great effect, such as, for example, the breakdown of bonds in DNA which results in the loss of a cell's ability to replicate and can even destroy bacterial cells (D'Oca & Bartolotta, 2018). Therefore, the cellular destruction caused by the rupture of the genetic material of a living cell is what allowed the destruction of insects, inactivation of parasites, delayed ripening and prevention of germination (D'Oca & Bartolotta, 2018).

However, despite the benefits for conservation and maintenance of food quality, the search for reliable analytical techniques to identify irradiated foods has been constant, in order to increase consumer acceptance and facilitate commercialization (D'Oca & Bartolotta, 2018). The methods already validated by the European Committee for Standardization (CEN) for standard identification of irradiated foods, can be differentiated between chemical and physical: EN 1785: Foodstuff-detection of irradiated food containing fat, gas chromatographic/mass spectrometric analysis of 2-alkyl cyclobutanones. EN 1786: Foodstuff-detection of irradiated food containing bone, method by Electron Spin Resonance (ESR) spectroscopy. EN 1787: Foodstuff-detection of irradiated food containing cellulose, method by ESR spectroscopy. EN 1788: Foodstuff-detection of irradiated food containing silicate minerals can be isolated, method by thermoluminescence (TL) (D'Oca & Bartolotta, 2018). However, the current methods cited to identify irradiated foods are time consuming, requiring several stages of sample preparation and extraction, as well as using expensive equipment that does not allow for large-scale analysis. In this context, new alternative technologies has been proposed for monitoring food quality using physical methods based on nuclear magnetic resonance in the time domain (TD-NMR) and near and midinfrared spectroscopies (Carvalho et al., 2019; Pereira et al., 2019; Santos et al., 2014, 2016).

TD-NMR is widely used for quantitative and qualitative analysis of food (Pereira, Carvalho, Cabeça, & Colnago, 2013). The low cost of analysis, ease of use of the instrumentation and especially the speed of analysis and the preservation of the samples integrity are determining factors in the application of the TD-NMR method (Colnago, Azeredo, Netto, Andrade, & Venâncio, 2011; van Duynhoven, Voda, Witek, & Van As, 2010). For food analysis, TD-NMR has been used to measure intact fruits (Flores, Colnago, Ferreira, & Spoto, 2016; Pereira, Carvalho, Cabeça, & Colnago, 2013), cheese (Chen, MacNaughtan, Jones, Yang, & Foster, 2020) and beef (Santos et al., 2014).

Near-infrared (NIR) and mid-infrared (MIR) spectroscopies have also been used for quantitative characterization of food matrices (Xie, Ye, Liu, & Ying, 2011). The advantages of the NIR and MIR techniques are also due to the short measurement time, little no or minimal sample processing (Lorenzo, Garde-Cerdán, Pedroza, Alonso, & Salinas, 2009) including the analysis in the final product and also in the monitoring of the process (Pereira et al., 2008). In the fruits analysis NIR and MIR have been used to determine sugars, organic acids, and polyphenols (Oliveira-Folador et al., 2018; Oliveira, de Castilhos, Renard, & Bureau, 2014).

The growing application of non-destructive technologies has been possible due to the efficient combination of non-destructive methods and chemometrics, which allows the development of models for quantitative purposes, determination of chemical concentrations in the analyzed samples (Croce et al., 2020). Data obtained can also be analyzed by a series of chemometric classification tools, such as, principal component analysis (PCA), linear discriminant analysis (LDA) or k-nearest neighbors (kNN). There is also the possibility of using non-linear classification algorithms, such as support vector machines (SVM), which are almost also a promising approach (Gerhardt et al., 2019).

TD-NMR, NIR and MIR are rarely used to identify irradiated foods. Most studies have used these methods to predict quality, physical-chemical parameters or adulteration using chemometric data processing (Cortés-Estrada et al., 2020; Lemos, Machado, Egea-Cortines, & Barros, 2020; Santos et al., 2016).

Therefore, the aim of this study was to evaluate the use of simple, rapid and non-destructive TD-NMR, NIR and MIR technologies, associated with machine learning methods using unsupervised (PCA) and supervised (SVM) algorithm to classify orange juice in irradiated or non-irradiated and also by radiation dose received.

2.2. Material and Methods

2.2.1. Samples

Thirty liters of orange juice were obtained from a local supplier. Orange juice was extracted from cultivar *Pera Rio* using industrial extractor. After extraction juice was homogenized and bottled into previously cleaned 99 polyethylene terephthalate (PET) bottles of 300 mL.

Instrumental analysis of TD-NMR, NIR, MIR and physicochemical characterization were performed on three juice bottles on the same day of extraction. Until the irradiation processes, the other samples were stored for 12 hours in a refrigeration chamber at 5°C and 90% RH.

2.2.2. Gamma irradiation treatment

The 96 juice bottles were equally divided into four styrofoam boxes (18L) containing the control tags, 2 kGy, 4 kGy and 6 kGy. The irradiation process was carried out at the Energy and Nuclear Research Institute (IPEN / USP) in the city of São Paulo (SP, Brazil). The samples were submitted to doses of 2, 4 and 6 kGy using a multi-purpose gamma irradiator, equipped with a Cobalt 60 source. At the time of the process, the dose rate of the radiator was 4kGy / h. To check the doses applied in each box, dosimeters Amber 3042 Batch Y Type 3042 and Batch A Type Gammachrome YR were used. At the end of the process, the samples were stored at 5 °C and subjected to physical-chemical and instrumental characterization at 3, 7, 14 and 21 days after irradiation.

2.2.3. Time-domain nuclear magnetic resonance (TD-NMR) analysis

Each orange juice bottle containing samples was analyzed in a TD-NMR equipment (Spin lock Magnetic Resonance Solution, Cordoba, Argentina) equipped with a permanent 0.23-T (9 MHz for ^1H), 10-cm-diameter Halbach magnet and a 1-L single coil probe. The CPMG sequence (Santos et al., 2014) was used for ^1H transverse relaxation time (T_2) measurement using the following parameters: 90° and 180° pulses of 32μs and 64μs, echo time (τ) of 5ms, 1500 echoes, and 8 scans. The measurements were performed in at 21 ± 1 °C. The samples orange juices were further submitted to NIR and MIR spectroscopies analyses.

2.2.4. Infrared spectroscopies analysis

Orange juice spectra were measured in MicroNIR Onsite W spectrometer (900-1700nm NIR) equipped with liquid accessory side-vial-holder operating via Bluetooth connection with Android 7.0 smartphone, 10 ms integration time, 150 scans. MIR spectra were acquired in a Cary630 spectrometer (Agilent, Walnut Creek, CA, USA) equipped with DialPath accessory, suitable for measurements on liquid samples. Spectra were collected at 2cm^{-1} resolution, 64 scans. Three replicates were measured per sample, but only the average signal of the 3 acquisitions per sample was used for statistical treatments, counting a total of 99 average spectra. The samples were further submitted to physicochemical analysis.

2.2.5. Physicochemical analysis

Total Soluble solids (TSS) was measured in Brix degrees, with an Atago refractometer, model RX-5000 (AOAC, 1990). The hydrogen potential (pH) was measured in 20 mL of juice under agitation using a pHs-3B pH meter. Total acidity was obtained by titrating 10 mL of juice in 10 mL of distilled water, with 0.1 N NaOH solution, the values being expressed in% citric acid (Instituto Adolfo Lutz, 1985). At end, Ratio was calculated by the relation (soluble solids/titratable acidity) (AOAC, 1990).

2.2.6. Data processing

The TD-NMR, NIR and MIR data were processed using the Unscrambler X 10.5.1 software (Camo Analytics, Oslo Science Park, Gaustadalléen, Oslo). For the Principal Components Analysis (PCA) the TD-NMR data were pre-processed through normalization by max (0–1) and mean center. NIR data were submitted to standard normal variation (SNV) and first derivative with Smoothing (Savistky-Golay, 2^o degree polynomial with 5 points window size). In the MIR spectra, it was also applied first derivative with Smoothing (Savistky-Golay, 2^o degree polynomial with 61 points window size).

The pre-processing used for the classification models were different only for the TD-NMR data, in which it was changed the normalization by max for first derivative with Smoothing (Savistky-Golay, 2^o degree polynomial with 21 points window size) aiming noise reduction.

2.2.7. Chemometric approach

TD-NMR, MIR, NIR and physicochemical data were analyzed by an exploratory analysis using PCA. This unsupervised algorithm allows the identification of most important variables that explain the new arrangement of samples in principal component space. In the score's scatter plot, it was possible to find groups by chemical similarities and in the loadings plot it was possible to understand why that samples are similar or different.

After PCA analysis, the knolement of samples variation for each instrumental data was trained and validate using supporte using support vector machines algorithm. The Unscrambler X software allow us to train SVM models for classification performing a grid optimization to achieve the highest cross-validation accuracy combining the hyper parameters, such as, constraint C and kernel scale parameter gamma. The SVM classifier was optimized for the radial basis function (RBF), using cross-validation algorithm, based on, 7 random segments from the full dataset. The SVM results are presented by train and test segment's validation accuracies.

2.3. Results and discussions

2.3.1. Physicochemical characterization

Table 1 shows the average values and standard deviations of physicochemical data of the studied orange juices according to the treatment and storage time. Orange juice was also analyzed on the same day that it was produced, showing the following characteristics: TSS = 11.38, pH = 3.72, TA = 0.862 and Ratio = 13.19. Gamma irradiation resulted a small decrease in the pH of samples, with lowest value for 6kGy and highest value for non-irradiated orange juice. Similar results have been reported in different food matrices submitted to the irradiation process (Bashir & Aggarwal, 2016b; Wani et al., 2014). Total soluble solids did not show significant variations. The titratable acidity mainly of the control samples suffered a greater effect from the storage period. The highest TA value was found in the samples

submitted to 6kGy of gamma irradiation. Finally, higher Ratio values were observed in the control samples.

Fig. 1A and 1B show the score and loadings plots using principal components 1 (PC1) and 2 (PC2) for non-irradiated orange juice, irradiated in doses 2,4 and 6KGy analyzed during 21 days, with regard to the physicochemical data. Fig. 1A presents a good separation between the non-irradiated and irradiated orange juice. The PCA of the non-irradiated and irradiated orange juice showed 65 and 21% of variance in PC1 and PC2, respectively. This discrimination was more influenced by PC1 due to the effects that total acidity, Ratio and pH of the samples non-irradiated and irradiated played on PC weights, locating samples having low values on the left side of PC1 and those with high values on the right (Fig. 1 B). Thus, the control samples were characterized by the higher values of pH and Ratio and the samples submitted to the irradiation process characterized by the highest total acidity.

Table 1. Average values of the total soluble solids (TSS), pH, total acidity (TA) and Ratio in orange juice as a function of treatments and storage time

Analysis	Storage time (days)	Treatment			
		Control	2kGy	4kGy	6kGy
TSS(°Brix)	3	11.38±0.17Aa	11.51±0.12Aa	11.44±0.14Aa	11.53±0.06Aa
	7	11.30±0.09Aa	11.44±0.15Aa	11.47±0.20Aa	11.46±0.13Aa
	14	11.31±0.14Aa	11.49±0.10Aa	11.56±0.15Ba	11.57±0.02Ba
	21	11.26±0.13Aa	11.36±0.17Aa	11.39±0.19Aa	11.41±0.10Aa
pH	3	3.44±0.14Aab	3.40±0.27Aab	3.48±0.05Aab	3.35±0.05Aab
	7	3.70±0.09Aab	3.49±0.09Bab	3.54±0.10Bab	3.50±0.08Bab
	14	3.43±0.07Aa	3.35±0.03Ba	3.37±0.02Aa	3.30±0.02Ba
	21	3.46±0.08Aa	3.29±0.03Ba	3.29±0.06Ba	3.27±0.03Ba
TA (g/100mL)	3	0.830±0.74Aa	0.841±0.03Aa	0.845±0.03Aa	0.918±0.3Ba
	7	0.789±0.05Aa	0.845±0.06Aa	0.827±0.10Aa	0.854±0.04Aa
	14	0.789±0.01Aa	0.865±0.03Ba	0.841±0.02Ba	0.904±0.04Ba
	21	0.753±0.03Aa	0.866±0.03Ba	0.873±0.04Ba	0.864±0.20Ba
RATIO (TSS/TA)	3	13.81±1.43Aa	13.71±0.61Aa	13.55±0.44Aa	12.57±0.47Aa
	7	14.37±0.91Aa	13.60±0.98Aa	13.90±0.79Aa	13.45±0.62Aa
	14	14.32±0.22Aa	13.28±0.58Ba	13.75±0.30Aa	12.82±0.62Ba
	21	14.97±0.54Aa	13.14±0.70Ba	13.07±0.63Ba	13.20±0.43Ba

Note: averages followed by the same uppercase letter in the row and lowercase in the column do not differ at the 5% probability level by the Tukey test.

Figure 1. PCA of physicochemical parameters of control samples and submitted for irradiation process (2, 4 and 6kGy) analyzed during 21 days (A). Scores of PCA of physical-chemical parameters (B).

2.3.2. Instrumental characterization using TD-NMR, NIR and MIR spectra

Fig. 2A present the score plot of PC analysis of the TD-NMR relaxation data. PC1, PC2 and PC3 corresponded to 92%, 7% and 0.38% of the data variance, respectively. For visualization purposes, it is presented PC2 by PC3. On the PC2 axis two groups were separated, the control samples lying on the negative and the irradiated samples on the positive sides of PC2, respectively. Fig. 2B shows the PC2 loadings plots that demonstrate the points most influential in the samples separation.

T_2 values were also calculated, showing initial values of 0.210 ± 0.0003 s in the same day of the extraction orange juice. During the storage, the control samples showed T_2 equal to 0.213 ± 0.0003 s. Orange juice samples submitted to an irradiation, stored and analyzed under the same conditions as the control samples showed a small reduction in T_2 . Orange juice irradiated with 2kGy showed T_2 equal to 0.204 ± 0.0002 s, with 4kGy equal to 0.199 ± 0.0002 s and 6kGy 0.198 ± 0.0002 s.

The difference between control samples with irradiated samples was very low, as we can observe in both PCA and T_2 data. This lower reduction of T_2 for irradiated samples could be associated with little changes in physical-chemical values such as Ratio (Flores et al., 2016). However, this is an interesting result on the point of view of multivariate analysis that can be used to maximize these little differences and use this variability for training classification models.

Figure 2. PCA of TD-NMR decay of control samples and submitted for different doses of irradiation (2, 4 and 6kGy) analyzed during 21 days (A). Scores of PCA of TD-NMR decay (B).

Fig. 3A shows the score plot of PC analysis of the NIR data. PC1 and PC 2 corresponded to 77% and 2% of the data variance, respectively. As it can be observed, all samples distributed randomly both as positive and negative score on PC1 and PC2. All samples of orange juice analyzed, strongly overlapped with each other and no clusters among different treatments were obtained. Fig. 3B shows the PC loadings plots that demonstrate the influence mainly of the absorption bands in 1400nm, but also of the absorption bands in 1300, 1500 and 1100nm. Regarding the observed absorption bands, it is possible to highlight the strong influence of the water absorption bands on the orange juice samples, due to the O-H bond at approximately 1450nm (Williams & Norris, 1987).

Monomeric organic acids typically have O-H bands in the first three overtones at 1445, 1000 and 800nm, respectively (Goddu & Delker, 2002). All of these absorption bands are very close to the strongest water absorption regions, which makes it difficult to visualize (Delwiche, Mekwatanakarn, & Wang, 2008). In this case, the difficulty in visualizing monomeric acids in the spectra obtained by MicroNIR may have influenced the overlap of treatments observed in the PC analysis. However, even using the benchtop NIR spectral range, in which it is possible to highlight the characteristic band of organic acids, it was not possible to observe the formation of groups in the PC analysis in dry fermented sausages treated with ionizing radiation at 0; 0.5; 1; 2 and 3 kGy (Varrà et al., 2020). The author attributes this overlap as a

consequence of the very low degree of modification of the physical and chemical characteristics of samples induced by the irradiation treatments.

Figure 3. PCA of NIR first derivative data of control samples and submitted for different doses of irradiation (2, 4 and 6kGy) analyzed during 21 days (A). Scores of PCA of NIR variables (B).

Fig. 4A shows the score plot of PC analysis of the MIR data. PC1 corresponded to 75% of the data variance, but for visualization purposes it is presented PC1 by PC4. PC analysis of MIR data shows two groups were formed, the control samples lying on the positive and the irradiated samples on the negative sides of PC4, respectively. However, samples submitted to different doses of irradiation appear overlapped in PC analysis, as well as their storage times. Fig.4B shows the PC4 loadings plots that demonstrate the peaks most influential in the control samples and irradiated samples separation.

Such PC loadings plots showed, the peaks most influential are located in 3650cm^{-1} is assigned to hydroxyl (O-H) stretching vibration of water, carboxylic acids, and phenolic compounds (Cortés-Estrada et al., 2020). Also the strong peaks from 3000 to 2800cm^{-1} are assigned to C-H bonds organic compounds. The absorption band at 2347cm^{-1} corresponds to C-H stretching associated with the ring methane hydrogen atoms (Dar et al., 2018). Highlighted peaks also observed close to 1790cm^{-1} and 1000cm^{-1} related to C=O and C-O groups, respectively. These peaks have been associated with polysaccharides and essential oils (Schulz & Baranska, 2009).

Figure 4. PCA of MIR first derivative data of control samples and submitted for different doses of irradiation (2, 4 and 6kGy) analyzed during 21 days (A). Scores of PCA of MIR variables (B).

2.3.3. Classification models

Based on the PC analysis results, the classes defined as control and irradiated were used to train the SVM models, allowing the use of instrumental data by TD-NMR, NIR and MIR to classify the samples according to the application or not of the irradiation process. The approach of these models aims to be a fast and qualitative method to detect irradiated foods.

The use of SVM models applied in the analysis of non-destructive instrumental data can provide a quick identification of irradiated foods, thus being able to avoid re-irradiation, verify compliance with the minimum levels of microbiological requirements and contribute to the control of the international market for irradiated foods on a large scale.

Currently, the use of SVM models is growing amidst work involving applications with real data, most of which are non-linear in nature (Chiamulera et al., 2021). To work with nonlinear data, SVM models use the kernel function, which maps the variables to higher dimensions, where it is possible to characterize the same data linearly (Chiamulera et al., 2021). SVM models operate through the construction of the optimal hyperplane, which consists of the margins that best represent the separation of classes (Gerhardt et al., 2019). Between the hyperplane and the support vectors between classes there should be as much distance as possible.

Thus, a grid search of the trained SVM model was performed with 70% of the data, using cross-validation, which is shown in Table 3.

Table 3 shows the orange juice non-irradiated and irradiated classification performance using the tuned SVM model and TD-NMR, NIR and MIR data. Physical-chemical data was also used in the SVM model to compare the performance of the classification model built with instrumental data. The best performance for the classification of non-irradiated and irradiated juices was obtained with MIR spectra that provided 89.89% accuracy. In second place, TD-NMR decay showed 79.79% accuracy, the same performance of physical-chemical SVM model. Finally, only NIR SVM model showed lower accuracy (72.72%) that physical-chemical SVM model.

The results achieved in the present work can be explained mainly by the capacity of the MIR spectra to detect differences in the total acidity of the samples (Oliveira-Folador et al., 2018). In this context, it is noteworthy that this physical-chemical parameter had a great influence on the separation of non-irradiated and irradiated samples (Fig. 1). In MIR spectroscopy, important peaks are located at 1635cm^{-1} , which are related to the presence of acids (Oliveira-Folador et al., 2018). Also relevant regions are highlighted in the identification of organic compounds between 1700 and 1500 cm^{-1} related to the stretching vibration of $\text{C} = \text{O}$, a functional group present in acids (Anguebes et al., 2016). These regions related to the presence of organic acids can be observed in the scores of PC analysis (Fig. 4) showing great influence on the separation of the non-irradiated and irradiated samples.

TD-NMR also showed a good accuracy in the SVM model classifying non-irradiated and irradiated juices. The fact that CPMG decay is governed by transverse relaxation (T_2) which is inversely correlated with sample viscosity (Flores et al., 2016) is important to explain the SMV model performance. TSS is a factor related to the viscosity of fruit juices, however it had no influence on the separation of the non-irradiated and irradiated samples, since the control samples and all treatments showed very similar TSS. Thus, it is believed that another factor related to the juice's viscosity may explain the result obtained. In this context, the irradiation process showed changes in the viscosity of the some food matrices, which can be related to the changes in the T_2 values of the irradiated samples (Khalid Bashir & Aggarwal, 2017; Khalid Bashir et al., 2017; Kumar et al., 2017). In addition, the irradiation process has the capacity to inactivate enzymes related to food conservation (Khalid

Bashir et al., 2021). For orange juice quality, pectin methylesterase (PME) is an enzyme with major impact on the product that leads to quality losses due to its adverse effect on clarification of orange juice, or gelation of concentrated orange juice, if not inactive (Basak & Ramaswamy, 2001). Therefore, the samples orange juice submitted to the irradiation process may have shown less clarification effect through the inactivation of the PME, differentiating it from the control samples by the viscosity.

Thus, the use of MIR and TD-NMR techniques combined with machine learning models such as SVM have a potential to be an alternative in identifying irradiated foods that currently use expensive and time-consuming methods.

Table 2. SVM models trained using MIR, TD-NMR, NIR and physico-chemical data.

Method	MIR	TD-NMR	NIR	Physicochemical
Pre-processing	1 DRV (61pts)	1 DRV (41pts)	SNV + 1 DRV (5pts)	Autoscale
Training accuracy (%)	92.93	96.97	72.73	79.8
Cross-validation accuracy (%)	89.89	79.79	72.72	79.79

Pts: window points. 1 DRV: 1st derivative 2^o degree polynomial

2.4. Conclusions

The present study demonstrates the feasibility of a simple and rapid detection of orange juice treated with irradiation using MIR spectroscopy and TD-NMR decay combined with chemometric tools. Although the preliminary analysis performed by the PCA analysis was not satisfactory enough, the results of the SVM models constructed with data from the MIR and TD-NMR showed an accuracy of 89.89 and 79.79% respectively in the classification of non-irradiated and irradiated samples. Spectral bands associated with organic acids were influential in discrimination samples using MIR data and the relation between TD-NMR decay and samples viscosity explained the changes in the T_2 values of the irradiated samples.

Therefore, the good performance of the classification models of non-irradiated and irradiated samples has the potential to control and detect irradiated foods, offering advantages of speed and ease of analysis on a large scale compared to traditional methodologies of food analysis. However, the use of non-destructive methods to classify the radiation dose used still requires an in-depth study with a comprehensive database.

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3. SENSORY PANEL TRAINING BY QUANTITATIVE DESCRIPTIVE ANALYSIS TO EVALUATE ORIGINAL ORANGE JUICE FROM THE BRAZILIAN CITRUS BELT

Abstract

The objective of this study is to form a trained panel for sensory evaluation of fresh, reconstituted and pasteurized orange juice in the citrus industry as well as to relate the sensory attributes among themselves and with the current physical chemical quality parameters. Three samples were selected based on different flavor characteristics, processing type and based on market to develop a quantitative descriptive analysis (QDA) for the sensory evaluation of fresh, reconstituted and pasteurized orange juice in the citrus industry. Eleven trained panel members developed a lexicon composed of 4 attributes of appearance and aroma, 8 of flavor and 2 of texture. Physical-chemical parameters related to the flavor of orange juice were also measured. Statistical analyzes such as ANOVA, PCA and correlation map were used to evaluate the results. The QDA results showed that aroma, freshness, citric, natural and citric taste were characteristic attributes to distinguish fresh orange juice. Reconstituted juice was characterized by the aroma cooked and overripe, taste cooked, overripe, bitter, fermented and astringent. Finally, pasteurized juice was characterized by the lower appearance viscous, yellow, lower taste acid and mouthfeel body and presence of cells. In the point of view of the physical-chemical parameters, fresh juice was characterized by the content of centrifuged pulp, hesperidin, and recoverable oil. Reconstituted juice was mainly characterized by titratable acidity and limonin. Pasteurized juice was characterized by the Ratio and acidity. QDA results and physical-chemical parameters are consistent and mutually complementing, through the use of tools such as PCA and correlation map in the exploration of the data, results obtained were accurate and applicable to the evaluation of the quality of orange juices.

Keywords: Orange juice, QDA, physical-chemical parameters, lexicon development, performance visualization, PCA, correlation maps.

3.1. Introduction

Orange juice is one of the most largely consumed fruit beverages in the world. Its consumption has increased and showed in the 2019/2020 harvest that Brazilian exports increased by 26.6% for the both FCOJ (frozen concentrated orange juice) and NFC (not from concentrate), compared to the same period last year (CITRUSBR, 2020). The good scenario is seen as well in the NFC orange juice Spanish exports that totaled 200 thousand tons in 2019, an increase of 12% compared to 2018, when 117 thousand tons of juice were shipped (CITRUSBR, 2020). In the China market the orange juice is expected grow up 5% per year and reach revenues of \$ 1 billion by

2026. On the other hand, in the U.S production is forecast to rise 1% to 4.9 million tons (USDA, 2020).

The contrast between orange juice and other fruit drink is due to the fact the orange juice contain high ascorbic acid content, carotenoid and phenolic compounds, and high nutritional value (Agcam, Akyildiz, & Evrendilek, 2014; Vervoort et al., 2011). Also, this type of beverage has a unique taste that is well liked by consumers (Kim, Lee, Kwak, & Kang, 2013a). However, the sensorial characteristics may vary according to the way the orange juice is presented to the consumer. Different thermal processes affect the flavor profile of orange juice from the sensory and the chemistry flavor (Kim, Jang, & Lee, 2018).

For example, the NFC juice has no sugar addition and is in its natural concentration (Gonçalves, 2009), but the thermal processing used to inactivate microorganisms and enzymes, and to prolong the shelf life may have a negative impact on juice quality, such as loss of flavor and aroma compounds (Janzantti, Machado, & Monteiro, 2011; Mastello, Janzantti, & Monteiro, 2015). The reconstituted juice, on the other hand, is obtained from FCOJ with the addition of water and should present the same quality parameters as the pasteurized juice (Gonçalves, 2009). In this way, during the reconstituted processing, sensory changes of aroma and natural juice flavor may occur, consequently influencing consumers (Janzantti, Machado, & Monteiro, 2011). As to fresh juice, it has no thermal processing (Gonçalves, 2009), thus maintain its natural freshness, however it doesn't have stability, which is guaranteed by thermal processing loses turbidity and characteristic color of citrus juices (Baker & Cameron, 1999; Broeck et al., 2000).

As result the physicochemical compositions of these types of orange juice is different and is related to sensory characteristics of the product. Beyond the most important physical chemical parameters for juice industry, like total soluble solids (TSS), pH, titratable acidity (TA) and maturation index ratio (TSS/TA) (Flores, Colnago, Ferreira, & Spoto, 2016) the monitoring of limonoids is important because they are related with the formation of bitterness, mainly in processed juice after extraction from oranges (Ozaki et al., 1991; Roy & Saraf, 2006; Zaare-Nahandi, Hosseinkhani, Zamani, Asadi-Abkenar, & Omidbaigi, 2008). These compounds are highly oxygenated triterpenoid that occur in high concentrations as aglycones and glucosides in citrus seed and fruit tissues (Silva et al., 2018). In this context, the bitter taste in the orange juice is also associated with the essential oils that are extracted in

the orange-squeezing process (Kimball, 1987), therefore, the bitter taste is more apparent in freshly squeezed orange juice (Dea et al., 2013; Kim, Kim, & Lee, 2016).

Hesperidin is a flavonoids and also prominent component in the composition of orange juice (Peterson et al., 2006), which has been reported exhibit a broad spectrum of beneficial health effects involving the prevention of cardiovascular disease (Roohbakhsh, Parhiz, Soltani, Rezaee, & Iranshahi, 2015). However, China's Light Industry has developed a commercial standard in which a hesperidin concentration above 250 mg/L was recommended as a marker to identify NFC juice (Zhang et al., 2020).

Many studies used sensory analysis for studying the flavor of orange juice, sensory characteristics and flavor chemistry. Kim et al. (2013) applied the descriptive analysis and acceptability test for evaluate the sensory characteristics of commercially available orange juices and identify drivers of liking for orange juices in Korea. Others authors observed correlation among sensory characteristics and furan levelin commercial orange juice products (Kim et al., 2016). Also, the correlation between sensory and instrumental flavor analyses was studied using trained panel and multivariate analysis, and thus the volatile compounds responsible for the desirable flavor characteristics of juices were identified (Kim et al., 2018). However, few studies have aimed the formation of the one trained sensory panel in the citrus industry for evaluating the sensory profile of orange juices of different types and commercial brands, determining the impact of different processing conditions in the sensory evaluation of orange juice, and identifying the correlation of sensory attributes with quality physical-chemical parameters. Accordingly, the objective of the current study is forming a trained panel for sensory evaluation of fresh, whole and reconstituted orange juice from commercial brand sat the beginning, middle and end of the harvest, as well as relating the sensory attributes among themselves and with the current physical chemical quality parameters.

3.2. Material and methods

3.2.1. Samples

Three samples were selected based on different flavor characteristics, processing type and based on market. Among selected juice samples, one sample

was freshly squeezed 100% orange juice (OJ 1), two were commercial orange juice, being the second reconstituted orange juice (OJ 2), obtained by diluting concentrated juice with drinking water up to 11.5° Brix, and other pasteurized orange juice (OJ 3). All samples were obtained of the extractors and process JBT Food Tech. The sensory evaluations of the samples were performed during the beginning, middle and end of the cultivar Pêra Rio. The OJ 1 juice used came from oranges of Pêra Rio cultivar harvested of commercial farms in the state of São Paulo, which they were processed on the same day of the sensory test at the JBT FoodTech company facilities. The OJ 2 samples were supplied by citrus industry and the OJ 3 were acquired in the local market.

3.2.2. Physicochemical analysis

The physical-chemical parameters were analyzed on the basis of which they exert the greatest influence on the sensory characteristics of orange juice. Soluble solids were measured in Brix degrees, with an Auto Abbe refractometer, model 10500/10501, Leica, the result was expressed in °Brix (USDA, 1998). Total acidity was obtained by titrating 10g of homogenized juice in 50 mL of distilled water, with 0.1 N NaOH solution, the values being expressed in % citric acid (AOAC, 1990). Ratio was calculated by the relation content of soluble solids/titratable acidity (USDA, 1998). Ascorbic acid content was obtained titling 35 mL of starch-acid solution and 25 mL of juice sample with 0.1 N iodine solution while under stirring until the first stable blue color appears (AOAC, 1999).

Recovered oil was analyzed in different types of orange juice by the Scott method, distilling a mixture of 25 mL of isopropanol, 25 mL of H₂O with defoamer and 25 mL of sample. After the end of the distillation, 10 mL of HCl with methyl orange solution were added to titrate the distillate with the 0.0247 N KBrO₃-KBr solution for the disappearance of the dye. The amount of titrant used was recorded (FMC Technologies, n.d.; Scott, 1966). Suspended pulp or Bottom Pulp was measured pouring 100 mL of juice through a 20 mesh screen, after screened juice was put on centrifuge tube and centrifuged for 10 min reaching a centrifugation force of 365xg, after centrifugation the pulp volume was read (USDA, 1998).

For the viscosity measure viscometer of low measurement capacity was used and the result was expressed in cP (JBT Corporation, n.d.).

Limonin content was determined in orange juice samples by high performance liquid chromatography (HPLC) following the methodology already used in citrus industries (Shaw & Wilson, 1984) with reverse phase column (Microsorb C18, 150 mm x 4.6 mm, 5 μ particle size) and UV-visible detector. Its concentration (ppm) was calculated from the absorbance of the sample based on a linear regression equation of the absorbance of the standard curve of the peak area in relation to the standard concentration of limonene.

Hesperidin was quantified by the Davis test (Davis, 1947) by centrifuging 50 mL of juice at 1400RPM for 10 minutes. Then, in a test tube with a cap, 25 mL of diethylene glycol, 0.5 mL of the sample supernatant and 0.5 mL of sodium hydroxide (NaOH) 4N were added. After homogenization and remaining at rest for 10 minutes, the 360nm reading on the spectrophotometer was performed.

3.2.3. Trained panel descriptive analysis of orange juice

The sensory panel trained by the quantitative descriptive analysis (QDA) (Stone & Sidel, 1998) adapted method. The training was conducted at the facilities of the citrus pilot plant of JBT FoodTech Company in appropriate place and conditions with disposable material and sanitized place. The study was evaluated and approved by the Research Ethics Committee of ESALQ / USP, process number 94432918.0.0000.5395. The volunteers were trained to evaluate the appearance, aroma, flavor and texture of OJ 1, OJ 2 and OJ 3. The process was started with the recruitment of the volunteers, followed by selection with the basic taste test and threshold test. After the panelists approved were forwarded for the training stage and the panel formed realized the samples final test.

Recruitment

Thirty-seven JBT FoodTech company workers, some experienced at orange juice evaluation and in the age group between 20-30 were guests through questions form to participate the trained sensory. Among them, twenty-eight were men and nine were women. The form included questions such as the frequency with which you consume orange juice, the type of juice most consumed and whether you have any respiratory disease. The ability to use the scale of the volunteers was also assessed.

Selection

First selection stage was made with basic taste recognition test, using chemically pure solutions for basic tastes: sweet (2%), acidic (0,07%), salty (0,2%), bitter (0,07%), umami (0,02%) and neutral. Thirty-seven panelists simultaneously tested 5 coded samples (50 mL) with serving temperature ambient in disposable cups and with random digit numbers. For approval and continuity in the second selection test, the panelist needed 100% correct answers in the first test otherwise, 100% correct answers in the second and third tests.

The second selection test, Threshold test, was carried out by evaluating the threshold for bitter taste of thirty panelists (eight women and twenty-two men), due to the remaining 7 panelists not being approved in the first test. The threshold test was performed for bitter taste, because this parameter is associated with the presence of diseases in citrus fruits (McClean, 1970) and the composition of the fruit (high acidity and low sugar) (Graca, 1991), thus having a very large impact on the flavor of orange juice (Plotto et al., 2010). Therefore, two different samples were prepared, the first with 0.7g of caffeine / liter of fresh orange juice was added and for the second, 1g of caffeine / liter of juice was added. Thus, triangular test (ISO, 2004) was conducted, in which the taster received three 50 mL three samples of orange juice, where two samples was the same concentration of caffeine and another one of different concentration. The samples were encoded by three-digit random numbers, informing that two of them were equal, then offered at room temperature and order of presentation as described by Meilgaard (2006). In total, 10 triangular tests were performed for each panelist, on different days. Only the tasters who scored more than 80% on the triangular test were selected, who participated in the next phase, which consisted of the development of descriptive terminology and training of the panelist.

Lexicon development

Sixteen panelists approved in the selection stages were asked (2 women and 14 men) to develop a lexicon based on the appearance, aroma, taste and mouthfeel

attributes present in the sample set in this study. During the sessions all orange juice samples, OJ 1, OJ 2 and OJ 3, were presented to the panel during the lexicon development stage at room temperature and 50 mL of each juice. Panelist individually evaluated samples using network method (Dutcosky, 1996) and wrote down whatever characteristics they found in each sample. Hedonic and quantitative terms, for example good, were not used. After all samples were evaluated, the panel leader collected all the descriptors and organize with the objective of grouping similar terms that best described the orange juice samples for establishing the juice's sensory profile. Once the panel agreed with the descriptors started to be suggested the potential references. Different references were provided to the panelists, some have been proposed based to experience of some panelists and others based on previous studies (Kim, Lee, Kwak, & Kang, 2013b; Kim, Jang, & Lee, 2018; Kim, Kim, & Lee, 2016). All reference samples were tested and discussed among the panelists until an agreement was reached on the most appropriate references that represented the characteristics of orange juices.

During the sessions, the definitions of the attributes were also discussed and refined in order to avoid redundant terms and gross overlap between descriptive terms. At the end, the list of terms, definitions and references was defined by the panel.

Panelist training

Over 5 days total, many 1.5h sessions were made for the appearance (4), aroma (4), taste (8) and mouthfeel (2) attributes evaluation. All reference samples were produced on the same day and presented in groups regarding appearance, aroma, flavor and mouthfeel. The panelists were invited to read the attribute and reference definition sheets, to remember and discuss among the panelists. They proved the reference samples in the order from lowest to highest attribute intensity. Panelists had a break and returned on the afternoon shift to evaluate the orange juice in triplicate for each sample, in this moment the lexicon developed in the previous stage was provided to each panelist to help them evaluate the samples more accurately. At this stage, the ability of the panelists to use a 9cm unstructured scale to measure the intensity of each attribute was also practiced. The criteria of discriminative power, repeatability and consensus with the panel were used to select

panelists and form the sensory team. Analysis of variance (ANOVA) was made at the 5% level of significance and panelists who had good discriminative power of attributes ($pF_{\text{samples}} < 0.3$), good repeatability ($PF_{\text{repetitions}} > 0.5$) and consensus with the other panelists were selected for the final team (Damásio & Costell, 1991).

Samples definitive test

Panel, was formed by 11 panelists all men's, between 5 panelists who did not carry out the final tests, 2 did not present good discriminative ability and good repeatability, therefore they were not part of the panel. The other 3 panelists did not remain in the study for external reasons. Several days of definitive tests were carried out, so for the OJ 1 juice which corresponds to the fresh juice, it was necessary to use oranges at the beginning, middle and end of the harvest of the Pêra Rio orange. 50 mL each samples (OJ 1, OJ 2 and OJ 3) were offered to the panelists in disposable cups and ambient temperature, with monadic presentation in a randomized order. Panelists analyzed samples in triplicate monadically presented with short intervals between tests, using a 9cm intensity scale where 0= low and 9=high.

3.2.4. Statistical analysis

The performance the panelist was analyzed using PanelCheck 1.4.0 available in open access (<http://www.panelcheck.com>). ANOVA was conducted to test for differences in the appearance, aroma, taste and mouthfeel attributes between 3 different types of orange juice (OJ 1, OJ 2 and OJ 3) at the 5% level of significance. Samples, panelists, and replication were used as sources of variation, using Fisher's protected least significant difference (LSD) at the 5% level of significance to determine the significant difference between samples. Principal component analyzes (PCA) were conducted based on the results of the QDA and physicochemical analysis to observe the differences in sensory quality of orange juice samples. All PCA analyses were performed using The Unscrambler 10.5.1. The correlation between sensory attributes and physical-chemical parameters was assessed with Python software.

3.3. Results and discussion

3.3.1. Descriptive terminology development

In this study, was conducted a serie of descriptive analyses of OJ 1, OJ 2 and OJ 3 were conducted and 47 attributes that describe the juices appearance, aroma, taste and mouthfeel sensory characteristics were obtained through the network method (Fig. 1). Fifteen attributes describe the appearance, eleven the aroma, sixteen the taste and seven the mouthfeel, which suggested that the appearance and taste of orange juice is more complex in terms of its sensory qualities. Most attributes cited by each panelist had a similar definition, as well as, some discrepant terms presented by individual panelists, such as dry, salty, listed in the aroma and flavor attributes, respectively (Fig. 1). Thus, the panelists together with the leader, discussed the grouping of similar terms and the exclusion of discrepant ones, with the exception of the aroma and sweet taste, which was excluded from lexicon as it did not show consensus among panelists during training sessions. At the end, they agreed on 18 useful terms to describe the sensory characteristics of orange juices for the final lexicon, the definitions and references of each term are listed in Table 1. Overall, the generated lexicon (Table 1) was similar to the vocabulary described in other studies (Kim, Lee, Kwak, & Kang, 2013; Kim, Jang, & Lee, 2018).

Figure 1.Sensorial disk of OJ 1 (fresh orange juice), OJ 2 (reconstituted orange juice) and OJ 3 (pasteurized orange juice).

Table 1. Definition and references of orange juice sensory profiles listed in the final lexicon.

Description		Definition	References (0=none, 9=very)
Appearance	Yellow	Yellow color shade of fresh natural orange juice	JBT official showcase None: OJ6 Very: OJ1
	Homogeneous	Orange juice without visual phase separation	None: Finisher juice without pasteurization left in the refrigerator and a spoonful of concentrated juice. Very: Orange nectar.
	Presence of cells	Orange juice that has vesicles in its appearance	None: Powdered orange flavored juice. Very: Juice after extraction.
	Viscous	Characteristic related to the texture of the juice being more viscous, concentrated, thick or dense visually perceived	None: Pasteurized orange juice + water (1:1). Very: Concentrated orange juice + water (1:2).
Aroma	Freshness	Characteristics of freshly extracted orange juice aroma.	None: Powdered artificial orange juice. Very: Orange fresh cut.
	Cooked	Characteristic associated with an overheated or over-processed citrus product	None: Freshly extracted juice. Very: Pasteurized juice cooked in microwave (200 mL for 10 minutes).
	Citric	Characteristics of citrus fruits, such as orange, mandarin, grapefruit and lemon	None: Fresh juice + water (1:2). Very: Fresh juice with added aroma (5 mL of Oil Phase + 5 mL of essential oil).
	Overripe	Aroma associated with excessively ripe fruits or juices stored for excessive periods of time	None: Fresh juice. Very: Over ripe orange
Taste	Acid	Sensation of acid taste on the tongue which increases salivation	None: Fresh orange lime juice. Very: Fresh juice with addition comestible of citric acid (4,825g to 750 mL of juice).
	Bitter	Refers to the bitter taste characteristic of the orange, peel, seed and albedo fractions taste.	None: Fresh orange juice + water (1:3). Very: Fresh juice with caffeine (1,125g to 750 mL of juice).
	Cooked	Refers to the flavor of boiled, boiled orange	None: Freshly extracted juice. Very: Pasteurized juice cooked in microwave (750 mL for 30 minutes).
	Astringent	Feeling of drying or puckering on any of the mouth surfaces	None: Pasteurized juice + water (1:3). Very: Green banana.
	Natural	Characteristic of freshly extracted juice flavor that presents freshness	None: Powdered orange juice. Very: Freshly extracted fresh juice.
	Citric	Characteristics of citrus fruits, such as orange, grapefruit tangerine and lemon	None: Fresh juice +water (1:2). Very: Juice with comestible commercial orange essence.
	Overripe	Flavor present in excessively ripe fruits or juices stored for excessive periods of time	None: Fresh orange juice. Very: Over ripe orange juice
	Fermented	Characteristic flavor of fermented orange, spoiled juice	None: Fresh orange juice. Very: Natural orange juice left out of the refrigerator.
	Mouthfeel	Body	None: Fresh orange juice + water (1:1). Very: Fresh orange juice with addition of crushed cells in the blender.
			None: Orange nectar. Very: Juice after extraction.

3.3.2. Results of the QDA

Evaluation of panel performance

The training, when appropriate, allows the panelists correctly evaluate the samples and reach agreement with the panel, to obtain results that can be analyzed statistically (Ellendersen & Wosiacki, 2010). Thus, testing the consistency of the scoreboard is important and for this, indicators such as discriminative power, repeatability and consensus are used (Meilgaard, Civille, & Carr, 2006). To test the panel performance, the results were test using *Panelcheck* 1.4.0 and the results are shown in Fig. 2 and 3. Fig 2 shows the multivariate analysis method Tucker-1, which allows overview of assessor and panel performance with several attributes. The position of the points on the graph (Tucker-1) provides information about the performance of an individual or the panel as a whole. In this case, the greater the number of important systematic information a panelist presents, the closer it will be to the external ellipse and also, the closer the dots are, the higher the consistency of the whole panel (Tomic et al., 2010). The performance of the 11 panelists can be considered very good for all attributes. In particular for appearance attributes (yellow color; presence of cells and viscous), aroma (freshness; cooked; citric) flavor (cooked and natural) and texture (body), the performance of the panel was very satisfactory, since most or all panelists appear closer to the external ellipse. These same attributes also showed greater agreement in the evaluation of the 11 panelists, as they appear well grouped close to the external ellipse.

Other important information about the panel performance is presented in Fig 3. First, the mean square error (MSE) plot is used to visualize the repeatability of each panel, and the second, F plot (based on the F value), shows the evaluating of the panel's ability to discriminate each attribute (Li, Luo, Wang, Fu, & Zeng, 2019). It was possible to observe that the MSE values were low, indicating that the panel has a good capacity to detect differences. The values found are also similar to the values found by other authors in assessing the performance of sensory teams (Tomic et al., 2010). The F values most of them exceeded the horizontal line at the 1% significance level, and the horizontal line at the 5% significance level, which indicated that the panels had a good sense of distinction for most attributes. That is, based on the relatively low MSE values (good repeatability) and relatively high F values (good

sample discrimination), the panel's performance was good for evaluating the samples.

Figure 2. Eighteen identical Tucker-1 plots with each plot highlighting one of the eighteen attributes used in the profiling.

Figure 3. MSE plot visualizing the repeatability of each panel (A) and F plots visualizing the panels ability to discriminate between the tested samples for each attribute (B).

ANOVA of QDA results

Mean intensity scores of the 4 appearance and aroma, 8 taste and 2 mouthfeel attributes of the samples in the lexicon are listed in tables 2 and 3. For the appearance attributes, OJ 1 showed a higher intensity of cell presence. In contrast, the OJ 2 juice presented a greater number of attributes such as yellow, homogeneous and viscous. And OJ 3, less intensity of the viscous attribute.

Observing the aroma attributes, the OJ 1 sample, as expected, showed more attributes of fresh fruit, such as freshness and citrus aroma. And the OJ 2 sample showed greater intensity of aroma attributes like processed orange as a cooked and overripe flavor. Observing the taste attributes, OJ 2 presented a greater intensity of bitter and acid flavor. These results are in line with other studies that reported a higher intensity of bitter and acidic flavors in “shelf-stable” orange juices (Lotong, Chambers, & Chambers, 2003).

The flavor attributes related to fresh fruit such as citrus and natural flavor showed greater intensity in OJ 1 juice, as it did not undergo any thermal process. However, OJ 2 showed greater intensity of the astringent flavor. In this context, some acids present in orange juice, such as citric, malic and succinic acids (Kelebek & Selli, 2011) are known to be astringent in levels found in orange juices (Thomas Corrigan & Lawless, 1995). Therefore, it is important to highlight that OJ 2, which was judged by the panelists as the most astringent, was also evaluated with greater intensity of acid flavor, being in accordance with the information regarding the compounds responsible for the astringency of orange juice. The attributes of cooked, overripe and fermented flavor were differentiated by the processing conditions. OJ 2 had a higher intensity of these flavors.

Mouthfeel attributes were also differentiated by the processing conditions; OJ 2 presented more body and OJ 1 more presence of cells, as for the body attribute, OJ 2 to OJ 3, some values did not show significant difference ($P < 0.05$).

Table 2. Mean intensity scores and separation of appearance and aroma attributes for orange juices.

Sample	Appearance				Aroma			
	Yellow	Homogeneous	Presence of cells	Viscous	Freshness	Cooked	Citric	Overripe
OJ 1.1	4.9 ^d	3.8 ^g	3.1 ^{bc}	4.0 ^{dc}	5.8 ^b	0.7 ^h	4.1 ^{bcd}	0.5 ^h
OJ 1.2	6.4 ^{ba}	3.7 ^g	3.2 ^{ba}	4.6 ^a	6.6 ^{ba}	0.6 ^h	5.5 ^a	0.5 ^h
OJ 1.3	5.3 ^c	3.8 ^g	2.6 ^{bc}	4.5 ^{ba}	6.6 ^{ba}	0.5 ^h	5.4 ^a	0.4 ^h
OJ 1.4	5.9 ^{bac}	4.9 ^f	4.0 ^a	4.9 ^a	7.0 ^a	0.5 ^h	5.1 ^{ba}	0.4 ^h
OJ 1.5	1.8 ^h	5.6 ^{fe}	2.4 ^{dc}	2.8 ^{ef}	6.4 ^{ba}	0.7 ^h	4.8 ^{ba}	0.7 ^{hg}
OJ 2.1	6.4 ^a	5.9 ^{de}	1.9 ^d	4.4 ^{bac}	2.3 ^{hg}	4.2 ^b	2.6 ^{ef}	1.4 ^{ed}
OJ 2.2	2.7 ^g	6.2 ^{dec}	2.8 ^{bc}	3.7 ^{bdc}	4.1 ^{dce}	2.1 ^{fe}	3.9 ^{bc}	1.1 ^{egf}
OJ 2.3	6.5 ^a	6.5 ^{bdac}	0.8 ^e	4.8 ^a	1.6 ^h	5.1 ^a	2.3 ^f	1.9 ^{bc}
OJ 2.4	5.7 ^{bc}	7.2 ^a	0.6 ^e	3.8 ^{dc}	2.4 ^{fg}	5.2 ^a	2.4 ^f	2.6 ^a
OJ 2.5	3.9 ^{ef}	6.7 ^{bdac}	1.0 ^e	3.5 ^{ed}	2.8 ^{fge}	3.2 ^{dc}	2.2 ^f	1.7 ^{dc}
OJ 3.1	1.9 ^h	6.5 ^{bdac}	0.8 ^e	2.2 ^{gf}	3.9 ^{dc}	2.4 ^{dfe}	3.3 ^{ecd}	1.1 ^{edf}
OJ 3.2	3.8 ^{ed}	6.4 ^{bdec}	0.9 ^e	2.5 ^{ef}	3.4 ^{dfe}	2.8 ^{dce}	2.6 ^{ef}	2.0 ^{ba}
OJ 3.3	2.0 ^h	6.8 ^{bdac}	0.5 ^e	1.8 ^g	3.6 ^{dce}	1.2 ^{gh}	3.5 ^{bc}	0.7 ^{hgf}
OJ 3.4	3.3 ^{gf}	7.0 ^{bac}	0.5 ^e	2.2 ^{gf}	3.9 ^c	1.6 ^{gf}	3.0 ^{efd}	0.8 ^{hgf}
OJ 3.5	6.3 ^{bac}	7.0 ^{ba}	0.8 ^e	3.6 ^{ed}	3.1 ^{dfce}	3.4 ^c	2.5 ^{ef}	2.1 ^{bac}

Note: Means with different superscripts within a column are significantly different ($p \leq 0.05$) according to Fisher's protected least significant difference test (LSD). Because the OJ samples were analyzed by the sensory panel during the beginning, middle and end of harvest of the cultivar Pera Rio, the number next to the acronym OJ corresponds to the sample evaluation period.

Table 3. Mean intensity scores and separation of taste and mouthfeel attributes for orange juices.

Sample	Taste								Mouthfeel	
	Acid	Bitter	Cooked	Astringent	Natural	Citric	Overripe	Fermented	Body	Presence of cells
OJ 1.1	3.5 ^{cb}	1.2 ^{ed}	0.6 ^{hg}	1.5 ^{cebd}	6.4 ^a	5.1 ^{bc}	0.4 ^e	0.7 ^g	3.8 ^{bdec}	1.4 ^b
OJ 1.2	3.4 ^{cb}	0.8 ^e	0.9 ^{hfg}	1.1 ^{ced}	6.4 ^a	5.3 ^{ba}	0.6 ^{ced}	0.9 ^{gfe}	4.1 ^b	1.3 ^b
OJ 1.3	3.8 ^b	1.4 ^{ced}	0.6 ^{hg}	1.0 ^{ed}	6.5 ^a	6.0 ^a	0.6 ^{ced}	0.9 ^{gfe}	3.7 ^{bdac}	1.0 ^{cb}
OJ 1.4	3.1 ^{cbd}	1.2 ^{ed}	0.5 ^h	1.2 ^b	6.9 ^a	5.8 ^{ba}	0.4 ^e	0.8 ^{gf}	4.0 ^{bdac}	2.1 ^a
OJ 1.5	3.0 ^{cbd}	2.1 ^{cb}	0.9 ^{hfg}	1.4 ^{cebd}	6.5 ^a	4.8 ^{bc}	0.5 ^{ed}	0.8 ^{gf}	2.3 ^{fg}	1.4 ^b
OJ 2.1	4.8 ^a	2.0 ^{cb}	4.1 ^b	1.7 ^{cbd}	3.0 ^{ed}	4.5 ^{bc}	1.2 ^{cb}	1.5 ^c	4.4 ^{bac}	0.8 ^{cd}
OJ 2.2	5.4 ^a	1.5 ^{cbd}	2.5 ^d	1.6 ^{cb}	3.9 ^{cbd}	5.1 ^b	1.3 ^b	3.0 ^a	3.8 ^{bdac}	2.1 ^a
OJ 2.3	3.0 ^{cbd}	2.0 ^{cb}	5.3 ^a	1.6 ^{cebd}	2.3 ^e	3.0 ^{ef}	2.2 ^a	1.4 ^{dc}	4.6 ^a	0.7 ^{cd}
OJ 2.4	3.8 ^b	2.3 ^b	5.0 ^a	2.8 ^a	2.6 ^e	3.3 ^{ef}	2.5 ^a	2.1 ^b	3.6 ^{dec}	0.5 ^d
OJ 2.5	5.5 ^a	5.3 ^a	3.0 ^{cd}	2.9 ^a	2.9 ^{ced}	3.9 ^{ecd}	1.1 ^{cb}	1.2 ^{dfce}	3.5 ^{de}	0.9 ^{cd}
OJ 3.1	3.0 ^{cbd}	1.7 ^{cbd}	2.2 ^{ed}	1.7 ^{cbd}	4.0 ^b	3.4 ^{ef}	1.0 ^{cbd}	1.3 ^{dce}	1.9 ^g	0.5 ^d
OJ 3.2	2.4 ^{cd}	1.0 ^{ed}	3.3 ^{cb}	1.1 ^{ced}	3.3 ^{ed}	2.8 ^f	2.1 ^a	1.3 ^c	2.8 ^{fe}	0.5 ^{cd}
OJ 3.3	3.2 ^{cbd}	0.9 ^e	1.4 ^{efg}	0.9 ^e	4.2 ^b	4.1 ^{bcd}	0.6 ^{ced}	1.0 ^{gdfe}	2.0 ^g	0.4 ^d
OJ 3.4	2.4 ^d	0.8 ^e	1.5 ^{ef}	1.2 ^b	4.5 ^b	3.8 ^{efd}	0.7 ^{ced}	1.1 ^{gdfe}	2.1 ^g	0.5 ^d
OJ 3.5	2.4 ^d	1.5 ^{ced}	2.6 ^d	1.1 ^{cebd}	3.8 ^{cb}	3.5 ^{efd}	2.2 ^a	1.3 ^{dce}	3.7 ^{de}	0.7 ^{cd}

Note: Means with different superscripts within a column are significantly different ($p \leq 0.05$) according to Fisher's protected least significant difference test (LSD). Because the OJ samples were analyzed by the sensory panel during the beginning, middle and end of harvest of the cultivar Pera Rio, the number next to the acronym OJ corresponds to the sample evaluation period.

3.3.3. PCA analysis of the QDA and physicochemical results

The Principal components analysis (PCA) also confirmed (Fig. 4) sensorial differences between the OJ1, OJ 2 and OJ 3, but mainly between OJ1 and OJ 2. Fig. 4A shows 49% of the total variability in the dataset, with PC1 31% and PC2 18% of the variability. OJ 1 and OJ 2 were greatly differentiated by PC 1, where OJ 1 was located on a negative PC1, and OJ 2 located on a positive PC1. On the other hand, OJ 3 appeared positively charged in PC2, with some samples appearing mixed with OJ 1 and OJ 2. This overlap of the OJ 3 over the other juice samples, can be explained by the fact that some days of sensory analysis, according Anova results the OJ 3 samples did not show significant difference ($p < 0.05$) with the OJ 2 samples, in some attributes such as cell presence, homogeneity and viscosity (Table 2 and 3). In the same way that OJ 3 did not present significant difference in some days of test with OJ 1, for the attributes of yellow color and astringency for example.

Related to the citrus flavor and aroma attributes, fresh aroma and natural flavor, these were negatively loaded in PC1, while the overripe and cooked aroma and flavor attributes were positively loaded in PC1 (Fig. 4B). Appearance and texture attributes such as presence of cells, viscous and body, respectively, were negatively loaded in PC2 (Fig. 4B). That is, the results shown in figures 4 and 5 are in accordance with the sensory differences found between the samples in the ANOVA analysis. OJ 1 was characterized by fresh and citrus aroma, natural and citrus flavor (Fig. 4B). OJ 2 characterized by the overripe and cooked aroma and flavor and a little also for the bitter and fermented taste. And OJ 3 was strongly associated with the appearance attributes (viscosity and presence of cells) and texture (body), that showed less intensity of these attributes in relation to other juices. And a little by the acid flavor and yellow color with lower values in relation to OJ1 and OJ 2.

On the other hand, PCA of the physical-chemical analyzes (Fig. 5) differentiated OJ 2 from OJ 3 through PC 1, responsible for 35% of the variation of the dataset, and PC 2 separated OJ 1 from OJ 2 and OJ 3, being responsible for 19 % of the dataset. The OJ 1 which was characterized by the sensory panel with a greater intensity of citrus and fresh aroma and natural and citrus flavor, in this PCA was also more characterized by the content of centrifuged pulp, hesperidin, recovered oil and strongly characterized by the lower soluble solids value ($^{\circ}\text{Brix}$) (Fig. 5B). Bottom pulp or centrifuged pulp consists of suspended material which is present

in fresh juice, which contributes to the natural flavor of the OJ 1 juice (Redd, Hendrix, & Hendrix, 1986). The oil content in the juice contributes to its flavor, and must be in appropriate quantities to ensure the aspect of freshness that was found in OJ 1 (Redd et al., 1986). And the lower TSS ($^{\circ}$ Brix) influenced the high intensity of the citrus aroma and flavor attribute evaluated in the OJ 1 sample (Flores et al., 2016).

The OJ 2 sensorially was characterized by the aroma and the overripe and cooked flavor, but also a little by the bitter and fermented flavor, was differentiated in its physical-chemical composition by the acidity, limonin and centrifuged pulp (Fig. 5B) and for the low values of Ratio and recovered oil. In this context, it is believed that acidity contributes to the intensity of the fermented flavor, and limonin in the bitterness of OJ 2 juice (Chandler & Kefford, 1966; Manners, 2007; Manners, Jacob, Andrew III, Schoch, & Hasegawa, 2003). Limonin is a limonoid and is responsible for bitter taste in the citrus juice, such as orange, grape etc. In the juice extraction, the limonite β tasteless precursor from the juice sacs comes in contact with the acidic component of the juice and is gradually lactonized to form bitter limonin (Saraf et al., 2019). The threshold of limonin was reported to be 6mg/L in orange (Guadagni, Maier, & Turnbaug, 1973), however, studies indicate that the limonine threshold could be modified by adjusting the pH with the addition of citric acid or sucrose. In model solution or orange juice, the highest limonine threshold (lowest bitterness) was observed at pH 3.8 (Guadagni et al., 1973). In this context, it is important to note that OJ 2, which had a higher intensity of bitter taste by ADQ, was characterized in PCA (Fig 5B) mainly by limonin and acidity. The recoverable oil content of OJ is recurrent of the thermal processing that FCOJ juices undergo and the low Ratio may have influenced on the overripe taste that OJ 2 presented.

Finally, OJ 3 characterized by the sensory team as less intense viscosity attributes and presence of cells related to the appearance and body attribute related to texture, was also characterized by the lower content of centrifuged pulp (Fig. 5B). The OJ 3 was judged by the sensory panel as the one with the lower acid taste, according to the physical-chemical characterization made where it was differentiated by the lower titratable acidity.

Figure 4. Principal component of QDA data (A) and scores of PCA (B). The attributes preceded by the letters: Ap-corresponding to the appearance attributes, A- Aroma, T- flavor and M-mouthfeel.

Figure 5. Principal component of physicochemical data (A) and scores of PCA data.

3.3.4. Relationship between the QDA and physicochemical results

Figure 6 shows the correlation map between the sensory attributes and physical-chemical parameters. The squares that have a darker color mean that Pearson's correlation coefficient between the variables is closer to 1, while those with white color mean that Pearson's correlation coefficient is closer to -1. Thus, the correlation map demonstrated that the attributes of freshness and citrus aroma, natural and citrus flavor and parameters related to Ratio and recoverable oil correlate with each other and together they have information in common regarding the sensory quality of OJ 1 juice, since these were the parameters of greatest influence in the characterization of OJ 1 observed in the PCA of sensory and physical-chemical attributes. On the other hand, it is possible to observe the formation of another group of similar characteristics that contains information in common regarding the OJ 2 juice, they are the attributes of homogeneity, overripe and cooked aroma, overripe, cooked, fermented, bitter and astringent flavor and the Brix value. Finally, a large group of similar characteristics were formed, referring to acid flavor, presence of cells (appearance and mouthfeel), viscosity, yellow color (appearance), body (mouthfeel) and physicochemical parameters of acidity, limonin, hesperidin, centrifuged pulp, vitamin C and viscosity, to explain the quality of OJ 3 juice.

Therefore, the correlation map confirmed the sensorial characterization of the samples obtained by the Anova analysis and also confirmed the sensorial and physical-chemical differences between OJ 1, OJ 2 and OJ 3 observed by the PCA.

Figure 6. Correlation Maps of the sensory attributes and physical-chemical parameters.

3.4. Conclusion

A sensory panel was trained to evaluate the quality of fresh, reconstituted and pasteurized orange juice with the QDA method. The panelists demonstrated good performance in the evaluation of the samples, being able to obtain accurate and reliable results in the evaluation of the sensory profile of orange juice. Overall, 4 appearance and aroma, 8 taste and 2 mouthfeel terminologies were developed and evaluated by eleven trained panelist. The QDA results showed that aroma, freshness, citric, and taste natural, citric were characteristic attributes to distinguish OJ 1 (fresh orange juice). The OJ 2 (reconstituted juice) was characterized by the aroma cooked and overripe, taste cooked, overripe, bitter, fermented and astringent.

And OJ 3 by the lower appearance viscous, yellow, lower taste acid and texture body and presence of cells.

The QDA results and physical-chemical parameters are consistent and mutually complementing, through the use of tools such as PCA and correlation map in the exploration of the data, results obtained were accurate and applicable to the evaluation of the quality of orange juices.

This study developed a terminological lexicon that can assist in the evaluation and quality control of orange juices with great sensory differences.

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4. APPLICATION OF NEAR AND MEDIUM INFRARED TO THE SENSORY EVALUATION OF ORANGE JUICE

Abstract

Two instrumental techniques, medium and near infrared spectroscopy (MIR and NIR) have been used to predict and classify orange juice sensory descriptors. The reference sensory values were provided by a taste panel trained to assess sensory quality of orange juice by the QDA method. Predict models were developed using partial least squares analysis (PLS) and, with the best results, the classification models by partial least squares discriminant analysis (PLS-DA) were constructed. PLS models using MIR spectra showed the potential to predict some attributes in orange juice such as yellow color, freshness aroma, bitter and natural flavor, with R^2 higher 0.5 and error between 10-17%. They also showed correlation with the descriptors related to the presence of cells, citrus and cooked aroma, acid and cooked flavor (R^2 between 0.39-0.43 and error between 9.54-19%). The PLS-DA models built with the best results obtained in the predictive models were able to discriminate the samples for the descriptors yellow, taste natural, aroma freshness, aroma and taste cooked with predictive abilities between 70% and 87%. And thus, demonstrating to be more applicable for the evaluation of the sensory quality of orange juice than the PLS prediction models. MIR method combined with multivariate analysis has the potential to be a quick and non-destructive analysis of sensory attributes in orange juice.

Keywords: Orange juice, mid-infrared spectroscopy, near-infrared spectroscopy, sensory analysis, multivariate analysis.

4.1. Introduction

Orange juice is one of the most requested beverages by consumers due the fresh attributes presented by its bioactive compounds namely, flavonoid, limonoid, carotenoid and ascorbic acid (AA) (Charfi, Boujida, Abrini, & Senhaji, 2019) and also has a unique taste that stands out among other fruit juices (Kim, Lee, Kwak, & Kang, 2013). The nutritional benefits associated with orange juice consumption such, reduction risk of obesity, decreased risk of contracting urinary stones (Wabner & Charles, 1993), and high vitamin C content (Kim, Kim, & Lee, 2016), also have been reported.

In this context, orange production was 35.3% higher than that of the 2018/2019 season and recorded record productivity of 1,045 boxes per hectare (CITRUSBR, 2020a). This increase in relation to the previous harvest shows the biennial orange production cycle, when larger harvests are alternated with smaller harvests (CITRUSBR, 2020a). However, large producers such as Florida showed a

reduction of 1% in the harvest of oranges with a drop of 1 million boxes and production of 71 million boxes, according to the new forecast made for the 2019/2020 harvest (CITRUSBR, 2020b). Therefore, it is noted the importance of monitoring the quality of orange juice both on a large scale in a scenario of high productivity, and in maintaining product quality in times of lower production.

The freshly squeezed fresh orange juice maintains the freshness characteristics of the orange, however it does not have great durability. For this and for reasons of accessibility and logistics, many fruits are processed or preserved industrially to reach target consumers (Wahia et al., 2020). NFC (Not From Concentrate) orange juice manufacturing process involves squeezing fresh oranges, followed by the centrifugation and pasteurization process steps (Kim, Jang, & Lee, 2018). In addition, FCOJ (Frozen Concentrate Orange Juice) require the partial water removal. In sequence the reconstituted juice, obtained from FCOJ with addition of water, must comply the same quality parameters of pasteurized juice (NFC).

Thermal treatments applied to the conservation of orange juice, however, affect the flavor and chemical profile of the product (Kim et al., 2018). Previously, the quantitative descriptive analysis (QDA) was used to identify quality sensory attributes of different types of orange juice (Kim, Lee, Kwak, & Kang, 2013). In this study, 6 samples of reconstituted juice and 1 sample of fresh orange juice, conditioned in plastic, glass and cardboard cartons were analyzed by a trained sensory panel. The sensory characteristics of the juices were grouped, where group 1 samples were characterized by high in natural citrus flavors such as orange peel, orange flesh, citrus fruit, and grapefruit, whereas group 2 samples were characterized by processed orange-like flavors such as over-ripe, cooked-orange, and yogurt. Likewise, QDA also showed orange juices that require refrigerated storage had a high intensity of orange peel flavor and stable juices on the shelf had a high intensity of cooked orange flavor (Kim et al., 2018).

The main characteristics responsible for the flavor and aroma of orange juice are also identified by instrumental analysis (Baxter, Easton, Schneebeil, & Whitfield, 2005; Bi, Sun, Lao, Liao, & Wu, 2020). Compounds such, terpinolene, octyl acetate, carveol, carvone, linalyl acetate and δ -elemene, identified by the Headspace Solid Phase Microextraction (HS-SPME) technique, characterized non-processed orange juice, while ethyl butanoate, octanal, 1-octanol, linalool, ethyl 3-hydroxyhexanoate,

nootkatone and ethyl octanoate characterized processed orange (Mastello, Janzanti, Bisconsin-Júnior, & Monteiro, 2018).

Studies also show that sensory analysis can be interpreted together with a set of instrumental data to obtain complete food quality (Kim et al., 2018, 2016), and the relationship between both methods has been explored (Borràs et al., 2016a, 2016b, 2015). Instrumental data were combined with the response of sensory descriptors trained to analyze olive oil quality. Several predictive models were built using partial least squares regression (PLSR), where the attributes of fruity, bitter, fusty and musty showed better predictive performances (Borràs et al., 2016b).

However, most studies have evaluated the use of instrumental techniques such as mass spectrometry (MS) (Aishima, 2004), gas chromatography (GC) (Taylor & Linfoth, 2003) and ^1H nuclear magnetic resonance (NMR) spectroscopy (Malmendal et al., 2011) to predict sensory descriptors, which are destructive techniques, high cost and do not allow for large-scale analysis. In this context, medium and near infrared analysis can be an alternative for measuring food quickly, non-destructively and at a lower cost (Bureau, Cozzolino, & Clark, 2019; Nicolai et al., 2007; Schulz H., 2009; Viegas, Mata, Duarte, & Lima, 2016).

Infrared spectroscopy has been very developed in agriculture in the last 40 years, with great development in the late 80's, in the measurement of cereals, fruits, meat, among others (Bellon-Maurel & McBratney, 2011). This technique is based on the property of the molecules to absorb infrared light and to have a wide variety of vibrational movements characteristic of its composition when absorbing this infrared light (Oliveira, de Castilhos, Renard, & Bureau, 2014). The medium infrared spectroscopy (MIR) is in the range of $4000\text{-}400\text{cm}^{-1}$ and provides information on the fundamental molecular vibrational frequencies, on the other hand, the near infrared spectroscopy (NIR) is in the range of $2500\text{-}700\text{nm}$, containing information of overtones and their combinations (Shiroma & Rodriguez-Saona, 2009). NIR spectra are more sensitive to factors that influence light diffusion as physical structure and the presence of water in the sample, due the light diffusion is much greater in NIR range (Williams & Norris, 1987). However, compared to MIR the bands in the NIR spectra are less specific and thus, the MIR is highly sensitive to chemical composition of samples (Bellon-Maurel & McBratney, 2011). These differences between MIR and NIR spectroscopy show that one of them depending on the objective may be more appropriate. Therefore, the aim of this study was to evaluate

the performance of MIR and NIR spectroscopy in the prediction and classification of sensory descriptors of different types of orange juice.

4.2. Material and methods

4.2.1. Samples

Eighteen sensory attributes, four of appearance and aroma, eight of taste and two of mouthfeel were analyzed by the sensory panel of the JBT FoodTech Brazil Company as described in the Chapter 2 of the current thesis. The samples selected for analysis were freshly squeezed 100% orange juice (OJ 1), two commercial orange juice, one reconstituted orange juice (OJ 2), obtained by diluting concentrated juice with drinking water up to 11.5 °Brix, and other orange juice pasteurized (OJ 3). All samples were obtained the extractors and process JBT Food Tech. OJ 1 used the fruits at the beginning, middle and end of harvest the Pêra Rio cultivar, which they were processed on the same day of the sensory test. The OJ 2 samples were supplied by citrus industry and the OJ 3 were acquired in the local market.

4.2.2. Physicochemical analysis

In addition to the characterization of the sensory profile and instrumental, the orange juice samples were also subjected to physical-chemical characterization for the main parameters related to sensory changes in the juices. Soluble solids were measured in Brix degrees, with an Auto Abbe refractometer, model 10500/10501, Leica, the result will be expressed in °Brix (USDA, 1998). Total acidity was obtained by titrating 10g of homogenized juice in 50 mL of distilled water, with 0.1 N NaOH solution, the values being expressed in% citric acid (AOAC, 1990). Ratio was calculated by the relation (content of soluble solids/titratable acidity) (USDA, 1998).

Recovered oil analyzed in different types of orange juice by the Scott method, distilling a mixture of 25 mL of isopropanol, 25 mL of H₂O with defoamer and 25 mL of sample. After the end of the distillation, 10 mL of HCl with methyl orange solution were added to titrate the distillate with the 0.0247 N KBrO₃-KBr solution for the disappearance of the dye. The amount of titrant used was recorded (FMC Technologies, n.d.; Scott, 1966). Suspended pulp was measured pouring 100 mL of

juice through a 20 mesh screen, after screened juice was put on centrifuge tube and centrifuged for 10 min after reaching a centrifugation force of 365xg, after centrifugation the pulp volume was read (USDA, 1998).

For the viscosity measure viscometer of low measurement capacity was used (JBT Corporation, n.d.). Ascorbic acid content was obtained titling 35 mL of starch-acid solution and 25 mL of juice sample with 0.1 N iodine solution while under stirring until the first stable blue color appears (AOAC, 1999).

Limonin content was determined in orange juice samples by high performance liquid chromatography (HPLC) following the methodology already used in citrus industries (Shaw & Wilson, 1984) with reverse phase column (Microsorb C18, 150 mm x 4.6 mm , 5 μ particle size) and UV-visible detector. Its concentration (ppm) was calculated from the absorbance of the sample based on a linear regression equation of the absorbance of the standard curve of the peak area in relation to the standard concentration of limonene.

Hesperidin was quantified by the Davis test (Davis, 1947) by centrifuging 50 mL of juice at 1400RPM for 10 minutes. Then, in a test tube with a cap, 25 mL of diethylene glycol, 0.5 mL of the sample supernatant and 0.5 mL of sodium hydroxide (NaOH) 4N were added. After homogenization and remaining at rest for 10 minutes, the 360nm reading on the spectrophotometer was performed.

4.2.3. Sensory analysis

Sensory analysis was performed by the trained panel according to the adapted ADQ method (Meilgaard, Civille, & Carr, 2006), also used by other authors for sensory evaluation of orange juice (Kim et al., 2018, 2016, 2013; Plotto et al., 2010). This study was evaluated and approved by the Research Ethics Committee of ESALQ / USP, process number 94432918.0.0000.5395. The stages of formation and training of the panel, as well as the validation of the sensory data are described in the previous chapter. Were used 50mL of each sample were taste in disposable cups and ambient temperature. All samples were coded with random three-digit numbers and offered to panelists at random and monadic presentation. Panel evaluation was performed in triplicate with short intervals between evaluations. The descriptors intensity was assessed using a 9cm unstructured scale. The 11-member panel evaluated a total of 15 samples on different days, obtaining 135 results of the

sensory profile of OJ1, OJ 2 and OJ 3. All samples were analyzed by the panel and instrumental methods of MIR and NIR in the same day. The spectra were acquired from rates collected from each panelist.

4.2.4. Instrumental analysis

MIR spectroscopy

A FT-MIR Cary 630 (Agilent, WalnutCreek, CA, EUA) spectrometer was used with DialPath accessory, suitable for measurements on liquid samples. Spectra were collected at room temperature over the range $4000\text{-}650\text{cm}^{-1}$, at 2cm^{-1} resolution and with 64 scans both for background and samples. 100 μL of each orange juice sample was homogeneously placed over the ZnSe multi bounce ATR crystal and the DialPath accessory was positioned in cell 1 to acquire the spectra. Spectral acquisitions were carried out with the MicroLab software. Three replicates were measured per sample.

NIR spectroscopy

The orange juice samples were also measured by near infrared spectrophotometer (NIR) MicroNIR Onsite W model (VIAVI, 2019), which has two tungsten lamps as its radiation source. Spectra were collected at room temperature over the range 900-1700 nm and integration time of 10 ms and 150 scans. SciR software (Solutions, 2019) was used for data acquisition, where they are exported already processed with standard normal variation (SNV), first derivative with Smoothing (Savistky-Golay, 2^o degree polynomial with 5 points window size). Three replicates were measured per sample.

4.2.5. Statistical analysis

Physicochemical characterization dataset were shown using plot pairwise relationship using Python 3.6 data science routines. By default, pairplot or kernel density estimation (KDE) plot create a grid of axis such that each numeric variable in data will be shared in the y-axis across a single row and in the x-axis across a single

column. The diagonal axis are treated differently, drawing a plot to show the univariate distribution of the data for the variable in that column.

Exploratory analysis in NIR and MIR data were performed after aligning spectrometer signals with their corresponding reference values for sensory attributes. Principal component analysis (PCA) was applied in order to understand the influence of each wavelength (loadings), extracting the variance of the initial NIR and MIR data in each principal component. In addition, for MIR data a PCA orthogonalization was performed, extracting the 20 first principal components of each spectrometer sample data aiming to improve classification models accuracy.

To perform partial least squares regression (PLS) and discriminant analysis (PLS-DA) was used Kennard-Stone algorithm to split initial dataset in two with 70% for training and 30% for testing (Kennard & Stone, 1969). PLS models for MIR were trained and tested using entire spectrometer data preprocessed as described in Table 1. PLS models for NIR were trained and tested using entire spectrometer data preprocessed with first derivative of Savitzky-Golay using 2-degree polynomial and 5 smoothing points. The number of factors was chosen according to the lowest cross-validation RMSECV. Best MIR sensory PLS parameters were chosen to build PLS-DA models using spectrometer orthogonalized data by PCA where classes were defined based on median of each sensory parameter chosen. The data structure was organized, analyzed, and evaluated using software The Unscrambler X 10.5.1 (Camo Analytics, 2019).

4.3. Results and discussion

4.3.1. Physicochemical characterization

Figure 1 shows the kernel density pairplots for the orange juice physicochemical parameters evaluated by the sensory panel. It is possible to compare the analyzed parameters between the three types of juice. Orange juice samples show high different normal distribution for parameters TSS, recovered oil and hesperidin.

OJ 1 juice presented higher hesperidin content, mainly compared to OJ 3 (NFC). Studies evaluating the effects of orange juice storage also found a higher concentration of hesperidin in freshly squeezed orange juice than NFC juice, which

had been stored for some time (Zhang et al., 2020). However, the same study suggests that the decrease in the concentration of hesperidin in the juice is not caused by heat treatment or enzymatic degradation, but would gradually precipitate from orange juice during storage, which might arise from the decomposition of vitamin C. OJ 2 juice presented higher titratable acidity and also a higher limonin content. Limonin is primarily responsible for bitterness in orange juice, and the development of unpleasant flavors in orange juice is due to the processing of the beverage that results in the thermal degradation of the components (Lane, 2018). And so, before being bottled and marketed, the juice passes through a resin column to remove the bitterness (Lane, 2018). On the other hand, OJ 3 showed lower values of centrifuged pulp, viscosity and vitamin C compared to other juices. Bottom pulp when recorded 14% or more is an indication of high pressures used in extraction and finishing of the juice (JBT, n.d.). However, ripe fruit or fruit damaged by freezing can also result in large amounts of inferior pulp (JBT, n.d.).

Figure 1. Kernel density pairplots for the physic-chemical parameters of OJ1, OJ 2 and OJ 3.

4.3.2. MIR and NIR spectra interpretation

Fig. 2A shows the average of the MIR spectra of OJ1, OJ 2 and OJ 3 juices. The major components of orange juice are simple sugars (glucose, fructose and sucrose) and citric acid (Huang, Rasco, & Cavinato, 2009). Therefore, the region located between 1800 and 900 cm^{-1} is of great interest, as they are related to CO

stretching bonds in 1722 and CO stretching to 1226 cm^{-1} , related to the presence of carboxylic acid (Bichara, Lanús, Ferrer, Gramajo, & Brandán, 2011; Max & Chapados, 2004). Peaks located at 1135, 1105, 1054, 998 and 926 cm^{-1} are also noteworthy, as they are indicative of stretching and bending modes in sugars (Kačuráková & Mathlouthi, 1996).

The MIR and NIR measurements, especially for sugar analysis, have great sensitivity. The absorption zone in the MIR spectrum between 1500 and 900 cm^{-1} corresponds mainly to the presence of sugars and organic acids (Bureau et al., 2009). The CH and CO bonds, present in sugars and organic acids (Ścibisz et al., 2011), can be associated peaks at 1200 to 900 cm^{-1} and 1500 and a 200 cm^{-1} , respectively (Skoog, Holler, & Crouch, 2007). Regarding the NIR spectra, the band in 1190 nm is in agreement with the second and third CH overtone regions and the peak of 1790 nm overlaps with the first CH overtone region, both associated to sugar solutions (Osborne, Fearn, & Hindle, 1993).

Figure 2B also shows orthogonalization of the MIR spectra in order to avoid grouping the samples according to the day of analysis and remove spectral variations not related to attributes. For this, data on the first main component were excluded. However, orthogonalized matrices were used only to build the PLS-DA classification models. In order not to lose the number of spectral information, regression models via PLS used non-orthogonalized matrices.

Regarding the NIR spectra presented in Fig.3, absorption bands observed are the second (920 nm) and the third (720 nm) overtones of O-H stretching, and the third (910 nm) and the fourth (750 nm) overtones of C-H stretching, they are typically found in fruits and associated to starch and sugars (Golic, Walsh, & Lawson, 2003; Subedi, Walsh, & Owens, 2007). In the first three overtones at about 1445 , 1000 and 800 nm are present O-H bands related to monomeric organic acids (Goddu & Delker, 2002). However, all of these absorption bands are located very close to the strongest water absorption regions, making it difficult to visualize (Delwiche, Mekwatanakarn, & Wang, 2008).

Figure 2. MIR spectra of OJ1, OJ 2 and OJ 3 juice (A). MIR spectra of OJ 1, OJ 2 and OJ 3 juice after data orthogonalized (B).

Figure 3. NIR spectra of OJ 1, OJ 2 and OJ 3 juice.

4.3.3. Partial least squares analysis

Table 1 show the PLS models obtained for each descriptor using two instrumental techniques modeled individually. The best predictive results were obtained from the MIR data, and the best predicted descriptors were yellow, presence of cells (appearance), aroma freshness, citric and cooked, and flavor acid, bitter, cooked and natural. Descriptors such, yellow, aroma freshness, taste bitter and natural yielded correlations higher than 0.5 (R^2) and errors around 10% (RMSECV) for bitter, 14% for yellow and close to 17% for aroma freshness and taste natural. On the other hand, presence of cells, aroma citric and cooked, taste acid and cooked shows correlations between 0.39-0.43 and errors between 9.54-19%.

These results are consistent with the literature in the case of the bitter responses. In foods studied such as olive oil, the presence of non-volatile compounds characteristic of the phenolic fraction of olive oil is related to the taste of bitter taste, which is strongly associated with the signs of MIR (Angerosa et al., 2004; Kalua et al., 2007).

The correlation between citric aroma and acid taste are due to the fact that both descriptors are associated with the presence of organic acids present in orange juice, and these in turn, showed specific peaks in the MIR spectra (Bureau et al., 2019). The main organic acids found in fruits are citric, quinic, tartaric and malic acid. The analysis of the spectra of these pure acids dissolved in water, showed in the located region between $1800-800\text{ cm}^{-1}$ defined peaks of carboxylate salts due to the antisymmetric and symmetrical stretch of CO_2 in the range of 1650 to 1450 and 1450 to 1360 cm^{-1} , respectively. However, these gradually replace the $\text{C}=\text{O}$ (1720 cm^{-1}) and $\text{C}-\text{O}$ (1220 cm^{-1}) stretching modes as the acid is de-protonated (Bichara et al., 2011; Kozhevina, Skryabina, & Tselinskii, 1980; Max & Chapados, 2004; Smith, 1998). Therefore, among many applications of MIR analysis, stands out the quantification of free organic acid (citric acid, malic acid, tartaric acid), titratable acidity, concentration of vitamins (ascorbic acid) (Bureau et al., 2019).

The performance of the aroma freshness and natural flavor descriptors can also be explained by the relationship between the MIR technique and quantification of organic acids. However, the freshness of orange juice is also associated with the oil content, which when in adequate amounts ensures the freshness aspect of the juice (Redd, Hendrix, & Hendrix, 1986). In this context, MIR spectroscopy has shown

to be able to classify olive oil according to the freshness of the samples (Sinelli, Cosio, Gigliotti, & Casiraghi, 2007). This study can also predict the freshness of olive oil, providing information comparable to chemical modifications. The peaks resulting from the of CO stretching vibrations of aldehydes and ketones (1743 cm^{-1}), scissoring vibration of CH₃ from ethers (1363 cm^{-1}) and vibration of CO stretching of epoxides (1218 cm^{-1}) were pointed out as the main responsible for the variation of the spectra as alteration of the freshness of the samples. These may be related to a higher concentration of aldehydes, ketones and epoxides in oil samples stored than in fresh oils (Sinelli et al., 2007). This approach was also applied to characterize, differentiate and verify the authenticity of cold pressed lemon essential oils (EOs) (Mehl et al., 2014). In addition to instrumental methods such as Gas chromatography-flame ionization detector GC-FID, Nuclear magnetic resonance (^1H NMR), the authors used Fourier transform-mid-infrared spectrometry (FT-MIR) to assess the classification potential of 64 EOs from Argentina, Spain and Italy. Results were obtained capable of differentiating and classifying the lemon oil samples according to their geographical origin, highlighting the FT-MIR and ^1H -NMR methods as highly reproducible and fast.

PLS modeled with MIR data also showed a correlation of 0.59 (R^2) and an error of 14.82% with yellow color. The prediction of β -carotene through the acquisition of MIR spectra in passion fruit showed similar results ($R^2 = 0.55$), but higher error (36.07%) (Oliveira et al., 2014). In this context, the author suggests that the low concentration of carotenoids in the fruit may have influenced the performance of the predictive model.

Finally, the descriptor related to the presence of cells, although showing a correlation of 0.39, obtained a low error (9.54). This characteristic in orange juice is related to viscosity and the centrifuged pulp (bottom pulp) (Redd et al., 1986) that are due to the pectin content. In the MIR spectra pectin has a characteristic peak at 1606 cm^{-1} , attributed to the anti-symmetric stretching of the carboxylate anion (COO^-), due to the non-esterified carboxylic groups displayed by its structure (Heredia-Guerrero et al., 2014; Nogales-Bueno et al., 2017).

MIR spectroscopy related to sensory analysis has also been explored by other authors to predict the sensory score of traditional balsamic vinegar (TBV) (Versari, Parpinello, Chinnici, & Meglioli, 2011). The best results of the study were obtained with a correlation coefficient of 0.889 and 0.885, which allowed us to conclude that

the method is suitable for the rapid instrumental screening of the sensory quality of the TBV. In addition to the results obtained in the evaluation of the sensory quality of TBV, predictive models using MIR and Headspace-Mass Spectrometry (HS-MS) data showed good performance in the sensory evaluation of olive oil, especially for predicting the descriptors of quality (fruity and bitter) and defect (fusty and musty) (Borràs et al., 2016b).

Based on the results obtained in this study, it is believed that the performance of the NIR models was not satisfactory due to the accessory for transreflectance used in the measurements. Other collection modes, with other accessories can be tested in future studies.

Table 1. Prediction results for sensory descriptors.

Descriptor	Technique	Preprocess	RMSEC(%)	R ² CAL	RMSECV(%)	R ² CV	N Train	N Test
Yellow	NIR	1 DRV (5pts, order 2) + MC	22.55	0.118	19.65	0.275	84	36
	MIR	MC	7.11	0.926	14.82	0.598	88	37
Homogeneous	NIR	1 DRV (5pts, order 2) + MC	17.09	0.586	19.09	0.106	78	33
	MIR	2 DRV (61pts, order 2) + MC	18.50	0.486	18.26	0.348	78	33
Presenceofcells	NIR	1 DRV (5pts, order 2) + MC	14.45	0.234	15.54	-0.001	83	35
	MIR	1 DRV (61pts, order 2) + MC	17.74	0.242	9.54	0.398	86	36
Viscous	NIR	1 DRV (5pts, order 2) + MC	18.89	0.221	20.81	-0.175	83	35
	MIR	2 DRV (61pts, order 2) + MC	18.56	0.315	17.14	0.319	83	35
Aroma- Freshness	NIR	1 DRV (5pts, order 2) + MC	19.08	0.202	20.07	0.021	73	30
	MIR	2 DRV (61pts, order 2) + MC	15.09	0.668	17.70	0.501	84	35
Aroma- Cooked	NIR	1 DRV (5pts, order 2) + MC	14.90	0.354	19.99	-0.081	77	33
	MIR	2 DRV (61pts, order 2) + MC	16.21	0.469	15.74	0.407	80	33
Aroma- Citric	NIR	1 DRV (5pts, order 2) + MC	18.66	0.043	21.38	0.122	80	34
	MIR	1 DRV (61pts, order 2) + MC	20.92	0.24	19.38	0.312	89	37
Aroma- Overripe	NIR	1 DRV (5pts, order 2) + MC	29.15	0.354	39.11	-0.081	84	36
	MIR	MC	14.15	0.957	13.48	0.04	79	33
Taste- Acid	NIR	1 DRV (5pts, order 2) + MC	17.18	0.27	16.99	0.221	83	35
	MIR	MC	16.57	0.439	18.70	0.42	90	38
Taste- Bitter	NIR	1 DRV (5pts, order 2) + MC	11.63	0.502	15.20	0.185	82	34
	MIR	SNV+MC	13.33	0.243	10.44	-0.529	81	34
Taste- Cooked	NIR	1 DRV (5pts, order 2) + MC	17.05	0.121	19.79	0.007	75	32
	MIR	1 DRV (61pts, order 2) + MC	13.22	0.711	17.90	0.436	80	34
Taste- Adstringent	NIR	1 DRV (5pts, order 2) + MC	13.58	0.173	16.12	0.014	89	38
	MIR	SNV+MC	11.32	0.114	10.67	0.132	84	36
Taste- Natural	NIR	1 DRV (5pts, order 2) + MC	17.88	0.134	18.67	0.137	88	28
	MIR	2 DRV (61pts, order 2) + MC	13.44	0.734	17.99	0.544	86	36
Taste- Citric	NIR	1 DRV (5pts, order 2) + MC	18.64	0.121	19.49	0.119	81	34
	MIR	2 DRV (61pts, order 2) + MC	16.52	0.417	15.58	0.292	81	34
Taste- Overripe	NIR	1 DRV (5pts, order 2) + MC	18.35	0.168	21.40	-0.114	84	35
	MIR	MC	12.95	0.076	16.86	0.011	78	33
Taste- Fermented	NIR	1 DRV (5pts, order 2) + MC	11.98	0.039	12.83	0.007	81	34
	MIR	SNV+MC	12.90	0.04	8.30	-0.233	81	34
Mouthfeel- Body	NIR	1 DRV (5pts, order 2) + MC	20.92	0.139	18.08	-0.208	86	36
	MIR	2 DRV (61pts, order 2) + MC	18.39	0.224	17.20	0.138	84	36
Mouthfeel- Presenceofcells	NIR	1 DRV (5pts, order 2) + MC	10.21	0.626	18.26	0.136	87	36
	MIR	MC	11.95	0.103	11.76	-0.007	83	35

4.3.4. Partial least squares-discriminant analysis

The descriptors that showed the best predictive performance were also used to build classification models by Partial least squares-discriminant analysis (PLS-DA)

and their respective results are shown in Table 2. Interest was focused on classifying orange juice samples as light or dark yellow, with little or a lot of cell presence, and on classifying according to the intensity of the quality or defect attributes, that is, classify the juices as little or a lot of aroma freshness and citrus, but also as, little or a lot of cooked aroma. The flavor descriptors were also used to assess whether MIR analysis can be a quick method, capable of classifying attributes felt by the panelists taste. Analyzing if an orange juice has more intensity of natural flavor, than acid, bitter and cooked flavor, can say a lot about its sensory quality and even its acceptance in a quick and non-destructive way.

For the matrix X PLS-DA, models were developed using the orthogonal data in the matrix X, showed in the fig.2B, and since PLS-DA has the ability to predict the classes and not quantify the intensity of each descriptor, for the construction of the classes, the median values of each descriptor were calculated, establishing values below the median as belonging to class 0 and above belonging to class 1. The sensitivity and specificity parameters the closer to 1 indicate that very assertive models and errors between classes demonstrate the safety margin to discriminate a sample between two classes (Amodio, Ceglie, Chaudhry, Piazzolla, & Colelli, 2017; Biancolillo, Marini, & D'Archivio, 2020; Medina, Perestrelo, Silva, Pereira, & Câmara, 2019). Thus, it is possible to observe that the best classification models considering the mentioned parameters and the accuracy are the yellow color, taste natural, aroma freshness, aroma and taste cooked.

The performance of the classification models for the aforementioned descriptors indicate high potential of the MIR method associated with PLS-DA multivariate analysis to classify orange juice samples according sensory characteristics. In this way, it is possible to obtain a rapid and large-scale response on quality characteristics, regarding the appearance, freshness and natural flavor of the product, but also to classify the sample with characteristics of highly processed products, such as aroma and cooked flavor.

In this context, instrumental analysis by MIR has also shown promising results for identifying defects in olive oil. In this study, PLS-DA models were able to discriminate between defective and high quality oils, with predictive skills around 87% for mold defects and around 77% for wine, dust and rancidity defects (Borràs et al., 2015).

Table 2. Classification results for sensory descriptors.

Descriptor	Sensitivity (Cal)	Specificity (Cal)	Sensitivity (CV)	Specificity (CV)	ClassError (Cal)	ClassError (CV)	Accuracy (Cal) (%)	Accuracy (CV) (%)
Yellow	0.821	0.868	0.746	0.853	0.155	0.2	84.45	82.09
Appearance- Presofcells	0.746	0.781	0.648	0.578	0.236	0.386	76.3	64.45
Aroma- Freshness	0.896	0.662	0.836	0.588	0.221	0.287	78.52	74.08
Aroma- Citric	0.735	0.806	0.632	0.582	0.229	0.392	77.04	60
Aroma- Cooked	0.868	0.761	0.721	0.672	0.185	0.303	81.49	71.86
Taste- Acid	0.776	0.559	0.761	0.544	0.332	0.347	65.93	65.93
Taste- Bitter	0.824	0.522	0.765	0.448	0.327	0.393	66.67	60.75
Taste- Cooked	0.667	0.758	0.681	0.758	0.287	0.28	70.38	70.38
Taste- Natural	0.899	0.606	0.87	0.712	0.247	0.209	77.78	79.26

4.4. Conclusion

In this study a methodology using mid and nir-infrared (NIR and MIR) spectroscopy in combination with multivariate tools was used for the prediction and classification sensory descriptors in different types of orange juice evaluated a trained panel.

The MIR spectra showed the potential to predict some attributes in orange juice such as yellow color, freshness aroma, bitter and natural flavor, with R^2 higher 0.5 and error between 10-17%. It also showed correlation with the descriptors related to the presence of cells, citrus and cooked aroma, acid and cooked flavor (R^2 between 0.39-0.43 and error between 9.54-19%). The models built with the NIR spectra did not present satisfactory performance, which is believed to be due to the accessory used for the measurements, that was not the most appropriate, interfering in the results obtained.

The PLS-DA models built with the best results obtained in the predictive models were able to discriminate the samples for the descriptors yellow, taste natural, aroma freshness, aroma and taste cooked with predictive abilities between 70% and 87%. And thus, demonstrating to be more applicable for the evaluation of the sensory quality of orange juice than the PLS prediction models.

MIR instrumental approach in combination with multivariate tools demonstrated good performance in the classification of orange juice according to its sensory quality. Once fully validated, the MIR method could be an alternative to the

complex and time-consuming orange juice QDA analyze, because of the advantages in time and simplicity.

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5. ORANGE JUICE SENSORY EVALUATION WITH TD-NMR COMBINED WITH MULTIVARIATE ANALYSIS DATA PROCESSING

Abstract

This study represents a fast and non-destructive approach based on time domain nuclear magnetic resonance (TD-NMR) and multivariate analysis for sensory evaluation orange juice. The sensory characteristics of orange juice are affected by many factors such as thermal processing and physicochemical composition of the orange. Current methods for characterization of the sensory profile, requiring selection, training of panelists hindering rapid process intervention. The reference sensory values were provided by a taste panel trained to assess sensory quality of orange juice by the QDA method. Predict models were developed using partial least squares analysis (PLS) and with the best results were constructed the classification models. The PLS models did not show satisfactory performance for predicting the sensory descriptors of orange juice. However, the classification models based on TD-RMN data achieving 79% of accuracy in yellow color classification, and 72% in body classification orange juice. The results demonstrate that TD-NMR and classification models have the potential to quickly assess the color and body of orange juice.

Keywords: Orange juice, TD-NMR, sensory analysis, multivariate analysis

5.1. Introduction

Orange juice is one of the most appreciated by consumers among the other fruit juices due to its fresh-like attributes due to the presence of bioactive compounds like, flavonoid, limonoid and ascorbic acid (Charfi, Boujida, Abrini, & Senhaji, 2019). In the EU the retail sales reach 101 million liters, registering a 30.9% increase in purchases over the same period last year (CITRUSBR, 2020a). The same scenario is seen in the export of Brazilian orange juice, where there was an increase of 16% in the eleven months of the 2019/20 harvest compared to the same period of the previous harvest (CITRUSBR, 2020b).

Due economic, accessibility to consumers and logistical reasons, fruits like orange are processed or preserved industrially by pasteurization (Khandpur & Gogate, 2016). This commercial process includes squeezing fresh orange, centrifuging step and heat process (Kim, Kim, & Lee, 2016). For the fresh orange the temperature is set at 75° for 30s, while frozen concentrated juice is pasteurized at 95° for 30s, followed by freezing and thawing process (Gil-Izquierdo, Gil, & Ferreres, 2002). However, due the different thermal processing conditions the sensory

characteristics and physiochemical properties of orange juice can be affected (Gil-Izquierdo et al., 2002; Kim, Lee, Kwak, & Kang, 2013).

Previously, orange juice sensory characteristics were identified using quantitative descriptive analysis (QDA) (Kim, Lee, Kwak, & Kang, 2013; Kim, Jang, & Lee, 2018). This analysis method use the human assessors as measuring instruments (Yang & Boyle, 2016). For the formation of the panel, participants are selected based on their ability to discriminate sensory attributes, and then they are trained using product and attribute references. At the end, the panelists measure the intensity of the attributes using an unstructured scale, so that the data are statistically analyzed (Yang & Boyle, 2016). Because it is an efficient method of sensory characterization of foods, QDA has been chosen as an analysis tool in citrus, such as in the evaluation of the sensory profile of non-symptomatic and symptomatic oranges infected by diseases (Plotto et al., 2010). Others authors use the same method to compare sensory characteristics with furan level in commercial orange juice (Kim et al., 2016).

The effect of different types of processing on the sensory characteristics of orange juice can therefore be analyzed through trained assessors. However, in the citrus industry the time required for selection, training and formation of a sensory panel, makes it difficult to integrate this method with other quality analyzes carried out periodically.

The increasing demands of consumers, industries and regulatory agencies have been driven the interest and development of more accurate and faster techniques for assessing food quality (Carvalho, Morais, Lima, & Teixeira, 2019). In this regard, time-domain nuclear magnetic resonance (TD-NMR) has been pointed as an alternative non-destructive method for food quality analysis (Kirtil, Cikrikci, McCarthy, & Oztop, 2017; Santos, Pereira-Filho, & Colnago, 2016), since TD-NMR is rapid, simple technique and has a potential for online and in situ measurements (Santos et al., 2014).

TD-NMR technique, the ^1H relaxation (transverse relaxation, T_2) is monitored providing information about the mobility of the nuclei (Santos et al., 2016). In the fruits analyze, studies have showed that T_2 has good correlation with sugar content because it alters the mobility of the nuclei (Flores, Colnago, Ferreira, & Spoto, 2016; Pereira, Carvalho, Cabeça, & Colnago, 2013). In the food quality control, TD-NMR also has been demonstrated good results for evaluation of many food matrices

including cheese (Chen, MacNaughtan, Jones, Yang, & Foster, 2020), milk (Santos et al., 2016) and meat (Santos et al., 2014).

Among non-destructive techniques, TD-NMR is gaining prominence in determining the quality of citrus fruits. In the literature there are many works concerning the determination of the quality parameters in intact oranges using this technology, such as total soluble solids (TSS), pH, titratable acidity (TA) (Flores et al., 2016) and also in the study of the effect of conservation on storage (J. Zhang, Liu, Wang, & Ruan, 2012).

Although the TD-NMR technique is extensively studied in the food and citrus quality, little attention has been given to sensory parameters, specifically to the descriptors evaluated by trained panelists. Therefore, with the purpose of filling this gap, the aim of this study was to evaluate the use of non-destructive TD-NMR data, associated with chemometric models to predict and classify the sensory descriptors of different types of orange juice.

5.2. Material and methods

5.2.1. Samples

Samples obtained from different types of processes and consequently with very different characteristics were selected for this study. In this way, were analyzed by the sensory panel and instrumental analysis via TD-NMR, samples of 100% freshly squeezed orange juice (OJ 1), reconstituted orange juice (OJ 2), obtained by diluting the concentrated juice with drinking water up to 11.5°Brix and pasteurized orange juice (OJ 3). All samples were obtained the extractors and process JBT Food Tech. OJ 1 used the fruits at the beginning, middle and end of harvest the Pêra Rio cultivar, which they were processed on the same day of the sensory test. The OJ 2 samples were supplied by citrus industry and the OJ 3 were acquired in the local market.

5.2.2. Sensory analysis

The sensory attributes of the orange juice samples were analyzed by a sensory panel trained at JBT FoodTech Brazil Company. The entire training process,

formation of the sensory panel and data validation is described in the previous chapter. Using the adapted ADQ method (Meilgaard, Civille, & Carr, 2006), eighteen sensory attributes were evaluated, four attributors referring to appearance and aroma, eight referring to taste and two to mouthfeel. Each panelist assessor was offered 50 mL of each sample in disposable cups and at room temperature. All samples were coded with random three-digit numbers and offered at random and monadic presentation. Panel evaluation was performed in triplicate with short intervals between evaluations. A 9 cm unstructured scale was used to measure the intensity of the descriptors. In total 11 panelists evaluated 15 samples on different days than OJ 1, OJ 2 and OJ 3 totaling 135 responses. Sensory and instrumental by TD-NMR analysis were made in the same day. The decays were acquired from rates collected from each panelist. This study was evaluated and approved by the Research Ethics Committee of ESALQ/USP, process number 94432918.0.0000.5395.

5.2.3. Time-domain magnetic resonance (TD-NMR)

Minispecmq20 spectrometer (Bruker, Germany) was used which has a magnetic field of 0.47T, equivalent to 19.9 MHz for ^1H . The pulse sequence used was CPMG with the parameters: pulse of $90^\circ = 5.6\mu\text{s}$, pulse of $180^\circ = 11.4\mu\text{s}$, $\tau = 0.4\mu\text{s}$ time between the pulses, echo number = 30,000, recycle time = 5s and 8 scans.

The samples evaluated by each panel advisor were measured separately at a temperature of 21.5°C and a volume of 500 μL . A Specfit spectrometer (Fine Instrument Technology, Brazil) was also used in the measurements, which has a magnetic field 0.35T equivalent to 15 MHz for ^1H , also using CPMG pulse sequence with 90° pulse = $12\mu\text{s}$, $\tau = 1000\mu\text{s}$ time between pulses, acquisition time = $64\mu\text{s}$, recycle time = 5 s, echo number = 1500 and 16 scans.

5.2.4. Physicochemical analysis

The samples also were subjected to physical-chemical characterization for the main parameters related to sensory changes in the juices. Soluble solids were measured in Brix degrees, with an Auto Abbe refractometer, model 10500/10501, Leica, the result will be expressed in $^\circ\text{Brix}$ (USDA, 1998). Total acidity was obtained

by titrating 10g of homogenized juice in 50 mL of distilled water, with 0.1 N NaOH solution, the values being expressed in% citric acid (AOAC, 1990). Ratio was calculated by the relation (content of soluble solids/titratable acidity) (USDA, 1998). Ascorbic acid content was obtained titling 35 mL of starch-acid solution and 25 mL of juice sample with 0.1 N iodine solution while under stirring until the first stable blue color appears (AOAC, 1999).

Suspended pulp was measured pouring 100 mL of juice through a 20 mesh screen, after screened juice was put on centrifuge tube and centrifuged for 10 min after reaching a centrifugation force of 365xg, after centrifugation the pulp volume was read (USDA, 1998). For the viscosity measure viscometer of low measurement capacity was used (JBT Corporation, n.d.).

Recovered oil analyzed in different types of orange juice by the Scott method, distilling a mixture of 25 mL of isopropanol, 25 mL of H₂O with defoamer and 25 mL of sample. After the end of the distillation, 10 mL of HCl with methyl orange solution were added to titrate the distillate with the 0.0247 N KBrO₃-KBr solution for the disappearance of the dye. The amount of titrant used was recorded (FMC Technologies, n.d.; Scott, 1966). Limonin content was determined in orange juice samples by high performance liquid chromatography (HPLC) following the methodology already used in citrus industries (Shaw & Wilson, 1984) with reverse phase column (Microsorb C18, 150 mm x 4.6 mm , 5 μ particle size) and UV-visible detector. Its concentration (ppm) was calculated from the absorbance of the sample based on a linear regression equation of the absorbance of the standard curve of the peak area in relation to the standard concentration of limonene. Hesperidin was quantified by the Davis test (Davis, 1947) by centrifuging 50 mL of juice at 1400RPM for 10 minutes. Then, in a test tube with a cap, 25 mL of diethylene glycol, 0.5 mL of the sample supernatant and 0.5 mL of sodium hydroxide (NaOH) 4N were added. After homogenization and remaining at rest for 10 minutes, the 360nm reading on the spectrophotometer was performed.

5.2.5. Statistical analysis

The sensory panel formed by 11 panelists, evaluated 15 samples in total of OJ 1, OJ 2 and OJ 3. The sample evaluated by each member was also individually analyzed via TD-NMR.

Origin 8.1 (OrigiLab, Northampton, MA, USA) software was used for TD-NMR data processing. Decays obtained from two spectrometers were normalized between 0 and 1 and transverse time relaxation (T_2) was calculated with bi exponential adjustment. Subsequently, the matrix X was built with TD-NMR data and matrix Y was contained the reference analysis, that is, the response of each assessor member of the sensory panel. Principal component analysis (PCA) was used to observe the relation between TD-NMR data and the type of orange juice that was used as classes.

Considering 135 reference measurements and their respective instrumental data were divided into two sets, with 70% used for training and 30% used for testing using the Kennard-Stone algorithm (Kennard & Stone, 1969). Partial least squares regression (PLS) was made using software The Unscrambler X 10.5.1 (Camo Analytics, 2019) and number of factors was chosen according to the lowest cross-validation RMSECV.

Best TD-NMR sensory PLS parameters were chosen to build supervised classification models using Python 2.7 in the PyCharm 2018 development environment, where classes were defined based on median of each sensory parameter choosed. Panda 0.18.1, Numpy 1.11.1 and Scipy 1.0.1 libraries were used to organize and analyze the data. For the SVM calculations, an auxiliary package Scikit-learn 0.19.1 was used, also Matplotlib 2.2.3 and Seaborn 0.9.0 were used to create the graphics (Pedregosa et al., 2011). The SVM classifier was optimized for the radial base function (RBF) and was chosen based on previous performance tests. An adjustment function was applied to obtain the best SVM classifier, using a grid search to find the best combination of C constraint from the C regularization parameter box and kernel scale gamma parameter, in order to achieve the highest determination coefficient cross validation (R^2) and lower RMSECV for the training data set. The KNN classifier, with $k=3$ for yellow and viscous descriptors and $k=5$ for body also used as a method for supervised classification that find the k examples in the dataset closest to the unknown class, using Euclidean distances and selecting the predominant class. The classification accuracies of the SVM and KNN models were evaluated using the test dataset (Gerhardt et al., 2019).

5.3. Results and discussion

5.3.1. Physicochemical characterization

Table 1 shows the minimum, maximum, average and standard deviation values of physicochemical data applied in triplicate of the studied juices, according to the process of the juices. OJ 2 presented TSS similar to OJ 3, according to the quality standard norms for FCOJ (Gonçalves, 2009). The lower percentage of recoverable oil found in the OJ 2 samples, is due to the thermal processing used to obtain this product. OJ 2 also presented higher limonin value in relation to the other analyzed juice samples. On the other hand, OJ 3 presented lower hesperidin content, centrifuged pulp and viscosity. In this context, the evaluation of the effect of different processing and storage conditions on the content of hesperidin, found that the concentration of hesperidin in orange juice gradually decreases during storage, even when the juice was stored at low temperature (Zhang et al., 2020). Although the mean values for the hesperidin concentration of OJ 1 and OJ 2 are similar, OJ 1 showed higher maximum values (989mg / L). Thus, in the present study, hesperidin concentrations in samples that were subjected to thermal processing and storage (OJ 2 and OJ 3) were lower when compared to freshly squeezed juice (OJ 1).

Table 1. Chemical analyses of OJ 1, OJ 2 and OJ 3.

SAMPLE	STATISTICS	TA (%)	TSS (°Brix)	RATIO (TSS/TA)	%Oil	CP (%)	VISCOSITY (cP)	HESPERIDIN (ppm)	VITAMIN C (mg/L)	LIMONIN (ppm)
OJ 1	Minimum	0.55	8.86	13.87	0.011	10	5.95	325	327	0.59
	Maximum	0.65	11.01	20.03	0.027	14	6.22	989	403	4.14
	Average	0.63	9.93	15.96	0.017	12	6.09	559	364	2.59
	Std.	0.04	0.77	2.36	0.006	2	0.13	324	31.28	1.40
OJ 2	Minimum	0.66	11.64	12.24	0.005	10	6.85	170	213	1.39
	Maximum	0.96	11.70	17.59	0.008	14	8.32	846	555	10.35
	Average	0.83	11.67	14.38	0.006	12	7.63	561	327	4.08
	Std.	0.14	0.02	2.47	0.001	2	0.74	323	158.70	3.66
OJ 3	Minimum	0.52	11.01	15.26	0.007	6	2.47	287	209	0.93
	Maximum	0.73	11.36	21.54	0.015	9	5.93	470	256	4.53
	Average	0.63	11.20	18.26	0.013	8	3.87	385	238	2.10
	Std.	0.10	0.16	2.81	0.004	1	1.82	75	22.27	1.46

TA= Total acidity; TSS= Total soluble solids; CP= Centrifugate pulp. Std.= Standart deviation

5.3.2. TD-NMR signal decay interpretation

Figure 1 shows TD-NMR decays obtained with CPMG pulse of three types of orange juice, OJ 1, OJ 2 and OJ 3. In the TD-NMR measure the calculated T_2 value is inversely correlated with sample viscosity (van Duynhoven, Voda, Witek, & Van As, 2010). Therefore, the orange juice with lower viscosity shows longer CPMG decay (longer T_2) than that of the orange juices with higher viscosity. This case can be observed in Figure 1 based on the table 1, where the juice OJ 3, which has the lowest viscosity (3.87cP), presented the highest T_2 and vice versa, OJ 2 with the highest viscosity (7.63cP) obtained the lowest T_2 . Also, OJ 3 showed lower content of centrifuged pulp that which is one of the parameters related to the viscosity of fruit juices.

Figure 1. CPMG decay of OJ 1, OJ 2 and OJ 3 samples.

Fig. 2 show the score plot using principal component 1 (PC1) and 2 (PC2) for OJ 1, OJ 2 and OJ 3, with regard to the TD-NMR data. Fig. 2 shows a very good separation between juices. The PCA of OJ 1, OJ 2 and OJ 3 showed 93 and 7% of variance in PC 1 and PC2, respectively. OJ 3 is positive for PC1 and OJ 2 mostly, is

negative for PC1. This can be ascribed the difference of CPMG signal decay obtained by TD-NMR in juices with different viscosity.

Figure 2 .PCA of T_2 and A_2 values of OJ 1, OJ 2 and OJ 3 samples.

5.3.3. Partial least squares

Table 2 show the PLS models obtained for each descriptor using T_2 values. The best predicted descriptors were yellow, viscous and body. These descriptors showed correlation between 0.22-0.31 and errors around 20 and 23% (RMSECV). Also, viscous, body and really yellow are descriptors that are associated with the viscosity of orange juice, as demonstrated in the previous chapter in the correlations maps between sensory attributes and physical-chemical parameters. Thus, with viscosity being a parameter inversely correlated with T_2 (Fig. 1), the best predictive performances for the sensory descriptors of orange juice were those that are related to this parameter. However, the correlation for these descriptors with the TD-NMR decays could be greater if the samples included a greater range of viscosity.

To predict the viscosity of crude oils using NMR transverse relaxation time data, good PLS models were developed with a standard error of prediction of 0.161 logcP (de Oliveira Ramos et al., 2009). The results obtained in this study indicate that the technique of TD-NMR and PLS can be applicable in the viscosity measure of oils in a wide range. The viscosity of oils is dependent on the main fatty acids present

(Knothe & Steidley, 2005). In this context, even though the TD-NMR technique does not have sufficient resolution for the types of hydrogen couplings compared to the high-field NMR technique, it is able to acquire information from certain regions of the signal that may be associated with specific portions of acids fatty and smaller compounds (McDowell, Defernez, Kemsley, Elliott, & Koidis, 2019). Thus, T_2 decays reflect the differences in oil viscosity.

However, the viscosity of orange juice is related to the presence of molecules such as pectin that alter the distribution of particle size in the suspension, consequently increasing the viscosity of the juice (Aghajanzadeh, Kashaninejad, & Ziaifar, 2017; Croak & Corredig, 2006). TD-NMR also was used to predict the total pectin in intact orange where showed a good performed in the PLS models ($r = 0.76$ and error of prediction 5.76%) (Bizzani, Flores, Colnago, & Ferreira, 2017). The presence of paramagnetic ions, such as the iron contained in orange juice (Ladaniya, 2008), may have played an important role in achieving this result, as it may reduce the relaxation time of the water in some oxidation states (Ribeiro et al., 2010).

Table 2. Prediction results for sensory descriptors.

Descriptor	Intensity range	RMSEC (%)	R ² CAL	RMSECV (%)	R ² CV
Yellow	0.6-8.5	20.988	0.318	21.412	0.299
Homogeneous	0.6-9	28.044	0.116	28.811	0.086
Presence of cells	0.1-7.8	20.192	0.18	20.795	0.146
Viscous	0.2-8.1	22.395	0.226	23.160	0.188
Aroma- Freshness	0.2-9	28.544	0.008	29.467	0
Aroma- Cooked	0.1-9	26.333	0.066	26.867	0.047
Aroma- Citric	0.2-7.9	25.835	0.027	26.582	0
Aroma- Overripe	0.1-4.6	33.630	0.008	34.522	0
Taste- Acid	0.2-7.6	24.763	0.025	25.539	0
Taste- Bitter	0.1-9	18.656	0.028	19.189	0
Taste- Cooked	0.1-8.6	26.698	0.099	27.337	0.071
Taste- Adstringent	0.1-7.3	18.863	0.004	19.658	0
Taste- Natural	0.3-9	65.154	0.011	67.179	0
Taste- Citric	0.2-8.3	24.193	0.029	24.880	0
Taste- Overripe	0.1-4.3	35.349	0.023	36.535	0
Taste- Fermented	0.1-8	17.775	0.071	18.263	0.034
Mouthfeel- Body	0.2-7.1	20.085	0.287	20.704	0.26
Mouthfeel- Presence of cells	0.1-4.2	22.690	0.084	23.357	0.049

RMSEC: Root mean square error of calibration. RMSECV: Root mean square error of cross-validation.

R²CAL: determination coefficient of calibration. R²CV: determination coefficient of cross-validation.

5.3.4. Classification models

Based on the best PLS predictive models, the descriptors referring to yellow, viscous and body color were used to train the kNN and SVM models, allowing the use of TD-NMR decays to classify the intensity of these attributes. The approach of these models aims to be a fast and qualitative method for use in industrial processing plants, providing relevant information regarding the sensory aspects of orange juice.

For the construction of the classes, the median values of each sensory attribute were considered. Thus, the objective of the models was to classify the juice samples between greater or lesser intensity of yellow color, very or little viscous, very or little body.

The use of kNN or SVM models applied in the analysis of decay of TD-NMR can provide rapid assessment of sensory parameters, making it possible to describe characteristics that are analyzed through the perception of panelists on a large scale.

The kNN model is based on a distance function that measures the difference between groups, classifying an unknown sample from the validation set according to class associated with the majority of its closest k-neighbors in the training set (Teye et al., 2019). Fig 3 show kNN models for classification of yellow, viscous and body. The best performance was obtained for yellow classification (Fig 3A), that resulted in the training (70% of the data) in 79% accuracy, when the model was applied to 30% of the test dataset.

The SVM method is also a useful tool for analyzing data that is not regularly distributed or has an unknown distribution (Auria & Moro, 2008). However, its underlying concept is operated by the construction of the optimal hyperplane, which consists of the margins that best represent the separation of classes (Gerhardt et al., 2019). Between the hyperplane and the margin points between classes (support vectors) there should be the greatest possible distance. Hence, a grid search of the trained SVM model was performed with 70% of the data, using cross-validation to optimize the regularization constraint C and the gamma parameter of the kernel scale (Fig. 4A.1, 4B.1 and 4C.1).

Fig. 4 shows the classification performed using the tuned SVM model for the same descriptors used in the kNN method. The model provided 79 and 72% accuracy in the classification of yellow color and body, respectively (Fig. 4A.2 and 4C.2).

The accuracy of the classification results obtained using the two different models were very similar, with both models separating the orange juice samples according to the intensity of the yellow and body color. These results were obtained using T_2 values with bi exponential adjustment, avoiding problems of sample separation due to the two spectrometers used. The viscous descriptor, being an attribute of visual evaluation of the panelists, may have influenced the inferior performance of the classification models in relation to the other tested attributes.

In this study, the sensitivity of TD-NMR to liquid viscosity may have contributed to the good performance of the kNN and SVM models in the classification of orange juice according to color and body, since both attributes are also related to the viscosity of the juice.

In this way, the use of benchtop TD-NMR equipment combined with machine learning models such as SVM and KNN have potential to be an alternative to obtain some sensory aspects that are obtained by a lengthy process of selection and training of panelists.

Figure 3 .Computed decision boundaries in the kNN model for yellow (A), viscous (B), body (C) descriptors (k=3 model A and B, k=5 model C). (Stars means test set and circles training set).

Figure 4. Grid-search optimization of the SVM model aiming highest CV R^2 (A1, A2, A3) and computed decision boundaries in the SVM model (B1, B2, B3) based on Radial Basis Function kernel (Stars means test set and circles training set). (A=yellow, B=viscous, C=body).

5.4. Conclusions

TD-NMR relaxometry method was not efficient for prediction of sensory descriptors. However, combined with kNN and SVM models could be a useful method for classifying the yellow color and body descriptors in fresh, reconstituted and pasteurized orange juice. Accuracies of 79 and 75% were obtained for the kNN and SVM in yellow color classification, and 68 and 72% were obtained for the kNN and SVM in body classification. Therefore, TD-NMR coupled with machine learning models has potential for use in factory quality control laboratories as a screening method to monitor the color and body of orange juice.

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