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Development of low-density polyethylene films with lemon aroma

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ABSTRACT

The purpose of this study was to develop active films that impart flavour and to test them in the packaging of biscuits. The films were mechanically analysed, with respect to colour and water vapour permeability (WVP) to evaluate the changes in the films resulting from the active agents and conditioning time. We used low-density polyethylene with incorporated lemon essential oil (EO) and/or lemon aroma to create the films that were used in biscuits and evaluated over a 30 day period. The results showed that the films showed a lower elongation due to the incorporation of active agents, and they showed a reduction of tensile strength over time. In addition, the combined use of EO and aroma did not affect the WVP value. As for colour, flavouring films had a more yellow colour and were opaque. Sensory biscuits packed with flavouring films showed an average acceptance of approximately 8.0 based on aroma and taste. These films represent an innovation for the packaging industry, and, based on our results, we recommend the combined use of EO and aroma to develop films for flavouring.

1. Introduction

Citrus essential oils (EOs) contain 85–99 g/100 g volatile components and 1–15 g/100 g non-volatile components. The volatile constituents are a mixture of monoterpene hydrocarbons (limonene), sesquiterpene hydrocarbons and their oxygenated derivatives, which include aldehydes (citral), ketones, acids, alcohols (linalool) and esters (Sawamura et al., 2004; Vaio et al., 2010). The major chemical component of citrus oils is limonene, ranging from 45 to 76 g/100 g or lemon. Citral and linalool are thought to be the most potent aromatic compounds in citrus fruits, but they do not exceed 3 g/100 g in lemon oil. Fatty acids make up a negligible percentage (about 0.2 mL/100 mL) of citrus oils, and the major fatty acid in lemon oil is linoleic acid (Fisher & Phillips, 2008; Svoboda & Greenaway, 2003).

It is widely recognised that most of the essential oils have antimicrobial properties (Emiroğlu, Yemiş, Coşkun, & Candoğan, 2010; Fisher & Phillips, 2008; Suppakul, Sonneveld, & Bigger, 2011; Tsigarida, Skandamis, & Nychas, 2000). Individual components of EO, which are either extracted from plant material such as flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots (Bajpai, Baek, & Kang, 2011), or synthetically manufactured, are also used as food flavourings.

The ability of citrus oils to delay spoilage and add organoleptic qualities in food products may be interesting from a commercial point of view (Bajpai et al., 2011; Tunç & Duman, 2011). However, there are few studies evaluating EO compounds used to modify the sensory properties of foods (Gutiérrez, Batlle, Andújar, Sánchez, & Nerín, 2011; Kostaki, Giatrakou, Savvaidis, & Kontominas, 2009).

Food processing, heat treatment, concentration, evaporation, boiling, baking and the food matrix effect (Taylor, 2002) can result in a loss of flavour quality. To prevent this loss, active packaging materials can be used. Through of the incorporation of active agents in the polymer matrix, food can be aromatised by an interaction between the package and product. In addition to improving the sensory characteristics of foods, flavouring active packaging can be used to develop new products. From a processing line, you can obtain products of different flavours with the use of flavouring packaging in the conditioning stage. This is useful in a food industry

Abbreviations: WVP, Water vapour permeability; EO, Essential oil; LDPE, Low-density polyethylene; PP, Polypropylene; TS, Tensile strength; E, Elongation at break; RH, Relative humidity; PCA, Principal Component Analysis.

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that relies mostly on incremental innovation for new product launches; there is an increasing awareness in the industry that innovations are needed to remain competitive.

The transformation of cereal products from dough to biscuit, for example, is a very complicated process involving numerous mechanisms and many properties that must be controlled, such as colour, shape, aroma and crispness (Perrot et al., 2000). Biscuits are an important class of bakery products that are produced in a large variety of flavours. Every day, new types of biscuits, often with innovative flavours, are launched on the market. The degree of protection required by biscuits is determined to a great extent by their composition and the manufacturing process. However, in general, the shelf life of biscuits depends fundamentally on the barrier properties of the packaging materials used to preserve and protect the product from the ingress of atmospheric moisture and other agents that negatively affect flavour (Alves, Garcia, & Bordin, 1999).

Our objective was to develop low-density polyethylene flavouring films imbued with essential oils and/or lemon aroma. We sought to apply these films to the packaging of biscuits to evaluate the mechanical properties, water vapour permeability and colour of the films and the sensory properties of the biscuits packaged in the active films.

2. Materials and methods

2.1. Materials definer

Low-density polyethylene (LDPE, Braskem, Brazil), high-density polyethylene with a high absorption capacity (Accurel XP200, Braskem, Brazil), lemon essential oil (EO) and lemon heat resistant aroma (Duas Rodas Industrial Ltda., Brazil) were used to prepare the flavouring film. These films have the ability to aromatize food by diffusion of the active compounds added to the polymer matrix.

2.2. Experimental design

We used a complete factorial design with the following factors: level of EO/aroma (film 1: without EO and without aroma; film 2: with 10 mL of EO and 5 mL of aroma/100 g of polymer; film 3: with 5 mL of EO and 5 mL of aroma/100 g of polymer; film 4: with 10 mL of aroma/100 g of polymer) (Table 1) and observation times (0, 10, 20, 30 days). The experiment was conducted using a completely randomised design, and all samples were prepared and analysed in triplicate.

2.3. Preparation of the film

For the development of films with LDPE lemon flavouring, the resin Accurel XP200 was imbued with EO and/or lemon aroma. Subsequently, the blend (LDPE + Accurel XP200) was extruded using a monorosca extruder HaakePoly Drive (Thermo, Germany) with an extruded tube and five temperature stages (temperatures of 120, 130, 140, 150, and 160 °C, respectively).

Table 1
Flavouring films developed.

Films	Lemon	
	Essential oil (mL/100 g of polymer)	Aroma (mL/100 g of polymer)
1	0	0
2	10	5
3	5	5
4	0	10

2.4. Antimicrobial activity of EO

The antimicrobial activity of EO was evaluated by measurement of the inhibition zone sizes against *Staphylococcus aureus* (ATCC 6538), *Listeria innocua* (ATCC 33090), *Escherichia coli* (ATCC 11229), *Salmonella choleraesuis* (ATTCC 6539), *Pseudomonas aeruginosa* (ATCC 15442) (Fundação Osvaldo Cruz, Rio de Janeiro, RJ, Brazil) according to the Solid Diffusion Assays described by López, Sanchez, Batlle, and Nern (2005).

Strains of microorganisms were cultured over two nights to obtain nearly 10^8 viable cells mL^{-1}. The cultures were diluted in 0.1 g of peptone water/100 mL of solution to 10^6 cells mL^{-1} and inoculated in duplicate Petri dishes containing Mueller Hinton culture medium (Acumedia, Michigan). Filter paper (1 cm in diameter), previously sterilised by treatment with a UV lamp for 2 min in each side, was dampened with the essential oil of lemon and placed in the centre of each Petri dish. The dishes were incubated at 36 ± 2 °C for 48 h, and the diameters of the inhibition zones formed around the films were measured.

2.5. Experiment: application of films to biscuits

The flavouring films (primary packaging) were sterilised in a chamber with a UV lamp (Prodicil, 110 V, 254 nm) for 15 min and they were used to package biscuits (15 units). The biscuits wrapped in flavouring film were packed in polypropylene (PP) plastic bags (secondary packaging) that were sealed in sealing machine (Selovac® 200B, São Paulo, SP – Brazil) and stored at a controlled temperature of 20 ± 2 °C.

2.6. Analysis of films

2.6.1. Thickness

The thickness of the films was measured using a manual micrometre (Mituyoyo SulAmericana Ltda., Brazil, precision 0.002 mm), and the average of five measurements for each film was used to calculate the tensile properties. For water vapour transmission (WVT) calculations, the average of three thickness measurements of each sample was used (Kechichian, Ditchfield, Veiga-Santos, & Tadini, 2010).

2.6.2. Mechanical properties

The mechanical properties of the films were determined by the tensile test using a Universal Testing Machine (Instron, model 3367, USA) with the following parameters: a load cell of 1 kN and a speed of 50 mm min^{-1}. For each film, five samples with dimensions of 50 mm × 150 mm were analysed. The tensile strength (TS, MPa) and elongation at break (*E*, %) values were measured. TS was calculated by dividing the maximum load by the cross-sectional area of the film, and *E* was calculated by dividing the extension at the moment of rupture of the specimen by the initial length of the specimen and multiplying the result by 100 (ASTM, 2008).

Mechanical analysis were performed at 0, 10, 20 and 30 days of storage.

2.6.3. Water vapour permeability

The water vapour permeability (WVP) of the films was determined according to ASTM Standard Method 96-00 (ASTM, 2000), method E96, with some modifications. The test film was sealed in a permeation cell containing anhydrous calcium chloride. The permeation cell was then placed in a controlled temperature–humidity chamber maintained at 75% relative humidity (RH) and 25 °C to maintain a 75% RH gradient across the film. Because the RH inside of the cell was always lower than the outside, water vapour transport could be determined based on the amount of mass gained by the permeation cell. The samples were weighed until a constant weight was reached, and the weight values were plotted as a function of time. The slope of each line was calculated by linear regression ($r^2 > 0.99$), and the water vapour transmission rate (WVTR, g/h/m^2) was calculated from the slope of the straight line divided by the exposed film area (m^2). The WVP (g/(m s Pa)) of the film was calculated as follows:

$$\text{WVP} = (\text{WVTR}\cdot x)/3600(P_1 - P_2)$$

where x is the film thickness, and $P_1 - P_2$ represents the vapour pressure differential across the film.

The WVP of the films was measured at day 0.

2.6.4. Colour determination

Colour was measured using the Color Quest XE colorimeter (Huber Lab) and CIELab system with a D65 light source and an observation angle of 10°. The following parameters were used: opacity, $Y = (Y_b/Y_w)\cdot 100$, according the relationship between the opacity of the film superposed on the black standard (Y_b) and opacity of the film superposed on the white standard (Y_w), and b^* (yellowness).

Colour analysis were performed at 0, 10, 20 and 30 days of storage.

2.7. Analysis of the biscuit

2.7.1. Sensory analysis

The product was assessed for sensory acceptability at a central location. One hundred and two consumers of biscuits, between the ages of 15 and 60, evaluated the samples using a nine-point hedonic scale (1 = dislike extremely and 9 = like very much) (Stone & Sidel, 2004, p. 377). They used the scale to indicate how much they enjoyed or disliked the taste and aroma of lemon in the product. The three samples (A, B and C) were presented in a single session. We used the randomised complete block design.

Sensory analysis of biscuits was performed at 10 and 30 days.

2.8. Analysis of results

The data on the mechanical, thickness, colour and WVP properties of the films were subjected to an analysis of variance (ANOVA), and treatment means were compared using a Tukey test with a 5% p-value cut-off. These statistical analyses were performed using the software package SISVAR® (Ferreira, 2000).

To obtain the map of Internal Preference (Macfie & Thomson, 1988), the sensory analysis data were subjected to a Principal Component Analysis (PCA) based on the covariance matrix. The results are expressed as a biplot graph with the dispersion of the films and consumer sensory acceptability in the two first principal components. PCA was performed in Matlab version 7.5.

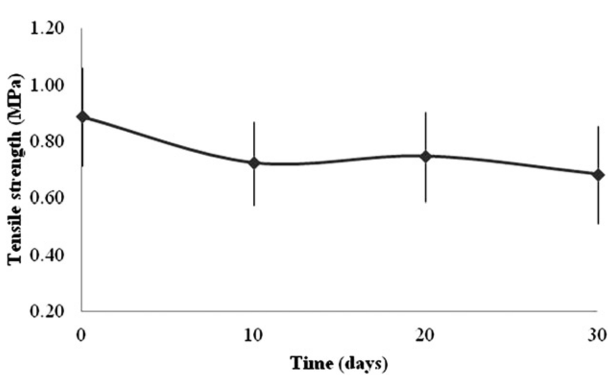

Fig. 1. Variation of tensile strength and standard deviation of the films imbued with essential oil and/or lemon aroma for 30 days after packing.

Table 2
Average values of percentage elongation at break[a] (%) of the films over time.

Film	Time (days)			
	0	10	20	30
1	212.47 ± 12.58^{aA}	3.99 ± 0.57^{bB}	4.34 ± 0.66^{aB}	27.36 ± 6.15^{aB}
2	6.44 ± 2.60^{cA}	7.92 ± 5.07^{bA}	9.52 ± 3.55^{aA}	5.89 ± 1.65^{aA}
3	4.66 ± 0.23^{cA}	5.33 ± 0.28^{bA}	4.75 ± 0.04^{aA}	5.50 ± 0.50^{aA}
4	107.41 ± 14.602^{bA}	119.71 ± 30.41^{aA}	4.57 ± 0.91^{aB}	5.94 ± 1.02^{aB}

The same lowercase (column) and uppercase (row) letters indicate differences that are not significant ($P > 0.05$), according to Tukey's test.
[a] The mean ± standard error.

3. Results and discussion

3.1. Antimicrobial activity of EO

We did not observe the formation of inhibition halos around the filters. Therefore, EO did not show antimicrobial activity, and the developed films were used as flavouring active packaging.

3.2. Analysis of films

3.2.1. Thickness

The level of EO and/or lemon aroma used did not significantly affect ($p > 0.05$) the thickness value of the films. The average value of the thickness for all films was 0.525 ± 0.06 mm.

3.2.2. Mechanical properties

The level of EO and/or lemon aroma added to the polymer matrix did not significantly affect ($p > 0.05$) the TS value of the films. The time factor was significant ($p < 0.05$), and, after 30 days, the treatments led to a reduction in TS (Fig. 1).

The force required to break the film decreased during conditioning of the biscuits. The contact between the product and packaging causes physical, chemical and structural changes in the polymeric materials. These changes occur due to the constitution of the product and may be caused by presence of oxygen or UV radiation and others. As a result, these changes can induce the process of polymer degradation, the migration of chemical compounds of low molecular weight and a reduction in functionality (Shimamura & Nakamura, 2009; Steinka, Morawska, Rutkowska, & Kukułowicz, 2006).

For elongation, the interaction of the factors studied was significant ($p < 0.05$). It is possible to observe that at time 0, the film without the addition of EO and aroma showed the highest value of E in relation to the other treatments (Table 2). The incorporation of

Table 3
Water vapour permeability[a] (WVP) and colour parameters[a] evaluated for the flavouring films.

Films	WVP (10^{-11} g m^{-1} s^{-1} Pa^{-1})	b^*	Opacity
1	1.78 ± 0.29^b	0.64 ± 0.16^b	10.55 ± 0.25^b
2	1.27 ± 0.04^b	0.78 ± 0.13^a	10.99 ± 0.36^a
3	1.66 ± 0.02^b	0.74 ± 0.07^{ab}	10.74 ± 0.25^{ab}
4	3.82 ± 0.70^a	0.75 ± 0.11^{ab}	10.76 ± 0.25^{ab}

The same lowercase (column) letters indicate differences that are not significant ($P > 0.05$), according to Tukey's test.
[a] mean ± standard error.

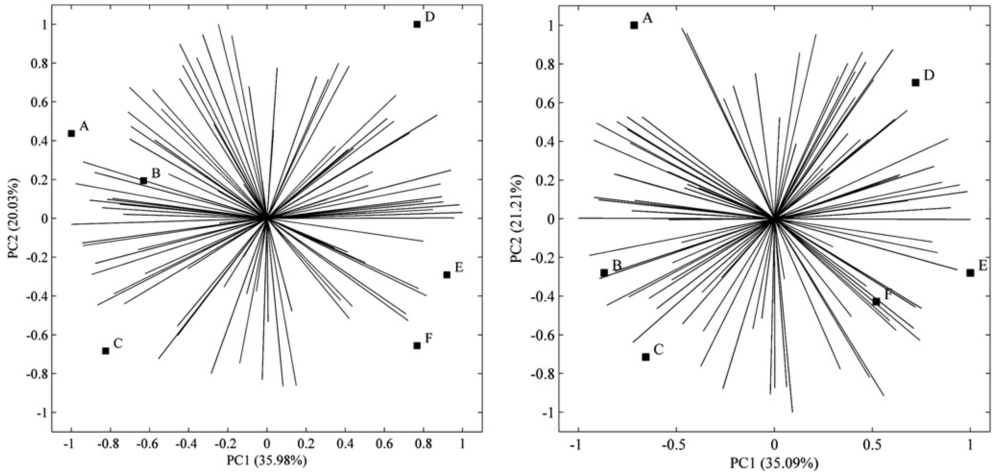

Fig. 2. PCA biplot of PC1 versus PC2 (scores and loads) for the sensory evaluation data of the aromas and taste of samples. A: biscuits conditioned for 10 days in film with 10 mL of EO and 5 mL aroma/100 g of polymer; B: biscuits conditioned for 10 days in film with 5 mL of EO and 5 mL of aroma/100 g of polymer; C: biscuits conditioned for 10 days in film with 10 mL of aroma/100 g of polymer; D: biscuit conditioned for 30 days in film with 10 mL of OE and 5 mL of aroma/100 g of polymer; E: biscuits conditioned for 30 days in film with 5 mL of OE and 5 mL of aroma/100 g of polymer; F: biscuits conditioned for 30 days in film with 10 mL of aroma/100 g of polymer.

EO and aroma caused microscopic changes in the structures of the films. The constituents of the active agents increased the intermolecular forces in the film, increasing the film stiffness and reducing the mobility of polymer chains. This led to a reduced capacity for elongation (extensibility) in the film.

We observed reduction of 49% in value of *E* for film 4, which was developed by adding 10 mL of aroma/100 g of polymer, which is polar, to the apolar LDPE matrix. This reduction could be explained by discontinuities in the polymer matrix introduced by the aroma incorporation and by changes in the polymer chain interactions in the presence of the aroma components, which lead to a weak mechanical response (Sánchez-González, Cháfer, Chiralt, & González-Martínez, 2010).

For other flavouring films developed in this work, there was a reduction in *E* of 96 and 97% for films 2 and 3 (10 mL of EO + 5 mL of aroma/100 g of polymer; 5 mL of EO + 5 mL of aroma/100 g of polymer, respectively) compared to the control. The apolar components of the lemon EO may have increased the strength of the links in the polymer chain and, consequently, increased the rigidity of the film.

Over time, significant changes ($p < 0.05$) in the values of *E* were observed only for films 1 and 4 (film without EO and without aroma and film with 10 mL of aroma/100 g of polymer, respectively) (Table 2). This shows that the lemon EO incorporated in the other treatments, films 2 and 3, acted to protect the films from alterations over time.

3.2.3. Water vapour permeability (WVP)

The results showed a significant effect ($p < 0.05$) level of EO and/or aroma on WVP. Components of the lemon aroma, such as alcohols and esters, have hydrophilic characteristics and water molecules diffuse preferentially in the hydrophilic phase (Sánchez-González et al., 2010). Furthermore the incorporation of 10 mL of aroma/100 g of polymer that has hydrophilic characteristics into the hydrophobic LDPE changed the structure of the polymer chains, resulting in a polymer matrix that was discontinuous and had a higher WVP (Table 3).

As shown in Table 2, films prepared with 5 mL of aroma/100 g of polymer (Films 2 and 3) showed no difference in WVP compared to the control, indicating that there is a limit for the addition of aroma within the studied interval. The addition of 10 mL and 5 mL of EO/100 g of polymer, respectively, in films 2 and 3 served to reduce the WVP in accordance with the hydrophobic nature of the EO and its high affinity for LDPE. The oil phase increases in the tortuosity factor for water transfer in the matrix, thus increasing the distance travelled by water molecules diffusing through the film and, consequently, reducing the WVP (Sánchez-González, Cháfer, González-Martínez, Chiralt, & Desobry, 2011).

3.2.4. Colour determination

For the parameters of colour, opacity and b^*, the level of EO and/or aroma in the film was significant. The addition of 10 mL of EO and 5 mL of aroma/100 g of polymer increased ($p < 0.05$) the values of b^* and opacity compared with the control film (Table 3).

The flavouring films showed a more opaque, yellow colouration and therefore were less transparent with respect to films that lack lemon EO and aroma.

3.3. Analysis of the biscuit

3.3.1. Sensory analysis

As shown in the biplot graph (Fig. 2), the first and second principal components (PC1 and PC2) together explain 56.01% and 56.30% of the variation found in the data analysis of the sensory attributes for aroma and taste.

All samples showed high acceptance by the judges with respect to lemon aroma and taste. With 10 days of conditioning, the samples had an average acceptance of 8.0 (really liked) for both aroma and taste, and at 30 days, the averages were 8.6 and 7.7, respectively, for aroma and taste.

After 10 days of contact between the food and the active film, the biscuits already had the taste and aroma of lemon. Therefore, considering the results of the sensory evaluation, it seems that the biscuits can be flavoured only by the incorporation of aroma into the films.

4. Conclusion

The addition of EO and/or aroma did not affect TS, but it reduced the percentage of elongation at break. The use of EO and aroma together protected the film from changes of *E* over time and avoided the reduction in WVP. The addition of only 10 mL of aroma/100 g of polymer increased WVP.

Sensorially, all biscuits were accepted with an acceptance average of approximately 8.0 for the aroma and taste attributes within 10 and 30 days of conditioning.

Considering the results of the characterisation of the films and sensory evaluation of the biscuits, we recommended developing flavouring films that use the EO and aroma of lemon to prevent changes in WVP and mechanical properties through time.

These films have great potential for application in the food industry, and future studies may also support the application of these films in other products. The study of the release of active agents may also lead to similar applications.

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