The Impact of Incorporation of Essential Oil of *Citrus aurantium* Peels on the Texture, Sensory Properties and Kinetics of Liberation of Aroma of Biscuits

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Abstract: The present study deals in the use of essential oil from *Citrus aurantium* peels as an aroma for its application in biscuits. Formulation was designed based on preliminary studies using Mix Design, with three compositions: flour, sugar and essential oil and one response: resistance to the deformation. Different parameters such as instrumental assessment for texture and physic-chemical measurement and sensory evaluation were determined for the selected recipe. The biscuits were packed in unit pouches of metalized polyester/poly laminate and stored at room temperature for three months. The profile of concentration of aroma compound in the matrix of biscuits based on headspace gas chromatography method, shows that biscuits with natural aroma (Essential Oils) retained aroma better than biscuits containing commercial one during period of storage. In addition, the analysis reveals the presence of molecules preventing the liberation of the principle compound of aroma.

Keywords: *Citrus aurantium*, essential oil, Biscuit, Sensory, Texture, aroma, headspace GC

1. Introduction

Aromas have been associated with physiological, psychological affective and behavioral effects (René et al., 2012). Aromas of citrus were considered among the most widely accepted by users as multifunctional agents, strongly stimulating the sense of taste (gustatory) and smell (olfactory). Thus, leaves, bark and orange blossom water are used as condiments in some traditional pastry recipes (Avry and Gallouin, 2003).

The Food and Drug Administration (FDA), focused that natural substances are recognized as "General Recognized As Safe "(GRAS) Smith et al., 2005. In addition, it issued the authorization to use *Citrus aurantium* as a flavoring agent in some medications (Ait Mohamed et al., 2005b; Jeff, 2002).

Orange fruits are the most popular ones for consumers throughout the world due to their pleasant flavors and nutritional value (The fruits are both consumed fresh and industrially processed. The pulps, which are rich in soluble sugars, significant amounts of vitamin C, pectin, fiber and different organic acids, are mainly used to process into juice. Orange peels containing abundant fragrant substances are extensively applied for processing into essential oils which are used commercially for flavoring foods, beverages, perfumes, cosmetics, etc. (Mayer et al., 2005)

Essential oils (also called volatile oils) are aromatic oily liquids obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruitsand roots). They can be obtained by expression, fermentation or extraction but the method of steam distillation is most commonly used for commercial production are one of the most important natural flavoring substances (Ali et al., 2012). The examination of the chemical composition of an essential oil flavoring considered GRAS is necessary to determine its chemical constituents and their range of concentrations and the presence of secondary components (Smith et al., 2005).

Analytical research on the aroma compounds in orange fruit has been carried out for many years. Many researchers have studied the volatile compounds of this fruit using different analytical methods (Jordânn et al., 2005). The major result of these studies indicates that the aromatic quality of the fruit is primarily dependent on the aroma active compounds.

Bitter orange peel contains a volatile oil with limonene (about 90%), flavonoids, coumarins, triterpenes, vitamin C, carotene, and pectin. The flavonoids have several useful properties, being anti-inflammatory, antibacterial, and antifungal (Jyotsna et Suryawanshi, 2011).

The presence of phytonutrient known as limonene in the oil of the peel of citrus fruits, led many agro-industries to use them as aromatic and nutritional ingredient.

In addition, a recent study of the effect of essential oils of *Citrus aurantium* bark on male Wistar rats treated at the rate of 1 to 15 mg of essential oil / Kg / day for 14 days showed no signs of toxicity or biochemical changes except the reduction of cholesterol levels.

Amazingly, we routinely throw out this most potent part of the orange. In fact, the orange peels were treated as waste materials and consequently may create environmental problems for local communities since the presence of biomaterials in orange peel.

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The aim of the study was to highlight the use of bitter orange peel by the establishment of innovative variety of healthy food through the formulation of a new cookie recipe with essential oil of bitter orange peel and to provide useful information about the most important potent odorants in this part fruit.

2. Materials and Methods

2.1. Plant Material

The peels of sour orange were harvested from the region of Nabeul (Northern Tunisia) during the month of February. Specimens were identified by Dr. Mohamed Larbi Khouja at the National Institute of Research in Rural Engineering, Water and Forest (INRGRREF, Tunis, Tunisia) and voucher specimens were deposited at the Herbarium of the Department of Botany in the cited institute.

The samples were then dried in the open air in the shade until constant mass and then stored for the isolation of essential oils (EO) and organic extracts. The dry matter content of plants was determined using an infrared dryer.

2.2. Extraction

2.2.1. Isolation of EO

A portion (100 g) of the dry plant material was subjected to hydrodistillation in a Clevenger-type apparatus as described in the European Pharmacopoeia and in the 10th edition of the French Pharmacopoeia. This operation was repeated three times for each sample. The EO was recovered, dried with anhydrous sodium sulphate and stored at 4 °C in the dark until its use.

2.2.2. Determination of chemical composition of EO by GC-MS

The volatile constituent’s analyses were achieved on a Hewlett-Packard gas chromatograph GC: 6890 series II and HP 5973 mass selective detector. The fused HP-5MS capillary column (l=60 m, φ=0.25 mm ID, 0.25 μm film thickness) was directly coupled to the mass spectrometer. The carrier gas was helium, with a flow rate of 1.2 mL/min. The oven temperature was programmed from 40 °C (1 min) to 280 °C (15 min) at 5 °C/min. The temperature of the injector port was held at 250 °C, the temperature of the detector was set at 280 °C. The mass spectrometer was operating (full scan-mode) in the EI-mode at 70 eV. The injected volume is equal to 1μL in the mode split. Compounds were identified by comparison of their KI (Kovats indices) relative to C5-C24 n-alkanes obtained on a non polar DB-5MS column, with those provided in the literature, by comparison of their mass spectra with those recorded in the library database Chem Station HP: HP Wiley 275L and reported in published articles and by co-injection of available reference compounds. The samples were analyzed in duplicate. The percentage composition of EO was computed by the normalization method from the GC peak areas. Results were calculated as mean values of two injections from each EO, without using correction factors. All determinations were performed in duplicate and averaged.

2.3. Effect of essential oils from sour orange peels on the sensory and textural properties of biscuits

2.3.1. Formulation methodology

The effect of essential oil incorporated in the cookie can be influenced by the amount of sugar. Seen that increasing concentrations of the latter have a structuring effect on the water, which in turn causes more volatile aromatic substances. Salting flavors can result ultimately in an increased olfactory perception (Mathlouthi and Reiser, 2003). Thus, the sugar / flour ratio is an important factor to master if you want to control the structure of the dough and regularity of the texture of the finished product. For the study of this, mix design approach was used.

2.3.1.1. Study Parametres

The optimization of variables affecting the formula biscuit is carried through a mix design. The measured response (Table 2) is the resistance to deformation. The factors studied are sugar, flour and essential oil dose.

<table>
<thead>
<tr>
<th>Table 2: Factors and Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factors</td>
</tr>
<tr>
<td>Sugar proportion X1</td>
</tr>
<tr>
<td>Flour proportion X2</td>
</tr>
<tr>
<td>Essential oil proportion X3</td>
</tr>
</tbody>
</table>

In our case, the proportions of all components are subjected to the lower and upper constraints (Table 3) based on preliminary tests.

<table>
<thead>
<tr>
<th>Table 3: Specifying limits of variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor</td>
</tr>
<tr>
<td>X1</td>
</tr>
<tr>
<td>X2</td>
</tr>
<tr>
<td>X3</td>
</tr>
</tbody>
</table>

2.3.1.2. Experimental matrix

Table 4 shows the proportions of the main components. These values are generated by the expert design software 9.

<table>
<thead>
<tr>
<th>Table 4: Matrix experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
</tbody>
</table>

2.3.1.3. Experimental and mathematical model

Mixtures plans offer a number of options for building simplices plans and simplices centered mixing variables. These plans can be improved by adding interior points and a centroid. In our case, the proportions of all components are subjected to the lower and upper constraints (Table 3).

2.3.2. Sensory evaluation

Sensory analysis was performed according to ISO 8586-1 (1993). Two types of tests were performed. Analytical Test
(Scoring Test on intensity scale) and hedonic test (Test of Evaluation of preferences)

2.3.2.1. Analytical test
To choose the best cookie formula, we organized an analytical panel. Each panel has 30 trained subjects. This test was used to compare the control biscuit: no aroma (C) and the two flavored cookies: cookie aroma with synthetic (S) and cookie with HE (N).

2.3.2.2. Test of Preferences
A naive panel composed of 30 people was questioned about the degree of acceptance of each descriptor of the product. Tasters noted the assessment of a given characteristic of each coded sample. The samples were coded with random three-digit numbers, simultaneously presented in a random order. The samples are assigned one by one, removing each sample after tasting as well as the completed form before proceeding to the next sample. The results should be in the form of codes corresponding to the sample chosen by the panel. The average data for each descriptor has been calculated. Then, the obtained values were processed by Excel.

2.3.3. Penetrometry
For the rheological study, the texture of biscuits was analyzed by means of a texture analyzer (TA.XT addition, Texture Analyser, Stable microsystem UK) linked to a computer. The test which was carried out is the penetrometry test. The texture analyzer was first calibrated with masses of 2 and 5 Kg then programmed to conduct a distance of 10 mm depth and a speed of 10 mm/s through a 2mm speed pretest/s and a speed test 3mm/s at an angle of 45° and a cylindrical probe P/5 (perpex conical probe) was used as a penetrating tool cookies placed in a concentric cylinder of diameter 30mm. The results are shown as curves whose ordinate of the maximum peak is the maximum deformation (in n/sec) (Tyagi et al., 2006).

2.4. Kinetics of release of aroma compounds
A single compound aroma was followed in this study: limonene. Experimental system: headspace method

The experimental system and the experimental tests were carried out in a cell represented by a bottle of inner cylindrical section 17.9 cm2 and a height of 7.6 cm. The total volume of the bottle is measured 135.6 cm3. A matrix volume of 24 mL was introduced into each vial. Analysis of the headspace in static conditions was performed manually. An amount of 1 ml of gas was collected in the bottle headspace (1 mL syringe, EMS) and injected into a gas chromatograph HP6890 chromatograph equipped with a DB-Wax column (J & W Scientific, length: 30 m, diameter 0.32 mm, thickness: 0.5 µm). We followed the aroma release kinetics by measuring the concentration of limonene in the biscuit side interface: the vials were maintained at 30 °C for various times during the period of strage (1day-115days). A single measurement was performed by bottle. Each measurement was repeated 4 times (4 different bottles).

3. Results and Discussions

3.1. Chemical Composition of EO from Citrus aurantium peels
By hydrodistillation of peels of C. aurantium L., yields (relative to dry weight material) of 1, 17% (w/w), respectively, were obtained. The high yield is in conformity with previous GC-MS studies of C. aurantium L. peel oil (Kirbaslar et al., 2009 and Njoroge et al., 2003). The results of analyses of peels EO by GC-MS are given in Table 1, where the compounds were listed according to their TR.

<table>
<thead>
<tr>
<th>No.</th>
<th>TR</th>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.87</td>
<td>Limonene</td>
<td>87.02</td>
</tr>
<tr>
<td>2</td>
<td>11.51</td>
<td>Linalool</td>
<td>6.24</td>
</tr>
<tr>
<td>3</td>
<td>14.08</td>
<td>α-Terpineol</td>
<td>6.74</td>
</tr>
</tbody>
</table>

The results of chromatographic analysis of EO of sour orange peels given in Table 1 show that the essence of the peel of C. aurantium L. is completely dominated by monoterpenes (100%). The main constituent of this fraction is the limonene (87.02%) which is used as a fragrance material in perfuming household products and as a component of artificial EO (Siddique et al., 2011). These results are in accordance with those obtained by Moraes et al. (2009) who found that limonene (97.5 to 98%) is the major constituent in the oils from the peel of Brazilian C. aurantium L.

3.2. Effect of essential oils from sour orange peels on the sensory and textural properties of biscuits

3.2.1. Formulation using Mix Design
Preliminary tests of incorporation of this oil in the cookie recipe have reduced the amount of butter incorporated, determined the quantity of eggs, and set the upper and lower limits of ingredients such as essential oil, flour and sugar. Formulation of a cookie containing peel EO of Citrus aurantium using mix design. In order to optimize the incorporation of the essential oil of bitter orange peel in the, a centered simplex containing 7 experiments was performed in which the doses of the various factors (flour, sugar, and essential oil) were donated by the Design Expert 9.0.3 software after fixing constraints.

The proportions of the different mixtures and their responses are summarized in the matrix of experiments (Table 5).

<table>
<thead>
<tr>
<th>Order</th>
<th>Flour</th>
<th>Sugar</th>
<th>Essential oil</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.6694</td>
<td>0.3294</td>
<td>0.0012</td>
<td>45.59</td>
</tr>
<tr>
<td>2</td>
<td>0.6388</td>
<td>0.36</td>
<td>0.0012</td>
<td>58.62</td>
</tr>
<tr>
<td>3</td>
<td>0.6395</td>
<td>0.36</td>
<td>0.0005</td>
<td>58.22</td>
</tr>
<tr>
<td>4</td>
<td>0.654362</td>
<td>0.344788</td>
<td>0.00085</td>
<td>53.58</td>
</tr>
<tr>
<td>5</td>
<td>0.7</td>
<td>0.299267</td>
<td>0.000733333</td>
<td>37.09</td>
</tr>
<tr>
<td>6</td>
<td>0.66975</td>
<td>0.32975</td>
<td>0.0005</td>
<td>44.36</td>
</tr>
<tr>
<td>7</td>
<td>0.7</td>
<td>0.2988</td>
<td>0.0012</td>
<td>39.11</td>
</tr>
</tbody>
</table>
The exploitation of ANOVA results indicate that the linear model is significant \( p = 0.0012 < 0.05 \). Indeed, more acceptable probability values, the value of the coefficient of determination further improves the descriptive quality of the model \( R^2 = 96.50\% \) which proves that 96.50\% of the variation tests are explained by this

**4. Mathematical Model**

Coefficients of the terms

Design Expert software estimates the values of various coefficients of the terms of the mathematical model summarized in Table 6 based on the effect of each factor to be able to predict any result in the experimental field by setting the recipe. These coefficients are used to apply a mathematical formula estimator of the response by the factors given by the following equation:

<table>
<thead>
<tr>
<th>Terms</th>
<th>Flour</th>
<th>Sugar</th>
<th>Essential oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coefficients</td>
<td>-67.3751</td>
<td>274.654</td>
<td>2197.08</td>
</tr>
<tr>
<td>probability</td>
<td>0.0012</td>
<td>0.0012</td>
<td>0.0012</td>
</tr>
</tbody>
</table>

\[
\text{Force N} = -67.3751 \times X_A + X_B + 274.654 \times X_C + 2197.08
\]

With:

\( X_A = \) amount of flour
\( X_B = \) amount of sugar
\( X_C = \) amount of essential oil

The optimal range is then defined by the area (50-55) in Figure 2. We used this optimal area to find a formulation of our sought consortium with the following compositions: 65.4\% flour, sugar and 34.4\% 0.085\% essential oil.

Indeed, a sensory analysis was made in order to choose the best cookie from three samples prepared as follows:

- Formula 1: cookie without aroma
- Formula 2: Biscuit with synthetic aroma
- Formula 3: Biscuit with natural flavor: HE

**3.2.2. Sensory Evaluation**

**3.2.2.1. Hedonic test**

Hedonic sensory approach was made by a panel of tasters asked to evaluate their assessments of the various sensory criteria biscuit for 3 samples based on a 7-point rating scale ranging from "I like" to "I hate a lot." Data were processed by analysis of variance (ANOVA) to evaluate the difference in assessment of the various sensory criteria for the 3 samples. Results are also well illustrated by the sensory profiles (Figure 3) that allow the visualization of differences of opinion between the 3 types of biscuit for each descriptor. Indeed, the statistical analysis of the data showed a significant difference between the degree of appreciation of color \( p = 0.003 \). The sensory profile shows that the panel found that the biscuit colors with essential oil are more preferred than other formulas.
Similarly, analysis of variance of sensory descriptors: smell and taste biscuit ($p = 0.000$ and $p = 0.0116$, respectively) revealed a large difference in preference between the three formulas. Indeed, this is well illustrated by the sensory profile, most of the tasters preferred the smell of the biscuit with curaçao oil, this can be explained by the fact that curacao oil contains a very high proportion of limonene well known by its organoleptic characteristics (Filipsson et al., 1998).

The appreciation notes of texture are also significantly different ($p<0.005$), although the levels of flour and sugar are approximately equal in the three formulas. Curacao oil could be responsible for this difference, since it appears that the texture of the cookies with EO was more appreciated than the biscuits with the synthetic aroma or flavor without cookies. The cookies did not have the same degree of appreciation by tasters ($p <0.005$). Indeed, the cookie incorporated with essential oil proved the highest appreciation by the taste panel. However, biscuit without aroma has not been much liked by the panel.

### 3.2.2.2. Test of Preferences

In order to confirm the results found by the hedonic sensory test, a panel of tasters is asked to classify the 3 formulas cookies in descending order in terms of preferences. The results of this test are shown in the histogram (Figure 4).

![Figure 4: Median of preferences of different biscuits (without aroma, with EO, and with synthetic aroma) at $p<0.05$ with error standard.](image)

Thus, as shown in Figure 4, our results are consistent with those of the hedonic test: the formula without aroma is the less preferred by the panel of tasters, the formula with synthetic aroma is rather preferred, unlike the formula with essential oil which is the most appreciated by the panel. In the rest of the work we performed a characterization of the quality of the optimal recipe filled biscuit.

### 3.2.3. Physicochemical characterization of the optimal recipe

In Table 7, are shown the results of the physico-chemical composition of the optimal cookie recipe.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.7 ± 0.02</td>
</tr>
<tr>
<td>water activity</td>
<td>0.58 ± 0.02</td>
</tr>
<tr>
<td>Proteins (%)</td>
<td>15.56 ± 0.002</td>
</tr>
<tr>
<td>Carbohydrates (%)</td>
<td>4.1 ± 0.01</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>7.14 ± 0.32</td>
</tr>
<tr>
<td>Peroxide value (µg O2 / g)</td>
<td>30.19 ± 0.50</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>36.96 ± 0.15</td>
</tr>
<tr>
<td>Minerals (%)</td>
<td>4.27 ± 0.01</td>
</tr>
</tbody>
</table>

The determination of physico-chemical characteristics of the biscuit allowed concluding that the cookie produced satisfies the international criteria imposed by standards. Indeed, pH, water content (less than 10%) and water activity (between 0.5 and 0.7) values are within the standards of a biscuit. This is promoting good conservation of biscuit because the growth of microorganisms is limited (Multon, 2002).

The biscuit peroxide index that provides information on the oxidation state has a value less than that fixed by the standard NF T 60-120 (1968) (<160 µg O2 / g) which proves that our biscuit has a good quality and suitable for consumption.

These results obtained from the physico-chemical study allow to calculate the caloric value of biscuits while taking into account the source of each component (fat, carbohydrate and protein) using the following formula (Feinberg et al., 1991):

Calorie (Kcal / 100g) = $8.93 \times 4.03 \times$ lipids + sugar + $3.87 \times$ proteins 

The calorific value of the cookie is then equal to 406.793 Kcal / 100g which is considered the average for a biscuit.

### 3.2.4. Study of rheological quality of optimized biscuit

The analysis of the resulting curves penetration test gave two textural parameters of the biscuit: hardness and resistance to deformation, their values are given in Table 8.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance to deformation (N/sec)</td>
<td>43.099 ± 0.234</td>
</tr>
<tr>
<td>Force (N)</td>
<td>52.826 ± 0.012</td>
</tr>
</tbody>
</table>

The resistive force to the deformation is a measure of the friability of the biscuits. As against the hardness gives an idea about the crispness of the biscuit Indeed, sugar is responsible for the hardening phenomenon of the cookie. The values of the hardness N and resistance to deformation
are consistent since the pressing force of maximum values indicates greater difficulty to cut cookies (Tyagi et al., 2006).

These results correlate with sensory analysis as this recipe shows average values of strength and resistance to deformation, which confers the highest degree of appreciation by the tasters.

3.3. Profile of concentration of aroma compounds in the matrix

Considering a diffusive mechanism in the transport of flavor compounds in the matrix, there is a delay of the transfer of natural aroma compounds in the biscuit. Thus, a concentration gradient of aroma compounds is established in the matrix.

Before initiating the release kinetics of aroma compounds from the biscuit into the headspace, a period of equilibration at 30 °C for 24 hours has preceded the experiment. This period was originally scheduled to reach thermal equilibrium and concentrations of aroma compounds between the two phases. No experimental data were collected during this period.

The curves represent the concentration of aroma compound (limonene) biscuit on the side interface in the cookie during period of storage (Figure 5). This concentration increases within two months, and then its growth slows to stabilize to 65000 mg/m³ at the end of a month for the cookie containing the EO. In this case we see, then, that physical balance is not fully achieved, contrary to previous work that found that the transfer of the aroma in a food matrix does not exceed 2 hours. This proved the existence of other natural aroma compounds (particularly oxygenated monoterpenes) making transfer of limonene slow, these compounds serve as a barrier to prevent release of limonene from the matrix to the headspace and the same volatilization of limonene in the headspace because it is expected to rapidly undergo gas-phase reactions with photochemically produced hydroxyl radicals, ozone, and nitrate radicals (Samuel, 2007).

In the case of the biscuit containing commercial aroma, the gas / biscuit interface runs out quickly then slowly to 13000mg/m³ at the end of the period of storage of the biscuit. The decrease of the concentration of limonene can be explained by the rapid evaporation of the limonene. This represents an economic gain. In fact, if the evaporation of limonene was slowed by the polymers present in the essential oils, this encourages manufacturers to use the natural flavor instead of the commercial flavor, to preserve the maximum aroma biscuits during their period of storage.

We have seen that release kinetics of the two aroma were different, which tends to confirm the existence of mechanisms slowing the sorption of limonene. These compounds are shown as factors limiting the transfer of limonene food matrix (cookie) to the air, changing the release kinetics. Thus, they act as a barrier to the release of the aroma. We see as prospects for achieving a model used to represent well the release of aroma compounds during storage and to predict concentrations at a given time t.

5. Conclusion

The approach mix design with three factors (sugar, essential oil and flour) allowed us to determine the constraints on the factors, to optimize the response (cookie force) and to restrict the experimental model in an optimum range. An hedonic sensory analysis was performed to evaluate preferences of consumer in terms of aroma (synthetic aroma, natural aroma, without aroma). It revealed that biscuits with EO of Curacao are the most preferred by consumers thanks to the strong smell and taste pronounced by sour orange essential oil. The profile of sorption of aroma compounds from biscuits into the air, during period of storage, showed that the evaporation of limonene in biscuits containing EO is much slower than in those containing commercial aroma.

In conclusion, the incorporation of the essential oil of Curacao is a successful and interesting operation, leading to a new cookie “clean label” and features sensory and nutritional characteristics, meeting the expectations of the consumer. As perspectives, this work could be completed by an analysis of fibers and vitamins, since oil curacao is known by their quality nutritional and several nutritional claims may be reassigned to such a product.
6. Acknowledgments

We thank Mrs. Ghribi Zeineb for her excellent technical assistance for the extraction of essential oils from *Citrus aurantium* peels (Clevenger).

7. Conflict of Interest Statement

The authors have declared that there is no conflict of interest.

Références


