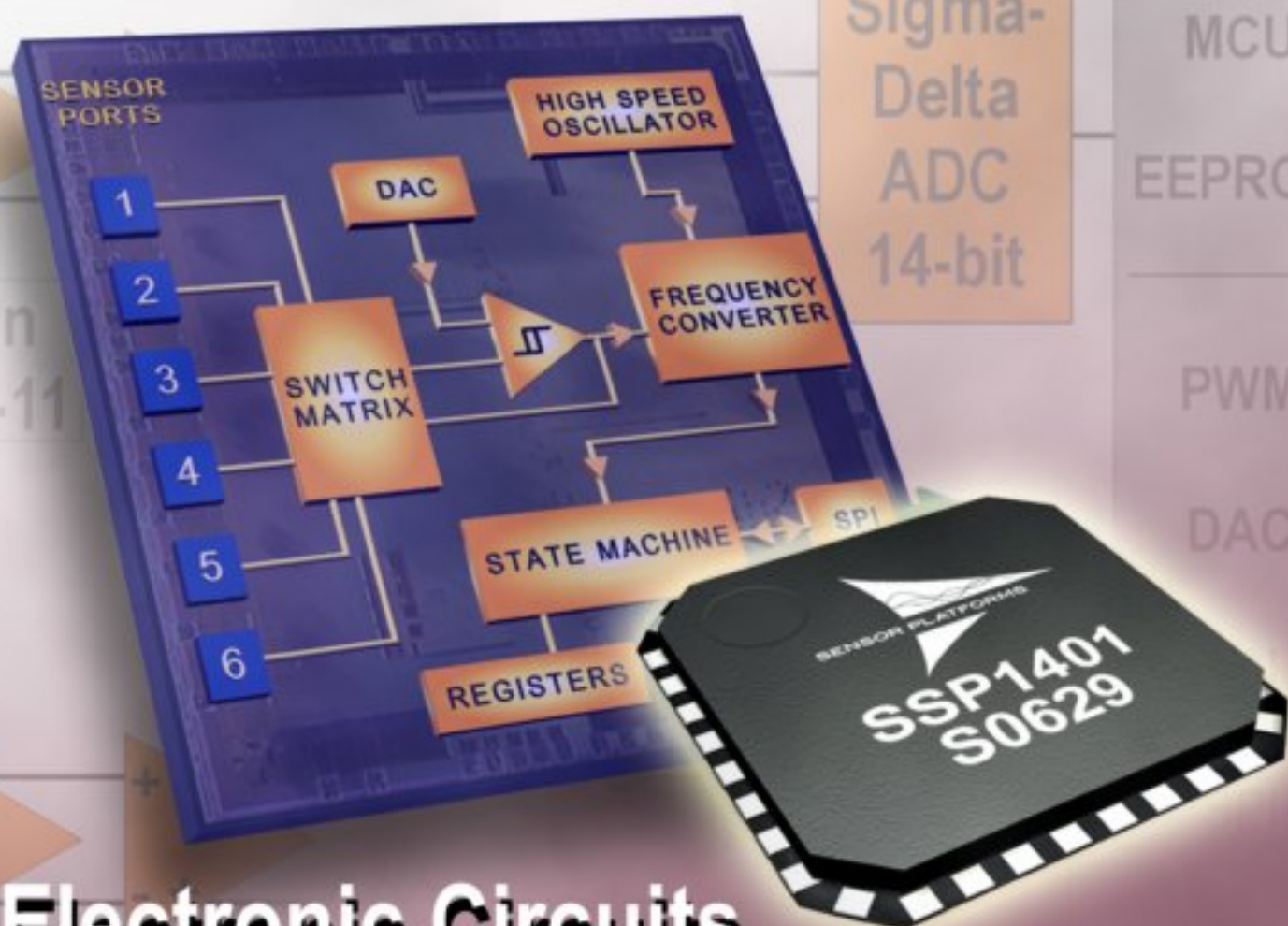


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Thermo-oxidation in Arauco and Arbequina Extra-virgin Olive Oil. Changes in Odour Using and Electronic Nose and SPME-GC-FID

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Abstract: Changes in odour of Arauco and Arbequina extra-virgin olive oil were monitored during frying by electronic nose and solid-phase microextraction–gas chromatography methodologies. Electronic nose data and volatile compounds were analyzed at intervals of 60 min (t_{60}) during 180 min of frying (t_{180}). Principal components analysis applied to electronic nose data showed one component, PC₁ which accounted 96.3 % of the total odour variation. SnO₂ sensors had a positive correlation with PC₁. Arauco variety corresponding to frying t_{120} and t_{180} had the highest positive correlation with PC₁. Analysis of variance results for volatile compounds showed an increase on production for: 3-methyl butanal, *n*-pentanal, *n*-hexanal, *n*-heptanal and *n*-nonanal at 60 min of frying for both varieties. Copyright © 2010 IFSA.

Keywords: Electronic nose, extra-virgin olive oil, solid-phase microextraction–gas chromatography, volatile compounds.

1. Introduction

The complex flavour, the combination of odour and taste of extra virgin olive is mainly produced by volatile and phenol compounds [1, 2]. Extra virgin olive oil is considered to be a stable oil, but it is susceptible to oxidation, because of this reason pleasant sensory characteristics of the oil change to

unpleasant ones (off-flavour) [3]. Although olive oil is not an important domestic agricultural product, olive oil flavor is of interest to several segments of the food industry. Food processors use olive oil in certain specialty food preparations because it contributes to the resultant flavor of the product, even though olive oil costs considerably more than the more common bland vegetable oils.

The chemical composition and physical properties of oil can be influenced by the growing conditions and agricultural practices to which the oil source has been subjected. Therefore, it is not feasible to describe the quality of these materials by a single chemical or physical factor. In practice, a selected group of properties is used to define and assess the quality of an oil. The limits for each of the selected properties are defined by the users and producers of fats and oils, and they are used as the specifications of the oil product for trade [4].

Extra virgin olive oil is considered to be stable but, it is susceptible to oxidation [3]. Oxidation process can be promoted by the presence of atmospheric triplet oxygen ($3O_2$) which reacts with lipid radicals and causes oxidation (a free radical chain reaction including different steps, initiation, propagation and termination). The primary oxidation products, lipid hydroperoxides, are stable at room temperature and in the absence of metals (iron and copper). Presence of high temperature and metals promote decomposition of lipid hydroperoxides to alkoxy radicals and then, form secondary products such as aldehydes, ketones, acids, esters, alcohols and short-chain hydrocarbons [5-8] reported that when oxidation process proceeds, some specific volatile compounds, such as *n*-nonanal, can be used as a marker of oxidation process in extra virgin olive oil.

Conventional analyses are usually used for flavour analysis but they result to be expensive, difficult and consume time. On the other hand, human assessments are used for flavour analyses. Trained panels can determine descriptors to better assess a certain product quality and detect modification due to taints, off-odours or off-flavours. Nevertheless, sensory panels are time consuming and have limitations related to human susceptibility and variability [9]. As stated by Piggott [10], each sensory character can be viewed as being a single and important part in the entire set of quality features. Because of these reasons, there is great interest in using an electronic nose for measuring odour.

The use of electronic nose based on different sensor technologies has been suggested for the rapid detection of quality-related volatile compounds for various food products. Gas sensors that are commonly used in electronic noses are non-selective toward individual compounds but show sensitivity toward certain classes of compounds. This property induces their potential for monitoring quality, associating it with varying levels of different classes of produced volatile compounds.

Electronic is characterized for being rapid, quantitative device, reproductive and objective. Several authors have reported application of electronic nose on different fields such as food industry, especially EVOO [11-13], medicinal plants [14], environmental [15], etc.

The aim of this work was to study the changes in odour caused by thermal oxidation (pan-frying). Varieties of extra-virgin olive oil (EVOO) were monitored during frying by an electronic nose approach complemented with the determination of volatile aldehydes content.

2. Materials and Methods

2.1. Extra-virgin Olive Oil Varieties Samples

Extra-virgin olive oil from Arauco (ARA) and Arbequina (ARB) varieties were properly obtained from fresh, mature fruits of good quality provided by an olive oil mill from Chacras de Coria (Mendoza, Argentina).

2.2. Pan-frying Process

For thermal oxidation, frying pans were filled with 600 mL of EVOO (ARB and ARA) and heated at 180 °C. Oil samples were obtained at the beginning of the experiment and then, three times at intervals of 60 min (t_{60}) during 180 min (t_{180}) for EN and volatile compounds determination. Samples were cooled at room temperature and stored under nitrogen atmosphere at -20 ± 1 °C until analysis. Frying procedure was repeated thrice and triplicate samples were taken each time.

2.3. Electronic Nose

An electronic nose MOSES II (Modular Sensor System) was used to discriminate odours of edible oils. MOSES II contains two modules of gas sensors, one of them composed of eight quartz microbalance sensors (QMB). This type of sensors consists of vibrating quartz crystals covered with polymeric selective coatings, on which gases are adsorbed. The initial vibration frequency (ν_0) of crystals decreases according to the mass increase because of the gases adsorption and the difference between ν_0 and the final frequency (ν_f) results proportional to the adsorbed gas concentration.

The other module of sensors (SnO_2) is composed by eight pure and doped semiconductive SnO_2 sensors. Doping with different elements increases SnO_2 selectivity for different gases. The SnO_2 surface conductivity changes as the semiconductor adsorbs oxidising or reductive gases [16]. The adopted configuration results very flexible for general purposes and convenient for a wide range of applications.

The EN provides data generally in bidimensional plots using the PCA statistical method. PCA algorithm makes use of the advantage that sensors are relatively non-specific and that it can combine the signals of all the sensors in a unique signal. Employing this method, similar odours tend to be grouped in clusters and the result is a bidimensional plot (axes are the components which contribute most to the odour as expressed in percentage).

2.3.1. Samples

Samples of 3 ± 0.005 g for each varieties of extra virgin olive oil were placed in five 10 mL glass vial equipped with a screw cap and silicon septum. Samples were stabilized at 40 °C for 10 min (incubation time) in an 86.50 Dani Headspace sampler and introduced into the MOSES II. Synthetic air was employed as carrier gas with a flow of 30 mL min^{-1} .

2.4. Volatile Aldehydes by Solid-phase Microextraction–gas Chromatography

Aliquots of 5 ± 0.05 g of EVOO (ARB and ARA) were placed into 10 mL headspace vials, adding 100 μL of internal standard solution ($100 \mu\text{g g}^{-1}$ 4-methyl-2-pentanone). Vials were sealed with crimp-top caps with TFE-silicone septa seals. The volatile compounds in the headspace of EVOO were extracted using Divinylbenzene / Carboxen / Polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm fibre [17].

The setting on the solid-phase microextraction (SPME) holder assembly scale was adjusted to 1.0 scale unit to ensure that the fibre was positioned in the headspace above the sample in exactly the same way from run to run. SPME extractions were carried out placing the sample vial in a water bath at 50 °C for 40 min. Then, the fibre was retracted into the needle assembly and removed from the vial.

The setting on the SPME holder assembly was changed to 4.4 scale units prior to injection into the gas chromatography (GC) injector port, which was fitted with a special insert for SPME analysis. For thermal desorption, the SPME fibre remained in the injector for 5 min. Three separate SPME extractions were collected from each sample.

2.5. GC Analysis of Volatile Compounds

A Shimadzu series 14B gas-liquid chromatograph, equipped with a flame ionization detector was used. The injector was operated in the splitless mode at a temperature of 260 °C and the detector at 280 °C. Nitrogen was used as carrier gas at a flow of 1 mL min⁻¹. A 30 m x 0.32 mm id x 1.00 µm ATM5 capillary column was used.

The following column temperature-programming sequence was used: an initial temperature of 40 °C was maintained for 5 min before being increased to 80 °C at 2 °C min⁻¹ then, it was raised from 80 °C to 150 °C at 4 °C min⁻¹ and, finally, from 150 °C to 280 °C at a rate of 10 °C min⁻¹ and held for 10 min. Peak identifications were based on comparison of retention time of unknown with authentic compounds.

The relative concentrations of individual compounds were determined by comparing the peak area of the compound in each chromatogram with that of the added 4-methyl-2-pentanone internal standard (100 µL of 100 µg g⁻¹ 4-methyl-2-pentanone solution) and considering the relative response factors of each studied compound.

The internal standard quantification method was based on that described by Vichi *et al.* [18] with the following modifications: standard mixtures with concentration in the range of 0.2–1.0 µg g⁻¹ for 3-methyl butanal, *n*-pentanal, *n*-hexanal, *n*-heptanal and *n*-nonanal and were analyzed under the conditions described earlier; the absolute response factors for each compound were then obtained from the slope of the linear regression of the total peak area as a function of the concentration and the relative response factors were calculated as the ratio between the absolute response factors of each compound to that of the internal standard. The relative concentrations are the average of three separate SPME extractions collected from each sample.

2.6. Statistical Analysis

Principal components analysis was applied, on EN data, to describe the relation between variables and their influence on frying time and EVOO varieties samples using the statistical software SPSS v. 12. A fixed effect factorial model, using general linear model (GLM) procedure (SAS 8.0, SAS Institute Inc., Cary, NC, USA) with two EVOO variety and seven frying times' levels, $y_{ijk} = \text{mean} + \text{EVOO variety}_i + \text{frying time}_j + (\text{EVOO variety} \times \text{frying time})_{ij} + e_{ijk}$ was applied on volatile compounds levels. Volatile compounds were reported as the mean value ± SD. Mean values were compared using the Tukey's test at a significance level of 0.05 [19].

3. Results and Discussion

3.1. Selection of Sensors of Electronic Nose (Edible Oils)

In a previous research Messina *et al.* [20] reported that using arrays of sixteen sensors (eight pure and doped SnO₂ and eight QMB) to evaluate the response of odour of edible oils in an Electronic nose

MOSES II, only SnO₂ sensor array showed higher response to these oils compared to the response of QMB array. Types of array of sensors are doped with different elements, increasing selectivity for different gases. When gases are selected by SnO₂ sensors, surface conductivity of the sensors changes as the semiconductor adsorbs oxidising or reductive compounds.

In the present research, doped SnO₂ sensors were used to analysis odour profile of different varieties of extra virgin olive oil.

3.2. Principal Components Analysis of Electronic Nose Data of Varieties of Extra-virgin Olive Oil at Different Frying Time

In Fig. 1 PCA shows the grouping of EVOO (ARA and ARB) as a function of different frying times. Each grouping of EVOO (ARA and ARB) at different frying times corresponds to a triplicate measurement (as described in ‘Materials and methods’). One PC₁ was found, accounting 96.3% of the total variation. PC₁ showed a positive correlation between doped SnO₂ sensors (S) and EVOO (ARA) for all frying times (60 (▲) min, 120 min (▲) and 180 min (▲)). Conversely, EVOO (ARB) showed a negative correlation with doped SnO₂ sensors for all frying times 60 min (●), 120 min (●) and 180 min (●).

Fresh samples for EVOO (ARA (▲)) and ARB (●)) were located on negative PC₁. EVOO (ARA) corresponding to frying time t₁₂₀ (▲) and t₁₈₀ (▲) showed the highest positive scores representing the highest sensors response (S). These results could be attributed to differences in the composition of each variety that may be related to changes in their odour.

Taurino *et al.* [21] and Cimato *et al.* [22] reported that extra virgin olive oil and olive oil from different regions of Italy were clearly differentiated using doped SnO₂ sensors.

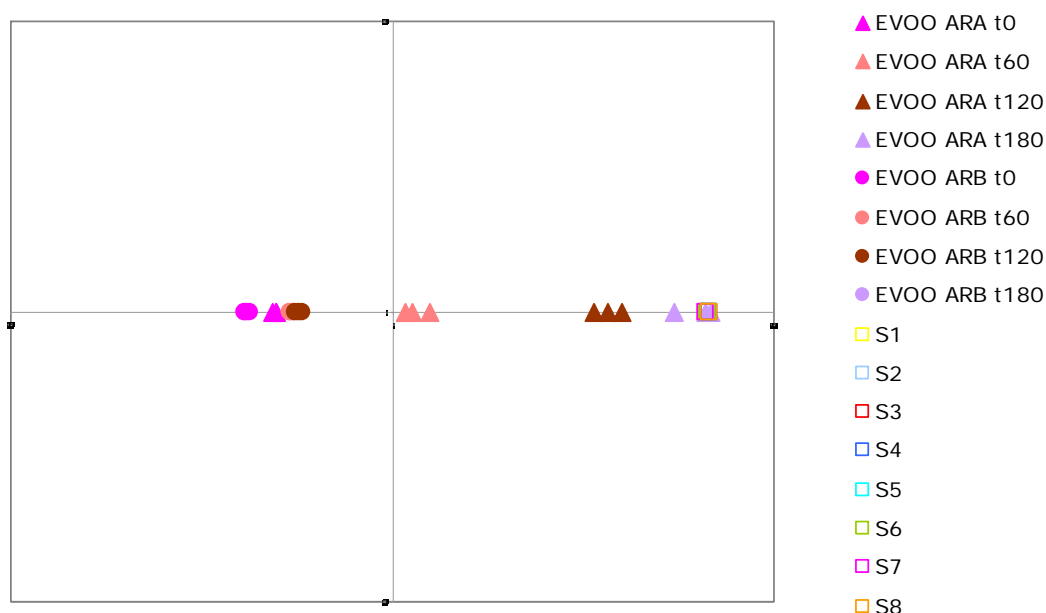


Fig. 1. Principal components analysis of electronic nose data corresponding to different frying time of extra-virgin olive oil (EVOO) for ARA (Arauco), at initial time (▲), 60 min (▲), 120 min (▲) and 180 min (▲) and ARB (Arbequina), at initial time (●), 60 min (●), 120 min (●) and 180 min (●) with different doping of SnO₂ (S1, S2, S3, S4, S5, S6).

3.3. Volatile Aldehydes of Extra-virgin Olive Oil Varieties

Volatile aldehydes produced during the frying process are shown in Fig. 2. Analysis of variance showed a significant ($P < 0.001$) effect on 3-methyl butanal production during frying. 3-methyl butanal showed maximal level of production ($P < 0.05$) at 60 min of frying for both varieties, EVOO (ARB) presented the higher levels throughout the experiment. This branched aldehyde was also detected in powder milk and fresh meat as an oxidation product [23, 24]. *n*-pentanal, *n*-heptanal and *n*-nonanal also showed maximum levels of production ($P < 0.05$) at 60 min of frying for both varieties. On the other hand analysis of variance showed a significant ($P < 0.001$) for *n*-hexanal.

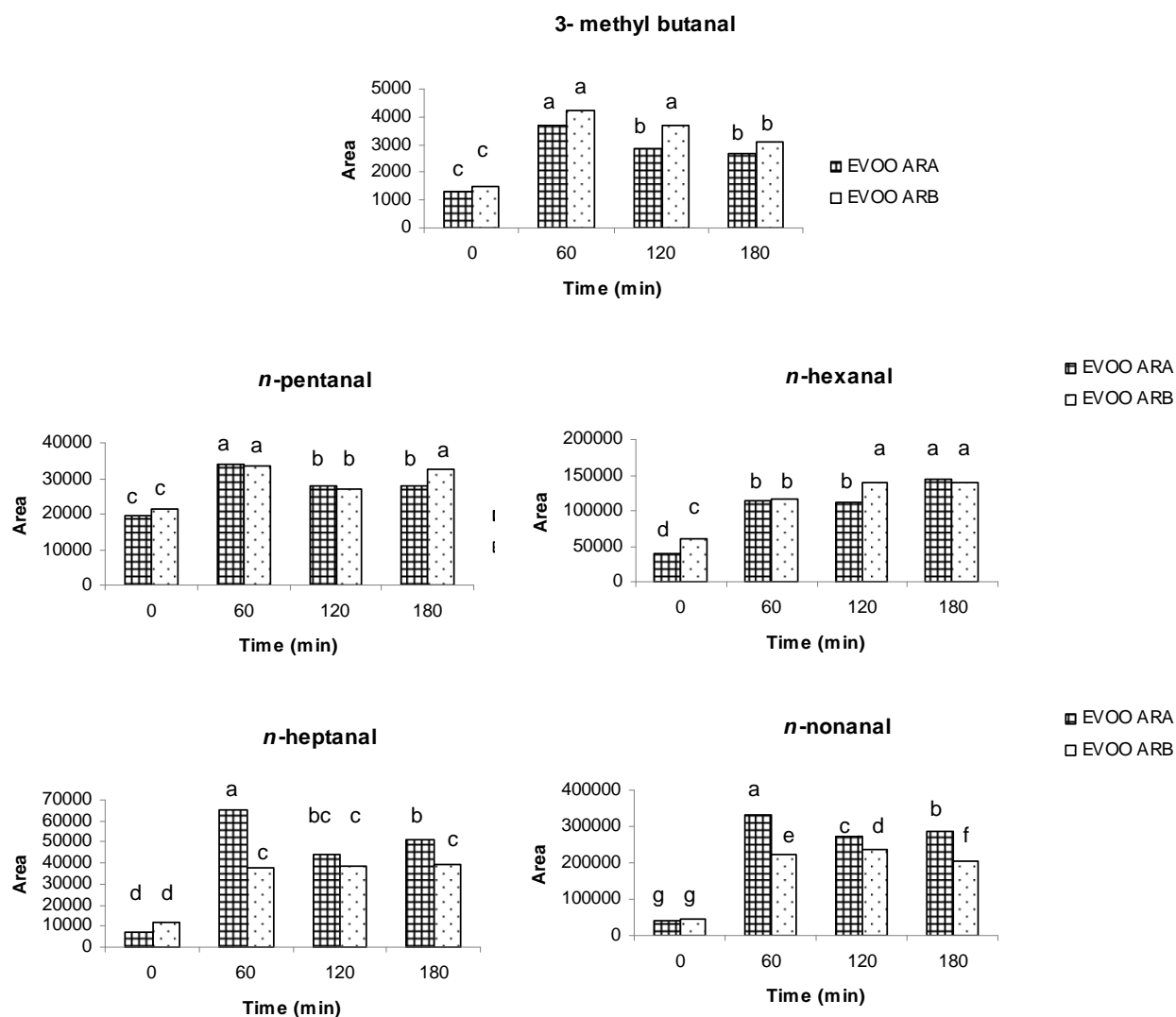


Fig. 2. Volatile aldehydes: 3-methyl butanal, *n*-pentanal, *n*-hexanal, *n*-heptanal and *n*-nonanal detected in extra-virgin olive oil (EVOO) of Arauco (ARA) and Arbequina (ARB) varieties during frying.

Both varieties showed a different behaviour related to the development of volatile production. EVOO (ARB) exhibited an overall higher production of volatiles. A remarkable significant difference ($P < 0.05$) in the production of *n*-hexanal was observed for EVOO (ARB) in comparison with EVOO (ARA). Other authors reported that the progress of oxidation process in vegetable oils was indicated by the increase of total volatile compounds and the concentration of some specific volatile compounds such as *n*-hexanal or *n*-nonanal [8].

Aparicio *et al.* [2], Vichi *et al.* [17] and Mildner- Szkudlarz *et al.* [25] showed that the effect of autoxidation led to the increased values of volatile compounds as product of fatty acids degradation. In accordance to Mildner-Szkudlarz *et al.* [25] and Vichi *et al.* [17], the results of n-hexanal showed the major production for both the varieties.

Morales *et al.* [8] and Aparicio *et al.* [26] reported that some volatile compounds in small amounts in extra virgin olive oil contribute to pleasant flavour but, when degradation process starts and levels of these volatile compounds increase, extra virgin olive oil turns into unpleasant flavour. *n*-hexanal has been reported by the mentioned authors as a pleasant flavour when levels are low in extra virgin olive oil, turning into unpleasent flavour at high levels.

4. Conclusions

In the last decade, odour research was focused principally on the identification of potent odourants, the determination of their odour relevance and their release in different foods. Nowadays, the development of the EN methodology, with a chemical sensory array, provides a powerful tool to analyze odour as a set of odourants present within a given sample. Sensory analysis, as branch of the food industry, will be benefited with the adoption of this methodology.

EVOO (ARB) and EVOO (ARA) showed different volatile production being clearly identified with the EN throughout the frying process, showing that EN approach represents an alternative powerful tool to traditional methods of odour measurements.

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Guide for Contributors

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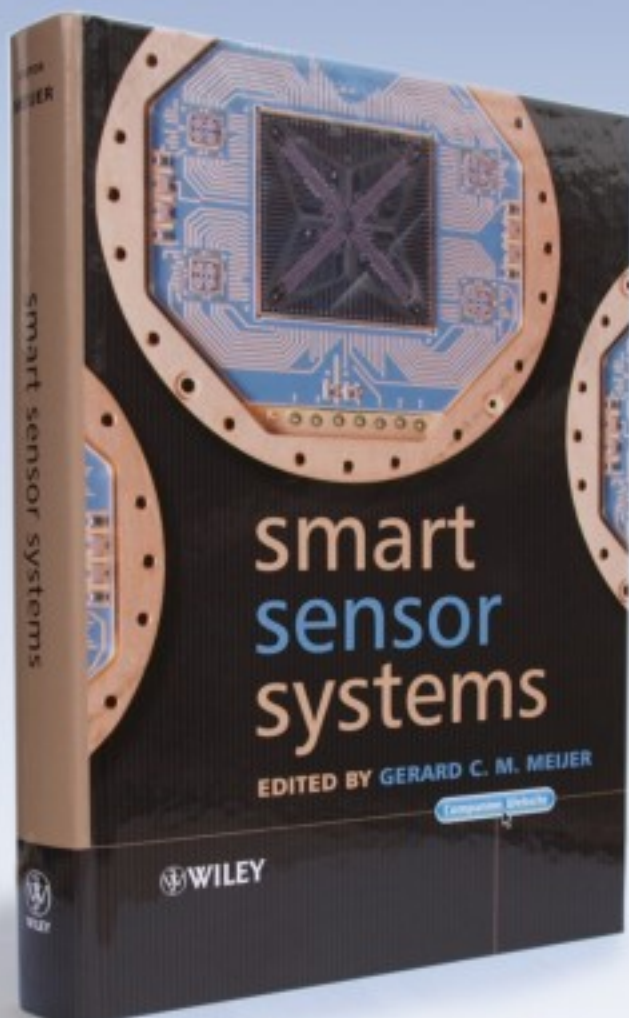
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