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Detection of Volatiles in Dark Chocolate Flavored with Orange Essential Oil by Electronic Nose

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Abstract
The aim of this study was the application of the electronic nose for detection of volatile release from dark chocolate flavored with orange essential oil during the storage. The detection of volatile in chocolate without and with different concentrations of orange oil (0–10 ppm) was performed using polyaniline (Pani) gas sensor array with different dopants. Orange oil and chocolate were analyzed in relation to water activity, moisture, and acidity. The chocolate maintained unchanged the water activity; however, the moisture and acidity presented alterations during the storage. It was verified a decrease in resistance response of the sensor array with the chocolate during the storage time. The sensor doped with TSA (toluenesulfonic acid) presented the higher sensitivity of the array. Principal component analysis (PCA) revealed five distinct groups corresponding to the volatiles released during the storage time (0, 20, 40, 60, and 100 days). This work demonstrates that the electronic nose technology with Pani gas-sensing array can be effective and successfully applied to discriminate different concentrations of orange essential oil during the dark chocolate storage.

Keywords Sensor array · Pani · Dopants · Storage · Volatiles

Introduction

The great competitiveness in the food market makes the industries improve quality, reduce safety mistakes, and monitor trends of the products. Chocolate stands out for its high consumption, where the global market estimated to ship over 9500 thousand tons in 2024, especially the dark chocolate market reached US$ 45.6 billion in 2018 (IMARC Group 2019). This high consumption is linked to the versatility on the preparation, and it uses in various manufactured products. Dark chocolate presents an intense flavor, which is very appreciated by the bittersweet. Also, it is an excellent source of flavonoids through antioxidant activity (Afoakwa et al. 2008).

Chocolate can be defined as a suspension of solid particles, sugar and cocoa powder, in a continuous fat phase (cocoa butter), which contributes to the aroma, taste, and color of the product (Sasaki et al. 2012). It should be melted quickly at temperatures close to 37 °C, otherwise, promote an aroma/taste release and a waxy residue (Beckett 2008). Chocolate is a source of protein, fat, carbohydrates, vitamins, and minerals.

The aroma is an extremely important sensory property and a major factor that affects quality perception and consumer's acceptance (Mirković et al. 2018). The use of essential oils in chocolates has advantage as the possibility of replacement of the artificial (flavors, odors, and colors) to natural. Natural products, particularly essential oils, are being targeted as potential preservatives due to the antimicrobial and antioxidant activity. In this context, the use of essential oils in chocolate presents new possibilities for exploring the use of these substances to make foods more natural and healthier (Albak and Tekin 2014; Dwijatmoko 2016; Ilmi et al. 2017).

Orange essential oil (Citrus sinensis) is composed of a mixture of compounds (terpenes, hydrocarbons, and oxidized compounds) considered chemically volatiles (González-Mas et al. 2019). The major compound is the limonene and the major sesquiterpene the valencene (Galvão et al. 2015). These compounds contribute to the aroma profile, gige the organoleptic characteristics of the fruit, and can be used to aromatize other products (Palazzolo et al. 2013; González-Mas et al. 2019). Also, it can be used as antimicrobial substance for food preservation. According to Espina et al. (2011), low concentration (0.2 μL/mL) of orange oil showed

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synergistic lethal effects on bacterial cells, inactivating more than 5 log units.

In the evaluation of food flavorings, sensory panels are used with trained staff to provide a profile of pleasure and satisfaction of the consumers. The sensory analysis can be limited by human factors as subjectivity and fatigue over long periods. In this sense, the electronic nose can be used to identify volatile compounds in food matrices in real-time, with high detection levels, fastness, and low cost. The electronic nose consists of a gas sensor array composed of different sensors to interact with a wide range of chemical classes and discriminate diverse volatiles compounds (Ballen et al. 2019; Graboski et al. 2018a, 2018b). Conductive polymer-based on gas sensors have been widely used in electronic nose due to their chemical structure and sensitivity. The gas sensors have low cost of fabrication, high sensitivity, and fast response when in contact with volatile molecules, and can be operated at room temperature. The use of different dopants in Pani films is very promising due to the increased conductivity, mechanical properties, and well-defined layered morphology, which have a pronounced influence on its charge-transport characteristics. The in situ polymerization technique can incorporate easily the dopant acid, so a variety of dopants can be used to construct the sensor array. Pani gas sensors have been applied successfully in humidity (Steffens et al. 2009; Steffens et al. 2013), aroma (Tiggemann et al. 2016, 2017), gummy candies (Ballen et al. 2019), female cattle fertile period (Manzoli et al. 2019), and fruits ripening detection (Manzoli et al. 2011).

In the present investigation, electronic nose composed by Pani gas sensor array was applied to detect release of the volatile compounds from dark chocolate with orange essential oil during the storage (1, 30, 40, 60, and 100 days).

Material and Methods

Chocolate Samples

Orange essential oil (Citrus sinenses var. Dulcis, Quinarí, Brazil) and dark chocolate (Harald, Brazil) were commercially purchased. According to the manufacturer, the dark chocolate contains the following ingredients: sugar, cocoa butter, cocoa powder, soybean lecithin, and polyglycerol polyricinoleate. The amount of orange essential oil added to the chocolate was based on preliminary tests and taking into account the antimicrobial activity (Espina et al. 2011). Also, the orange oil used is considered by the United States Food and Drug Administration (Electronic Code of Federal Regulations e-CFR 2019) generally regarded as safe oil (GRAS).

The dark chocolate flavored with orange essential oil was produced in an acclimatized room (20 °C). Initially, the dark chocolate was melted in a microwave oven (Consul, Brazil) at 45 °C and tempered. Next, the concentrations of orange oil (2.5, 5.0, 7.5, and 10 ppm, w/w) were added to the dark chocolate. Also, a control sample (without orange oil) was produced. The chocolate samples were molded in bars (10 g) using a polyethylene mold, cooled at 5 °C in a refrigerator (Consul, Brazil), packaged in polyethylene terephthalate (PET), wrapped in aluminum foil ,and maintained at 18 °C. The samples were stored in a place protected from light for 100 days. The samples were produced in three similar batches.

Electronic Nose

The electronic nose apparatus used in this work was described previously by Graboski et al. (2018). The apparatus was composed of a glass chamber (1 L), magnetic stirrer and heater (Fisatom, 714 model, Brazil), digital timer (Cronobio - SW2018), temperature and humidity commercial sensor (Sensirion Kit EK-H5, SHT2), glass holder (samples), and a unit with the gas sensor array. The data of the gas sensors (resistance) were obtained with a NOVUS acquisition system (Field Logger model).

The sensor array consisted of four doped Pani films. The gas sensors were obtained on a substrate of tracing paper (Schoellershammer 90/95 g). The graphite-based interdigitated electrodes were produced by the line patterning technique, according to Manzoli et al. (2011) and Steffens et al. (2010) over the tracing paper substrate. The sensitive layer (Pani film) was obtained by the in situ synthesis, in the emeraldine state, according to Graboski et al. (2018). The dopants used were chloride acid (HCl, Sigma-Aldrich, Germany), canforsulfonic acid (CSA, Sigma-Aldrich, Germany), toluenesulfonic acid (TSA, Sigma-Aldrich, Germany), and dodecylbenzenesulfonic acid (DBSA, Fluka) (Manzoli et al. 2011).

The response of the sensor array (resistance) to the volatiles from orange essential oil was evaluated at different concentrations (2.5; 5.0; 7.5, 10.0, and 12.5 ppm). These concentrations were inserted into the sampling chamber through a syringe (Hamilton). The response was obtained alternately in synthetic air flow (White Martins) for 5 min (baseline), and for 5 min in the volatile compounds (orange essential oil). Thus, each measurement was performed with synthetic air flow (100 mL/min) inserted in the glass chamber to obtain the baseline, next followed by the addition of the volatiles to be analyzed by the array of sensors (Ballen et al. 2019). The resistance values obtained in the e-nose not were submitted to any signal processing. This experimental sequence was repeated for all concentrations of orange essential oil. Also, to each orange essential oil concentration evaluated, the experiments were performed in triplicate.

The application of the electronic nose in the detection of the volatiles from dark chocolate flavored with orange essential oil was performed in the same way that the essential oil. The same procedure was repeated for all concentrations in increasing order of the amount of essential oil (0, 2.5, 5.0, 7.5, 10.0 ppm). For each analysis, 5 g of chocolate was used. The sample was inserted in a glass chamber, maintained at 35 °C, and submitted to agitation in a magnetic stirrer at 33 rpm. The release of volatile from dark chocolate flavored with orange essential oil was evaluated by the electronic nose during the storage time (1, 20, 40, 60, and 100 days). In the experiments, it was used one sample of each batch of chocolate, so there are three samples evaluated.

The Pani gas sensors with different dopants (HCl, TSA, CSA, and DBSA) were characterized in relation to sensitivity, limit of detection, and response time. The sensitivity and limit of detection were obtained from the analytical calibration curve (concentrations versus the maximum resistance). The response time was calculated as the time required for changing the sensor resistance (reached 90% of the equilibrium value) after the insertion of the volatile in the chamber.

Composition Analysis

The moisture, acidity, and water activity analyses were performed according to AOAC methodology (AOAC 2005). The chocolates with and without addition of orange oil were evaluated during 100 days of storage. The orange oil was also evaluated in full form. All the samples were analyzed in triplicate.

The moisture was determined by direct drying in an air recirculating oven (Fanem, model 320-SE, Brazil). Around 3.0 g of sample was weighed and dried at 105 °C for 4 h until constant weight.

Acidity (meq NaOH/100 g) was determined by the titration method using 0.1 M sodium hydroxide solution (Dinamica, Brazil).

Water activity was determined using a water activity meter (Lobtouch Novasina, China). The samples were cut in half and shredded into small pieces. Next, were placed in the sample holder of the equipment to perform the measurements. The experiments were conducted at 25 °C.

Chromatography analysis of orange essential oil followed the methodology described by Beneti et al. (2011) and analyzed in a gas chromatograph (CG-2010 Plus model; Shimadzu, Brazil) equipped with a polar column (Rtx-Wax, 30 m × 0.25 mm × 0.25 μm) and a flame ionization detector. The following temperatures were used: 60 °C (8 min), 60–180 °C (15 °C/ min), 230 °C (10 min), injector temperature at 230 °C, detector at 275 °C, with a 50:1 split ratio. Nitrogen and synthetic air (White Martins, 99.99% purity) were used as carrier gases, with a 1.5-mL/min flow rate. A diluted sample (1 μL) in dichloromethane (1:10; Merck, Germany) was injected. The sample components were identified by comparing their mass spectra.

Statistical Analysis

The mean composition results of the dark chocolate were evaluated by analysis of variance (ANOVA) at a 95% confidence level using Statistica 5.0 software.

The electronic nose responses obtained in triplicate at different concentrations of orange essential oil (2.5, 5, 7.5, and 10 ppm (w/v)) were treated using the principal component analysis (PCA) statistical method using OriginPro9.0 (© Origin Lab Corporation) software. The resistance values obtained during the storage time of the chocolate (1, 20, 40, 60, and 100 days) were also evaluated by PCA. By the PCA analysis, the signal of each Pani sensor of the array was transformed into variables, which are linear combinations of the original signals. These variables, called scores, were represented on a two-dimensional plane. In this plot, the similarities were evaluated between the dark chocolate samples measured by the distances between them in the plane. The closer together was described as more similar. Also, the discrimination of the days of storage is done by the score plot of PCA and the distribution of each sensor was done by the loading plot. The PCA algorithm was implemented in software for the different sensors (doped with HCl, CSA, TSA, and DBSA) by calculating the eigenvectors of a covariance matrix of data.

Results and Discussion

Orange Oil and Chocolate Composition

The orange oil presented water activity value of 0.54 ± 0.001, acidity of 1.32 ± 0.04 meq NaOH/100 g, and moisture of 78.18 ± 0.136%. Oils with low acidity value indicate an excellent storage life and can be used safely in food products (Kumar 2014; Putnik et al. 2017). The low acidity of the oils indicates a little magnitude of hydrolytic deterioration. Similar values of acidity were found in bergamot oilseed (Sicari et al. 2017), and lower than 2.0 in Brazilian citrus seed oils as rangpur lime and "sicilian" lemon (Reda et al. 2005).

According to da Silva et al. (2013) in a study of the chemical composition of the essential oil of Citrus sinensis L. and Citrus aurantiun L., the moisture content was 81.56 and 88.97%, respectively. These results were above to those found in the present study.

The results of chromatography analysis of orange essential oil are presented in Fig. 1. It can be observed from the peak area that d-limonene is the major substance, corresponding to 93.2%. Other identified substances were the myrcene, α-pinene, n-decanal, and n-octanal in a low amount. Limonene is a monocyclic monoterpene, 4-isoprenyl-1-methyl-cyclohexene (Badee et al. 2011), the main volatile constituent of citrus peel (Lu et al. 2014) and an aromatic substance (Nikfar and Behboudi 2014; Zhang et al. 2014).

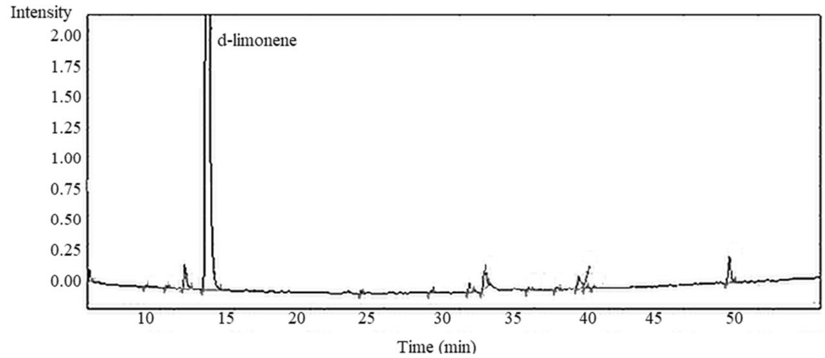


Fig. 1 Chromatographic profile of the orange oil

Orange essential oil (Citrus sinensis) is classified as a mixture of terpenes, hydrocarbons, and oxidized compounds. According to the International Organization for Standardization (ISO 2019), the essential oil of sweet orange must have a minimum of 93% and a maximum of 96% of limonene. The other remaining compounds refer to a mixture of other terpenes, and aliphatic aldehydes (Galvão et al. 2015).

In the food matrices, the essential oils can act as antioxidants and antibacterial, besides reproducing the flavor and odor of the plant used (Frassinetti et al. 2011; Chouhan et al. 2017). D-limonene presents biological activities, as antioxidant (Murali and Saravanan 2012) and antimicrobial (Lennartsson et al. 2012). So, it is considered a very interesting substance for addition to food stored at room temperature.

Table 1 shows the results of the water activity of dark chocolate without (0 ppm) and with different concentrations (2.5 to 10 ppm) of essential orange oil during the storage. In general, no significant difference ($p > 0.05$) was observed between the days of storage for the dark chocolates with and without addition of orange oil. The textural modifications suffered by foods, including chocolate, are directly linked to the structural changes over the storage time. Water is one of the main elements responsible for maintaining the texture characteristics of the food product due to the plasticizing effect. Thus, during the chocolate storage, the water activity remained unchanged, without structural changes such as the transition between the vitreous and gummy states (de Leite et al. 2005).

The water activity value must be lower than 0.60 for confectionery and chocolate to avoid the microbial proliferation and deterioration (Beuchat et al. 2013; Hartel et al. 2018). So, the water activity values found in the present study were lower than 0.55, making the product shelf-stable. Relative equilibrium is important to predict conditions of the product without chemical and microbiological deterioration during the storage (Sandulachi 2012).

Table 2 presents the results of moisture of chocolate during the storage time. It can be observed a significant difference ($p < 0.05$) between the 1 and 20 days of storage. In samples stored for 60 and 100 days, no significant difference was verified ($p > 0.05$). The increase in the moisture values during the storage can due to the migration and diffusion of water into dry products (Ghosh et al. 2004). The addition of orange oil caused an increase in the moisture content with significative difference ($p < 0.05$) between 1 and 40 days of storage, and after 60 days, the moisture content remained unchanged.

Low moisture values (0.5 to 3%) in foods present low spoilage during storage (Minifie 1989). So, values outside the technical recommendations can cause large losses in chemical stability, microbiological deterioration, or changes in overall food quality. Hygroscopicity is an undesirable property, in which the sugar has the ability to absorb moisture from

Table 1 Results of water activity analysis of dark chocolate during the storage time with and without the addition of orange oil

Water activity					
Time (days)	Concentration of orange oil (ppm)				
	0	2.5	5.0	7.5	10.0
1	$0.50^a \pm 0.05$	$0.51^a \pm 0.05$	$0.50^a \pm 0.03$	$0.52^a \pm 0.04$	$0.51^a \pm 0.02$
20	$0.54^a \pm 0.06$	$0.50^a \pm 0.02$	$0.55^a \pm 0.04$	$0.47^a \pm 0.02$	$0.48^a \pm 0.03$
40	$0.52^a \pm 0.03$	$0.47^a \pm 0.04$	$0.55^a \pm 0.03$	$0.47^a \pm 0.03$	$0.47^a \pm 0.05$
60	$0.50^a \pm 0.06$	$0.46^a \pm 0.04$	$0.54^a \pm 0.04$	$0.45^a \pm 0.04$	$0.49^a \pm 0.02$
100	$0.49^a \pm 0.04$	$0.45^a \pm 0.06$	$0.52^{a,b} \pm 0.05$	$0.45^a \pm 0.03$	$0.47^a \pm 0.03$

Mean values ± standard deviation followed by the same lowercase letters during the storage on the column do not indicate any significant difference at a 5% level (Tukey's test)

Table 2 Results of moisture (%) analysis of dark chocolate during the storage time with and without addition of orange oil

Moisture (%)					
Time (days)	Concentration of orange oil (ppm)				
	0	2.5	5.0	7.5	10.0
1	$0.9^{d} \pm 0.06$	$1.1^{d} \pm 0.03$	$1.2^{c} \pm 0.07$	$1.1^{c} \pm 0.05$	$1.3^{c} \pm 0.09$
20	$1.5^{c} \pm 0.08$	$1.4^{c} \pm 0.08$	$2.0^{b} \pm 0.09$	$1.9^{b} \pm 0.08$	$2.0^{b} \pm 0.07$
40	$2.0^{b} \pm 0.04$	$2.0^{b} \pm 0.02$	$2.1^{b} \pm 0.03$	$2.3^{a} \pm 0.05$	$2.2^{ab} \pm 0.10$
60	$2.1^{a} \pm 0.05$	$2.2^{a} \pm 0.04$	$2.2^{a} \pm 0.04$	$2.4^{a} \pm 0.11$	$2.3^{a} \pm 0.06$
100	$2.1^{a} \pm 0.08$	$2.2^{a} \pm 0.03$	$2.2^{a} \pm 0.04$	$2.4^{a} \pm 0.09$	$2.4^{a} \pm 0.10$

Mean values ± standard deviation followed by the same lowercase letters during the storage on the column do not indicate any significant difference at a 5% level (Tukey's test)

the environment. This hygroscopic characteristic of sugars directly affects the candies industries, causing losses in the product quality (Ghosh et al. 2005). So, the process should be carried out in a climate-controlled environment and package the product under appropriate conditions to maintaining the moisture content of chocolate bars (Ghosh et al. 2005).

The acidity results showed a significant difference ($p < 0.05$) between all days of storage (Table 3). The chocolates with 7.5 ppm of orange oil did not show significant difference ($p > 0.05$) between 1 and 20 days of storage. Chocolates with 10.0 ppm of orange essential oil presented high acidity. The acidity is due to the original oil acidity (1.32 ± 0.04 meq NaOH/100 g), and the increased acidity during storage may be associated with the hydrolysis of sucrose by the citric acid (Dar and Sharma 2011).

Electronic Nose

The maximum resistance response of Pani gas sensor array with different dopants (DBSA, CSA, TSA, and HCl) at different concentrations (2.5, 5.0, 7.5, 10.0, and 12.5 ppm) of orange essential oil is shown in Fig. 2. All gas sensors of the array distinguished the different concentrations of orange essential oil. All sensors presented saturation in the resistance signal at 12.5 ppm; thus, this concentration was excluded from the experiments.

Table 4 shows the sensitivity and limit of detection values for Pani gas sensors with different dopants exposed to orange essential oil. These values were obtained from the calibration curve (Fig. 2b). Pani sensors doped with TSA and CSA showed the high limit of detection and sensitivity for orange oil (Table 4). The response time demonstrates that the Pani HCl was faster in the detection of the orange oil (Table 4). The volatiles of orange oil come principally from limonene, considering that it is a major compound with considerable volatility (133 Pa steam pressure at 14 °C) that contribute to the citrus and floral aroma (Högnadóttir and Rouseff 2003). The good sensitivity of the sensors is attributed to the high surface area of the film. Also, it can be correlated to the conductivity of the dopant used (CSA > DBSA>TSA > HCl) that depend on interchain mobility, and the chain length (DBSA>CSA > TSA >

Table 3 Results of acidity (meq NaOH/100 g) analysis of dark chocolate during the storage time with and without addition of orange oil

Acidity (meq NaOH/100 g)					
Time (days)	Concentration of orange oil (ppm)				
	0	2.5	5.0	7.5	10.0
1	$1.59^{e} \pm 0.01$	$2.13^{e} \pm 0.01$	$2.43^{d} \pm 0.02$	$2.53^{e} \pm 0.01$	$2.59^{e} \pm 0.01$
20	$2.16^{d} \pm 0.01$	$2.27^{d} \pm 0.01$	$2.43^{d} \pm 0.01$	$2.48^{d} \pm 0.01$	$2.64^{d} \pm 0.01$
40	$2.22^{c} \pm 0.01$	$2.75^{c} \pm 0.01$	$2.83^{c} \pm 0.01$	$3.15^{c} \pm 0.01$	$3.23^{c} \pm 0.01$
60	$2.33^{b} \pm 0.01$	$3.04^{b} \pm 0.01$	$2.99^{b} \pm 0.01$	$3.28^{b} \pm 0.01$	$3.44^{b} \pm 0.01$
100	$2.37^{a} \pm 0.02$	$3.28^{a} \pm 0.02$	$3.31^{a} \pm 0.01$	$3.44^{a} \pm 0.01$	$3.65^{a} \pm 0.03$

Mean values ± standard deviation followed by the same lowercase letters during the storage on the column do not indicate any significant difference at a 5% level (Tukey's test)

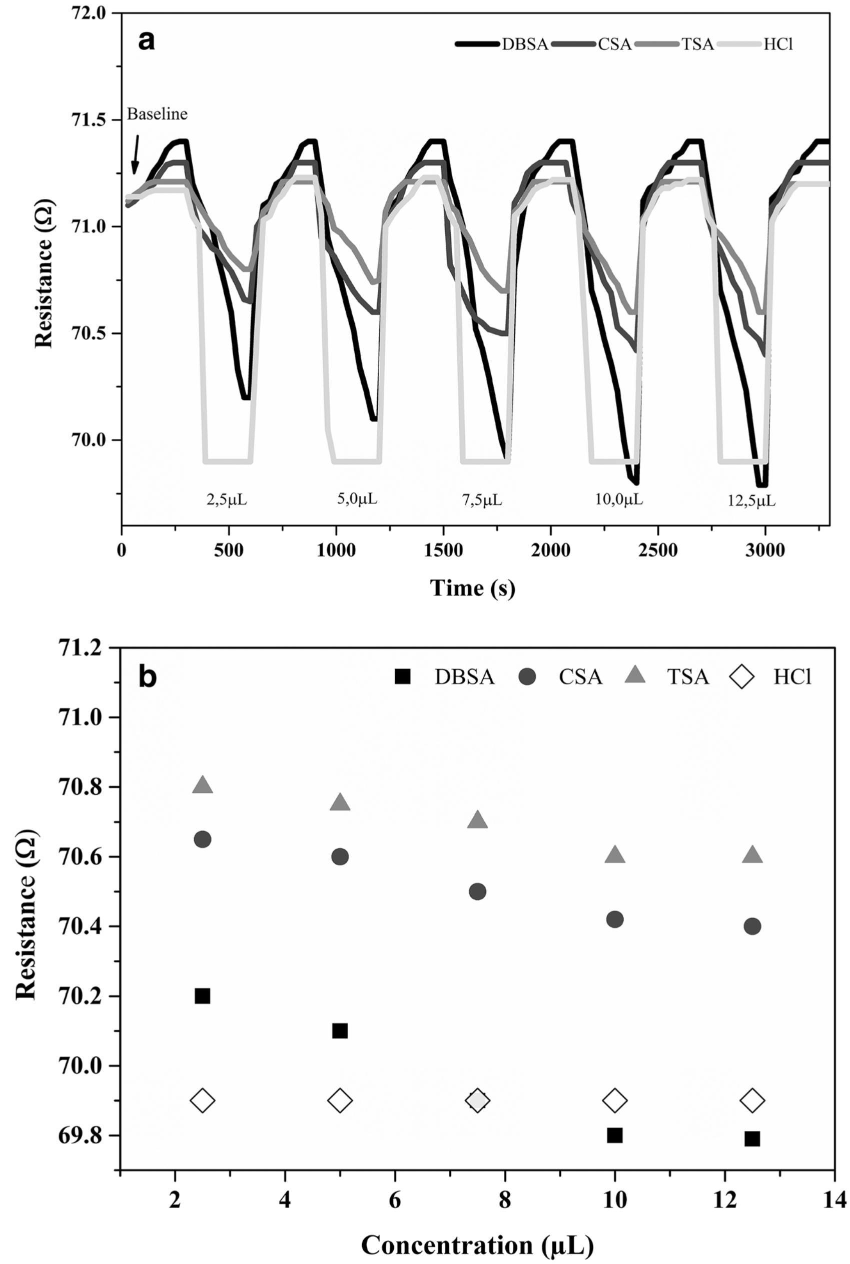


Fig. 2 Response of Pani film gas sensors with different dopants (DBSA, CSA, TSA, and HCl) exposed to different concentrations of orange oil (**a**) and response curve (**b**)

HCl) exerts more force against ordering and closing of the Pani chains (Sinha et al. 2009).

The size of the counter-ion may affect the mobility of charges present in the polymer chain and the free volume between them (Sinha et al. 2009), which influences the sensitivity and charge-transport properties. The addition of protons into Pani by the dopant (acid) has important effects on the conductivity. According to Kizildag and Ucar (2014) CSA is

Table 4 Pearson coefficient, limit of detection, sensitivity, and response time values for Pani gas sensors with different dopants (DBSA, TSA, CSA, and HCl) to the orange oil

Dopants	Pearson coefficient	Detection limit (ppm)	Sensitivity (mΩ/ppm)	Response time (s)
DBSA	0.965	16.755	0.052	147.97
TSA	0.982	7.108	0.026	119.27
CSA	0.972	7.322	0.025	83.74
HCl	0.800	111.611	0.066	54.25

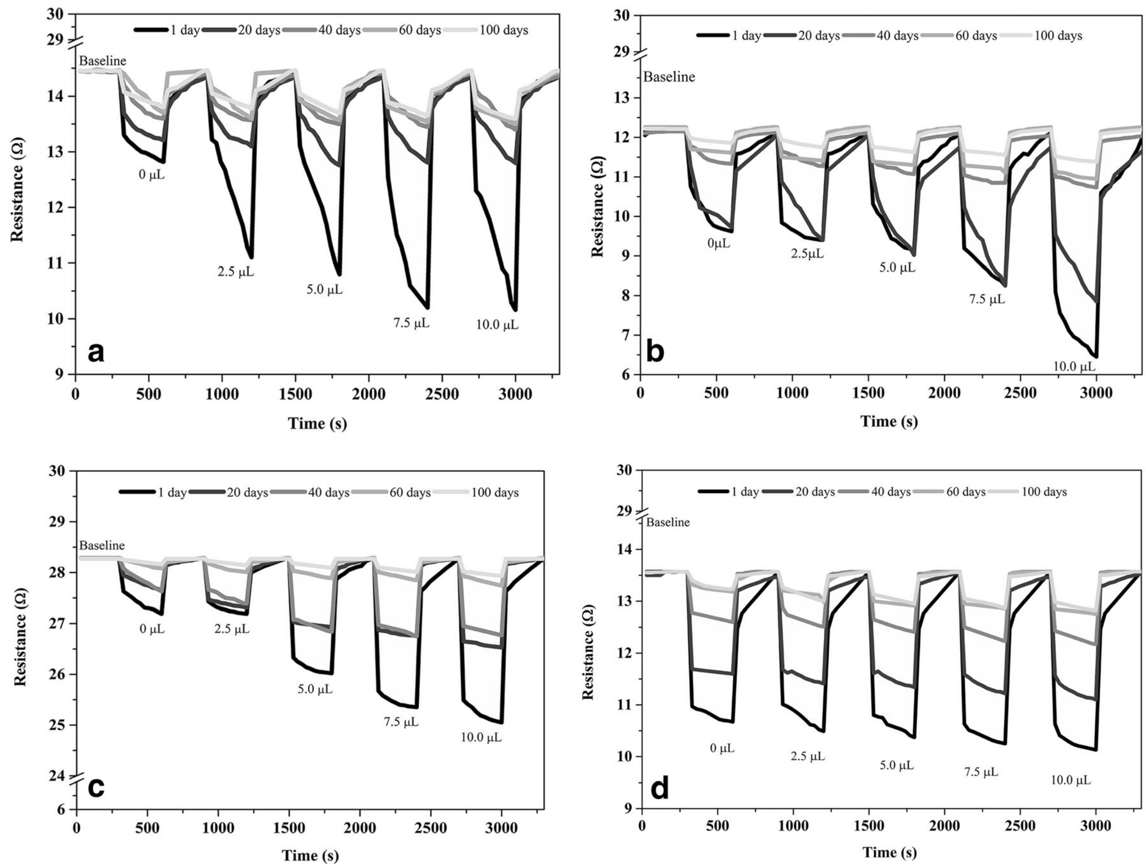


Fig. 3 Response of the Pani gas sensor array doped with (**a**) CSA, (**b**) TSA, (**c**) DBSA, and (**d**) HCl, exposed to chocolate with and without addition of orange oil during the storage

more acidic than DBSA, so the Pani film doped by CSA acid presents higher conjugation length and conductivity.

The fast response time of HCl sensor can be due to the morphology of the Pani film which allows the volatile molecules to easily diffuse into and/or out of the film, in relation to the other dopants. This was also observed by Qi et al. (2014) when used Pani deposited on a porous substrate. The possible reason for the fast response is the presence of lighter dopant ions (Cl−) in Pani doped with HCl, which promotes higher charge mobility. Tiggemann et al. (2017) also observed faster response time (3–11 min) for HCl-doped Pani sensor in relation to CSA and DBSA sensors in the detection of artificial aromas (strawberry, grape, and apple).

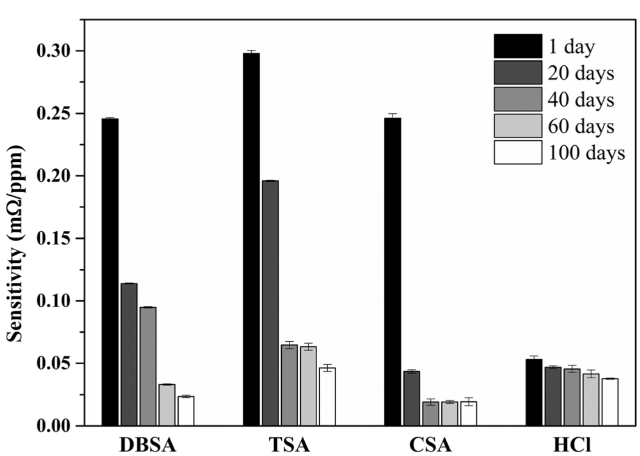


Fig. 4 Sensitivity values for Pani film gas sensors using different dopants (DBSA, TSA, CSA, and HCl) exposed to volatiles of the dark chocolate with addition of orange oil during the storage

The electronic nose experiments with chocolate were performed at 35 °C and relative humidity of 56% (± 4). The response was evaluated initially with dark chocolate without orange essential oil (0 ppm). Gas sensors presented response to chocolate without the addition of orange oil. Dark chocolate has volatile chemical compounds like pyrazines, amines and amides, acids, esters, and hydrocarbons (Afoakwa et al. 2008; Fernández-Murga et al. 2011) that was detected by the sensor array.

In Fig. 3, the resistances of the sensors decrease with the increase of the concentrations of orange essential oil in the chocolates. TSA, CSA, and HCl-doped sensors presented the low resistance to volatiles, which may be related to the dopant structure (Guenet 2008). The conductivity can be related to the length of the alkyl side chain, and the transport of electrons can be associated to the interchain transfer and intrachain coherence length (Zheng et al. 1996). The sudden drop on the resistance signal could be attributed to the

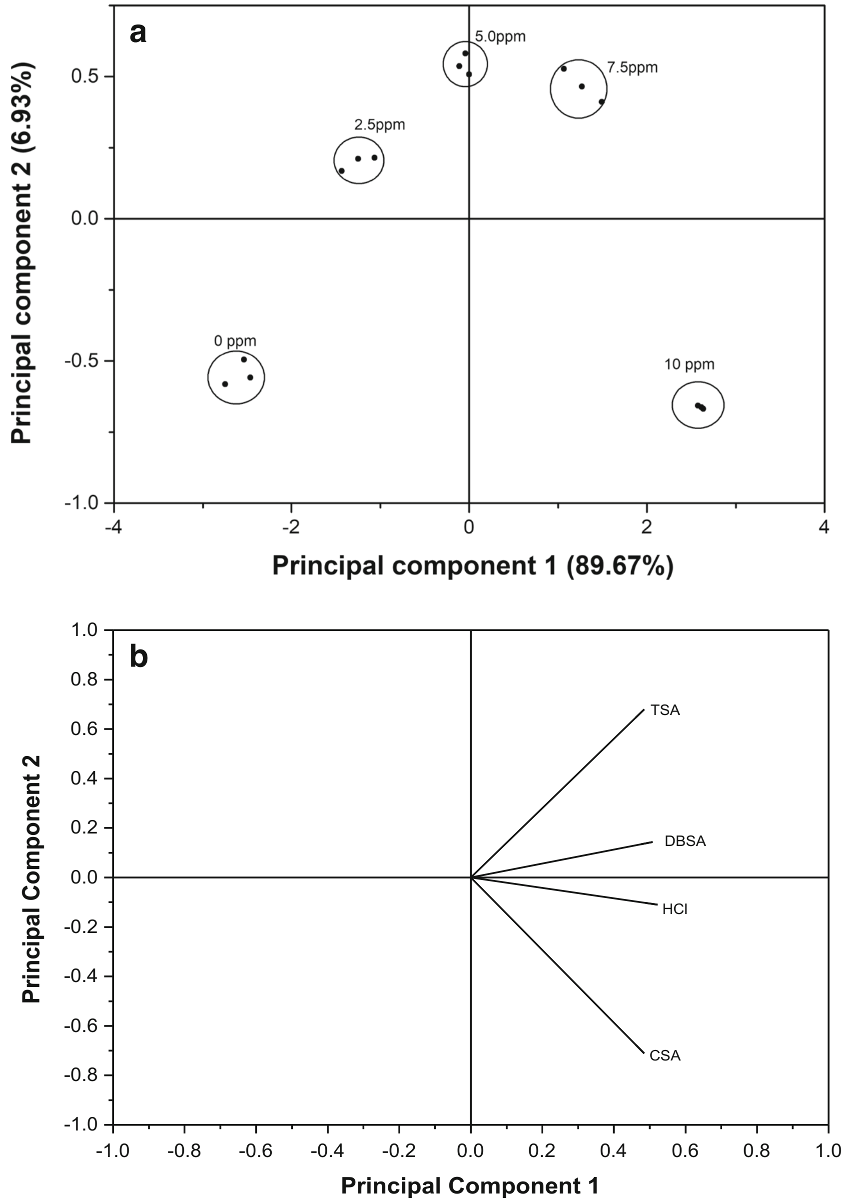


Fig. 5 PCA plot obtained from maximum resistance response of sensor array in the detection of the volatiles from the dark chocolate without and with the addition of different concentrations of orange essential oil (**a**), and loading plot of the gas sensor array (**b**)

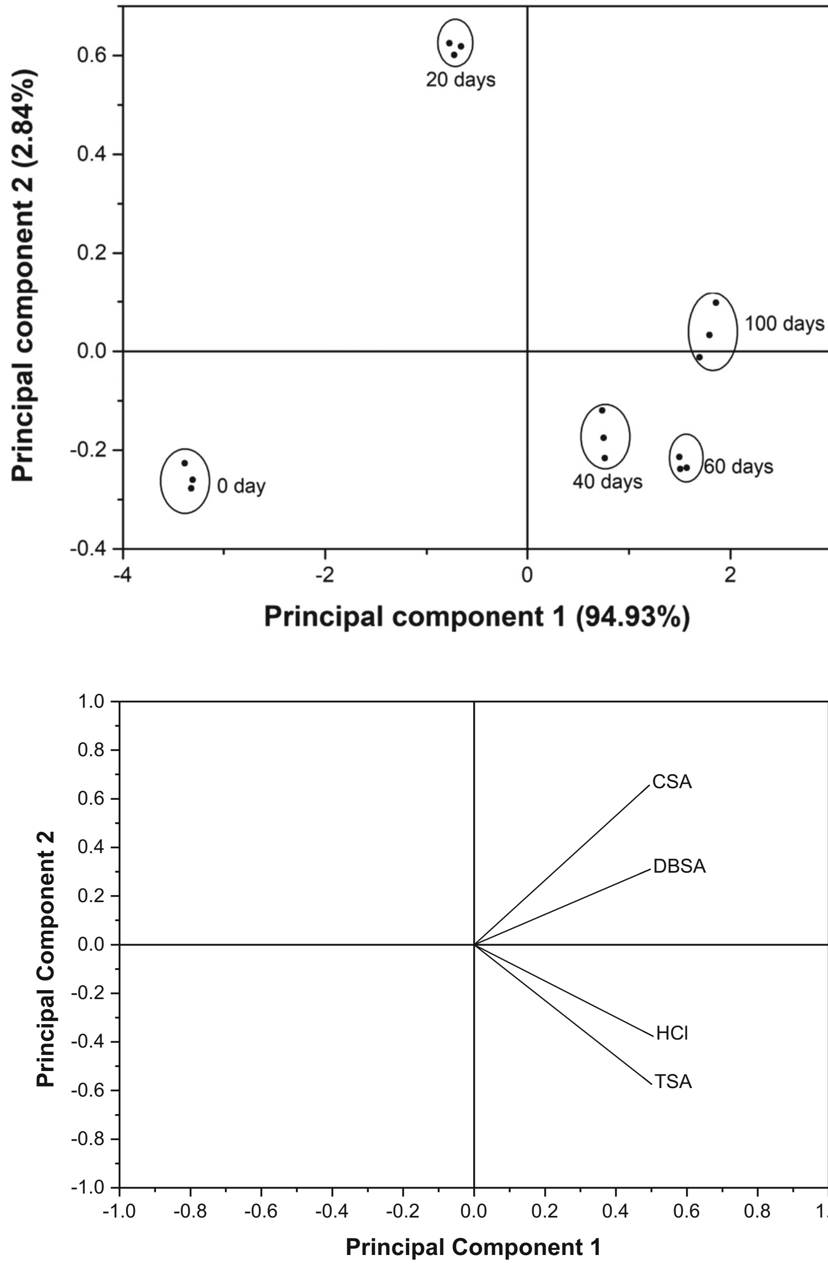
swelling of the Pani doped films when in contact with the volatile compounds that diffuse on the matrix. This response mechanism can increase the interchain distance in the Pani film and affects the flow of the electrons.

The resistance results of the sensor array decreased with the storage time of dark chocolate with different concentrations of orange essential oil. The loss of volatile compounds of the dark chocolate with storage is related to their constituents that can either block the volatile outflow through the barrier or increase the viscosity (Nightingale et al. 2012). Also, this result can be associated with the moisture values, because an increase of moisture was observed during the first and 40 days of storage.

The response to the volatile substances and concentrations occurs because each dopant used provides a kind of selectivity, which is called molecular recognition (Manzoli et al. 2010). Thus, sensors doped with different acids (CSA, TSA, DBSA, and HCl) exhibited different behaviors in the presence of the same specific substance. The TSA-doped sensor showed high sensitivity to the volatiles of the dark chocolate with orange oil (Fig. 4). The sensitivity values decrease for all sensors during the storage due to the volatile release.

Gas sensor responses were analyzed by principal component analysis (PCA) at different concentrations of orange essential oil (0, 2.5, 5.0, 7.5, and 10.0 ppm) added to the chocolate (Fig. 5a). The PCA showed 96.6% of the total information collected by the matrix in PC1 and PC2. PC1 presented the largest amount of information (89.67%); therefore, the concentration analysis should be based on this axis, although the small contribution of PC2 (6.93%) should be considered

Fig. 6 PCA plot obtained from maximum resistance response of the Pani sensor array in the detection of the volatiles from the dark chocolate during 100 days of storage (**a**), and loading plot of the gas sensor array (**b**)



for analysis. The high discrimination was observed between chocolate without orange oil (0 ppm) and the concentration of 10.0 ppm, being distinctly represented in the lower quadrant. Already, the concentrations 2.5, 5.0, and 7.5 ppm were discriminated but were closer.

The resistance results obtained by electronic nose experiments with chocolate during the storage (1, 20, 40, 60, and 100 days) were also evaluated by PCA (Fig. 6a). 97.77% of the total information was collected by the matrix (PC1 and PC2), and the PC1 has the largest amount of information (94.93%). The results demonstrate that the sensors' array used was able to discriminate the storage time of chocolate, being more evident a difference between 1, 20, and 100 days of storage. Lower discrimination was verified between 40 and 60 days.

The analysis of the contribution of each gas sensor (doped with CSA, TSA, DBSA, and HCl) of electronic nose array in the discrimination of different concentrations of orange essential oil (0, 2.5, 5.0, 7.5, and 10.0 ppm) and different days of storage was evaluated on the loading plot, presented in Figs. 5b and 6b, respectively. The loadings' plot showed the distribution of sensors in the positive side of the principal components. The sensors with high sensitivities (CSA and TSA) to the volatiles presented longer projections on the axes in relation to sensors doped with DBSA and HCl.

In literature works, the electronic nose was also applied in the discrimination of gummy candy volatiles during storage. The gummy candies were aromatized artificially flavored with apple, strawberry, and grape (Ballen et al. 2019). The developed electronic nose demonstrates great potential in the investigation and monitoring of volatiles and can be a powerful tool for the food industries to help in food quality control.

The results showed that the Pani gas sensor array doped with different acids was able to detect the volatile release from the dark chocolate during storage, presenting an excellent response to the concentrations studied (0, 2.5, 5.0, 7.5, and 10.0 ppm). In addition, dark chocolate with orange essential oil can be used as a flavoring agent, enhancing the health benefit with its biological activities as an antioxidant, anti-inflammatory, and antimicrobial.

Conclusions

Dark chocolate with orange essential oil indicated that there was no significant difference ($p > 0.05$) between the days of storage in the water activity analysis. However, it showed an increase in moisture and acidity values during the storage time. The chromatographic analysis of the orange essential oil demonstrates that d-limonene is the major substance present in the sample.

The sensors doped with CSA and TSA had the highest sensitivities, and thus felt more the presence of volatiles in orange oil. When the sensors were exposed with dark chocolate with different concentrations of orange essential oil, the best response was the sensor using the TSA dopant, followed by CSA, DBSA, and HCl. It was observed that there was a decrease in the response of the sensors during the storage days, related to the loss and release of volatiles.

Principal component analysis for different concentrations and storage time of the dark chocolate with orange oil showed good discrimination of orange essential oil at different concentrations. So, the different acid-doped Pani sensor array is a potential technique in the detection of volatiles during the food storage.

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Compliance with Ethical Standards

Conflict of Interest Elisiane Galvagni declares that she has no conflict of interest. Andressa Fritzen declares that she has no conflict of interest. Sandra Cristina Ballen declares that she has no conflict of interest. Adriana Marcia Graboski declares that she has no conflict of interest. Juliana Steffens declares that she has no conflict of interest. Clarice Steffens declares that she has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

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