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Warmed-over flavour analysis in low temperature–long time processed meat by an "electronic nose"

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Abstract

The ability of an electronic nose, comprising 32 conducting polymer sensors, to identify and classify warmed-over flavour (WOF) aroma in bovine *semitendinosus* muscle, processed by vacuum cook-in-bag/tray technology (VCT) and storage under refrigerated conditions, was evaluated. The VCT process employed low temperature–long time (50°C–390 min) thermal treatments. Multivariate analysis showed that VCT processed beef aroma profiles were sorted into two groups, one included samples stored for up to 20 days and the other included samples with 34 to 45 days of storage. WOF odour standard samples were recognised to have similar aroma as samples of the second group. Lipid oxidation results, measured by thiobarbituric acid reactive substances, showed an increment in oxidation level for samples stored for 34 days or more ($P < 0.05$). This study shows that electronic nose technology can be applied to WOF odour identification and classification in VCT beef meat, complementing chemical and sensory techniques used in this field. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Electronic nose; Aroma; TBARS; Cook-in-bag/tray technology; Warmed over flavour (WOF) odour; Meat

1. Introduction

Usually food contains complex mixtures of volatile compounds that cause a variety of odours, comprising often hundreds of chemicals components (Maul, 1998; Pearce & Gardner, 1998). For example more than 400 aromatic compounds were identified in cheese headspace, over 250 compounds in tomato, around 650 in coffee and over a hundred in fermented sausages (Hodgins, 1997; Stahnke, 1995). These large amount of chemicals produce several primary odours that results in different sensation due to their interaction with primary receptors (Piggott, 1990).

Up to the present, the analysis of characteristic food odours has been commonly carried out by human assessment and headspace/direct gas chromatography/mass spectrometry (GC/MS). Trained panels can determine aroma changes due to taints, off-odours and can develop flavour descriptors to better assess a certain product quality. These panels have limitations in terms of their availability and judge fatigue. Also especial care should be taken with the procedure employed and the statistical method used, in order to minimise subjectivity on the panel response and data variability between tests (Pearce & Gardner, 1998; Persaud, Khaffaf, Hobbs & Sneath, 1996). Instrumental techniques, like GC/MS for certain practices, have high operating cost, are time consuming (Przybylski & Eskin, 1995) and their results can not be directly related to sensory panel data (Hodgins & Simmonds, 1995). The employment of odour-sniffing ports is an useful approach, however it has the disadvantage of giving information concerning individuals compounds while food aroma depends on the complex interaction between all the volatile compounds within the matrix (Hodgins & Simmonds). Also, some of the extraction procedures can cause artefacts formation especially when fresh food volatile compounds are extracted using distillation techniques (Taylor, 1996). Therefore, neither the human nose nor chromatographic techniques have by itself the ability to both

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recognise and define the compounds responsible for a particular food aroma.

For these reasons, alternative analytical techniques are constantly designed with the purpose to mimic the human sense of smell or odour analysis. One example of this advanced technology developed to fulfil this objective is the electronic nose. Basically, this device is a sensor-based instrument designed to respond to the volatile compounds present in the headspace of a sample (Schiffman, Kermani & Nagle, 1997). This new technology has been successfully used to classify off-odours of agricultural product (Persaud et al., 1996), grains (Börjesson, Eklöv, Johnsson, Sundgren & Schnürer, 1996; Maul, Sargent, Huber, Balaban, Luzuriaga & Baldwin, 1997), sheepmeat (Braggins & Frost, 1997), pharmaceutical products (Schiffman et al.), ground beef (Spanier & Braggins, 1999), etc.

One particular off-flavour found in cooked meat is warmed over flavour (WOF). This term is used to describe the oxidised flavour that develops in meat following a thermal treatment after only a few hours of refrigerated storage (Tims & Watts, 1958). WOF includes odours and tastes commonly described as "stale", "cardboard-like", "painty", "rancid", "bitter" and "sour" among others (Love, 1988; St. Angelo et al., 1987). Processes which involve any action that disrupts the muscle fibre membrane, such as chopping, restructuring, or heating would enhance WOF of meat product (Mann, Reagan, Lillard, Campion, Lyon & Miller, 1989; Mielche, 1995; St. Angelo, 1996). This particular alteration is considered the main reason for the slow development of some cooked meat products (Hansen, Knøchel, Juncher & Bertelsen, 1995; Mason, Church, Ledward & Parson, 1990).

Vacuum cook-in-bag/tray technology (VCT), also known as *Sous Vide*, is a cooking process well established in the literature and industry (Church & Parsons, 1993; Creed & Reeve, 1998; Hrdina-Dubsky, 1989). This technology involves several steps, where the general concept is stated by the Sous Vide Advisory Committee (1991) as follows. The raw or partially cooked food is vacuum packaged into a laminated plastic pouch or container, cooked using a controlled temperature program, rapidly cooled and then stored at refrigeration temperature. In the following, VCT product will be used in reference to a product obtained employing VCT according to the concept detailed above. Applying this technology, it has been reported that food with better flavour, texture and nutrient retention than food conventionally cooked is obtained (Unger, 1985). Moreover, VCT offers the possibility of enhance shelf life of products during chill storage (Creed, 1998). Some aspects related to microbiological safety of VCT products are still under discussion, but they are mainly associated to products in which ingredients of different nature (i.e. vegetables and fish) are in the same package (Church & Parsons, 1993). It should be pointed out that the product in this study contains only beef meat.

Determination of thiobarbituric-acid-reactive substances (TABRS) is frequently used to evaluate the efficiency of different methods designed to reduce or retard oxidation development. Several researchers have investigated the development of WOF in various types of meat by the level of TBARS (Asghar, Gray, Buckley, Pearson & Booren, 1988; St. Angelo, 1996; St. Angelo, Vercellotti, Dupuy & Spanier, 1988). Smith and Alvarez (1988) investigated the stability of VCT processed turkey breast over 87 days. They observed that TBARS number increased in the first 68 days of refrigerated storage until it reached a value of 1.0 mg malonaldehyde (MDA)/kg meat and then decreased. Hansen et al. (1995) studied VCT processed *semitendinosus* muscles and they reported that lipid oxidation was low ($< 10\ \mu mol\ kg^{-1}$) and not affected by the type of spices added, age of raw material, heating and storage temperature. Also, it was well established that there is a good correlation between TBARS values and sensory panel evaluation scores (Mielche & Bertelsen, 1993; Spanier, Vercellotti & James, 1992; Stapelfeldt, Bjorn, Skibsted & Bertelsen, 1993). For example, White, Resureeccion and Lillard (1988) found that after seven days of storage their product reached the threshold for TBARS at which untrained panelist significantly detected off-flavour related to WOF. Thus, the TBARS test is an accepted technique to monitor meat flavour and to validate the performance of new technology in odour investigation.

The aim of the present study was to develop a fast analysis technique suitable for beef meat processed by VCT, using electronic nose technology, to detect and classify processed samples with WOF odour.

2. Materials and methods

The study described here is a part of a major project to develop new processes for ready to eat beef using VCT applying low temperature–long time (LT–LT) thermal treatments. The aim of this major project was to develop a beef muscle based product, with improved organoleptic properties and high cooking yield while preserving its microbiological safety (Vaudagna, Sánchez, Picallo, Margaría & Lasta, 1999).

2.1. Sample preparation

Bovine *semitendinosus* muscles purchased in a beef packaging plant 48 h after slaughtering were used as meat source. The muscles average weight was 1.7±0.5 kg with 1.5–2.0 w/w of intramuscular fat. They were fat trimmed and vacuum-packaged in cook-in bags (CN-510, Cryovac, Sealed Air S.A., Argentina).

The muscles were cooked-pasteurised in a computer-controlled water shower retort (Steriflow *Microflow,* Barriquand, France) using the basket-static mode (Vaudagna et al., 2000). Thirty-five muscles were processed in five runs of seven muscles each. In order to avoid microbial contamination due to handling, one of the seven muscles (temperature test sample) was used only for temperature control and it was not used for storage studies. The temperature was monitored by a T-type thermocouple fixed at the muscle's geometrical centre (slowest heating point) using a stuffing box device (Ecklund Harrison Technologies, Inc., USA). Time–temperature curves for both, temperature test sample and space inside of the autoclave, were recorded using a digital Multimeter Hydra 2625A Data Logger (John Fluke Mfg. Co., Inc., USA) and acquired by a personal computer through a RS232 card.

Cooking was performed at a temperature of 50°C for 390 min. After thermal treatment, samples were cooled in an ice–water bath for approximately 70 min until the sample reached 26°C in the geometrical centre. Ended the cooling step, shrink juice was eliminated and the muscles were again vacuum packaged and stored in the dark at 1.5±0.5°C for a period of 24 h. Then, the analyses were carried out at 0 (at the end of the initial 24 h period), 2, 4, 6, 13, 20, 34, 42 and 45 days of storage (storage time, St). At each St, three muscles were analysed. Each muscle was divided into proximal, medial and distal thirds, and one slice (approximately 200 g) from each of these three parts was collected to form a sample. This sample was reheated in a microwave oven for 9 min (1000 Watt, 80% power), cut and then divided in different aliquots of 10 g, one for TBARS analysis and one for E-nose analysis. Others aliquots were utilised for sensory evaluation and microbiological analysis, which results were presented by Vaudagna et al. (2000).

2.2. Electronic Nose analysis

In this investigation, an Electronic Nose (E-nose) AromaScan™ A32 (Osmetech PLC, UK) with a detector array of 32 conducting polymer sensors was used. The relative response of each sensor reflects the range of volatile compounds in the headspace of the sample during data acquisition. In this device, the acquisition cycle consists of a five-step sequence of actions to transport the headspace from the sample across the sensor array. The system allows a fast and accurate identification of unknown samples by odour analysis using recognition software that includes an Artificial Neural Network (ANN) (Ni & Gunasekaran, 1998).

2.2.1. Meat analysis

Each of the 10 g aliquots, prepared as described in Section 2.1, was cut into strips of approximately 1.5×0.8×0.4 cm and placed into a 50 ml screw-cap stoppered test tube.

The tube cap presented inlet and outlet ports. Polytetrafluoroethylene (PTFE) lines connected the test tube inlet to the reference air supplied by de AromaScan system and the test tube outlet to the analyser sampling port, using a dynamic stripping standard configuration. The dynamic stripping technique, using nitrogen (oxygen-free quality) as carrier gas, was selected to allow constant replenish of the head space and strip of volatile compounds from the sample by drawing off the air close to its surface (AromaScan, 1997). The aroma detection was made in one cycle: reference: 30 s, sample: 90 s, wash: 60 s, reference: 120 s and 2% *n*-butanol–water solution as cleansing agent. The duration of each step was found to be sufficient to establish a correct base line, to collect volatile compounds and to recovery up the sensors between sample analysis.

To eliminate temperature fluctuations during the runs and to avoid differences on headspace formation, during the analysis the sample temperature and the PTFE lines were maintained at a constant temperature of 50°C in a water bath with large thermal inertia.

2.2.2. Data repeatability test, water vapour influence and sampling period selection

The degree of the repeatability of the sensor response was determined by analysing the differences in the intensity of the response among three consecutive runs of each sample.

Determination of water influence was necessary because the polymer sensor array used has a high sensitivity to many polar compounds and, therefore, to water. When meat is being measured, water vapour will be generated in the headspace during the sampling due to the water activity in the meat. Thus to verify if the instrument could differentiate between water and meat profiles during the runs, blanks were analysed. The blank samples used were a saturated solution of NaCl with excess solids at a humidity value similar to the meat (Ockerman, 1986; Visser & Taylor, 1998).

To collect aroma information, different sampling time periods can be selected during data acquisition. Data can be analysed whether an equilibrium in the sensor responses is reached or not, in the last case differences between samples may be more pronounced (Lane & Wathes, 1998). The lack of equilibrium may be due to large molecular volatile with slow adsorption/desorption kinetics, although the contribution to the aroma profile from non-equilibrated sensors is still valid.

2.2.3. WOF odour standard

Standard samples with high-WOF odour were prepared from slices of *semitendinosus* muscles, cooked in boiling water during 10 min, cooled at room temperature, packed in polystyrene tray wrapped with poly-

ethylene and stored at 4°C for 3 days. The sample descriptor was generated in the training sessions of an eight-member trained panel with 8 years of experience in evaluating meat products. Using the standardised lexicon of meat descriptor for WOF (Love, 1988), judges described the standard samples off-odours with the terms "painty", "cardboardy", "boiled fish" and "stale". For sample evaluation, the judges used non-structure scale of 10 cm (WOF absence; extremely intense WOF) (Sánchez et al., 1999).

2.3. TBARS test

TBARS numbers were determined as suggested by Pensel (1990). Briefly, 5 g of samples were homogenised in an homogeniser (Stomacher™, Colworth, UK) with 50 ml of 20% TCA (trichloroacetic acid) in 1.6% metaphosphoric acid solution, filtrated, diluted to 100 ml with distilled water and then 5 ml of filtrate were mixed with 5 ml of fresh TBA solution. Extracts were left 15 h in the dark at room temperature for colour development. Colour was measured at 530 nm in a UV–VIS Spectrometer Lambda Bio20 (Perkin-Elmer Corp. Analytical Instrument CT, USA). The determined recuperation percentage in 1,1,3,3 tetraethoxypropane (TEP) enriched samples was 94.6%. TBARS values were corrected for cooking and storage loss to allow unbiased comparisons among different times of storage.

2.4. Statistical analysis

E-nose data were handle by the statistical software included in the system. Two methods were employed for aroma profiles analysis as dimensionality reduction techniques, an adaptation of one described by Sammon (1969) and principal component analysis (PCA) (Schaller, Bosset & Escher, 1998). In the aroma maps, data points from alike samples appear together in one domain and separated from another domains that represent samples that are found to be different (Visser & Taylor, 1998). The quality factors (QFs) give an estimation of the discrimination between each pair of clusters. They could be obtained from data as Euclidean distances or Malanobis number (Spanier & Braggins, 1999).

TBARS values were analysed using an analysis of variance performed with PROC GLM (SAS, 1999). Multiple comparisons among treatments were accomplished using Duncan' s new multiple range test (Montgomery, 1997).

3. Results and discussion

3.1. VCT processed beef product aroma

In Fig. 1 it is possible to observe a sample characteristic pattern profiles obtained by the E-nose, for a sample with 20 days of chilled storage. In the figure each line represents the response, change in resistance relative to the base resistance ($\Delta R/R$), of one sensor when the volatile compounds of the sample headspace reach it out. In Fig. 1, the different phases of the aroma detection cycle are indicated.

The results of sensor response repeatability test showed that the differences between the sensor intensities for three successive analysis of the same sample were always lower than ±0.56. These differences might be attributed to changes in odorant concentrations (successive repetitions, headspace-pressure variation) (Schiffinan et al., 1997). Nevertheless, the patterns have similar amplitude so the response intensity should be considered similar. Then, for subsequent analysis of the data, an average pattern of three consecutive runs was calculated and used as representative of the sample.

To choose the sampling period to collect data, two lapses of time during data acquisition at 30–40 s (steep stage of the curve) and 50–70 s (plateau part) were considered. It was observed using PCA analysis that data corresponding to different storage time were grouped in a similar cluster for the two periods considered. Thus,

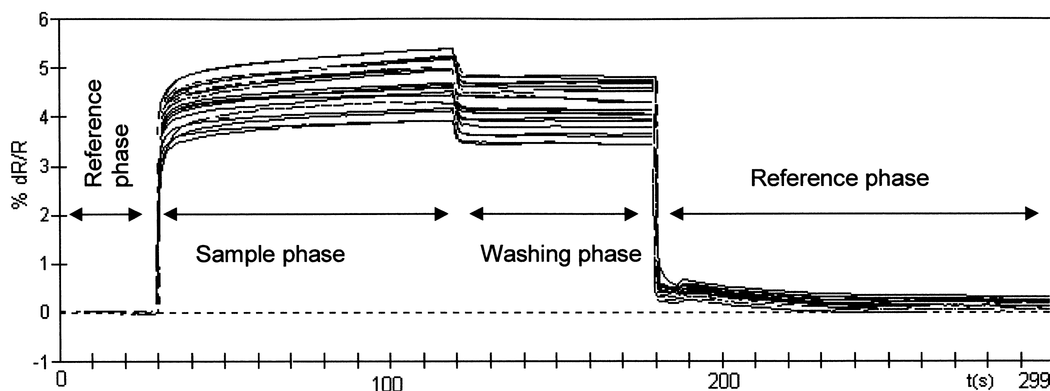

Fig. 1. Characteristic odour pattern profiles of VCT processed beef samples. In this figure the aroma pattern correspond to a sample with 20 days of refrigerated storage. Change in resistance relative to the base resistance of each sensor.

for further analysis the interval in the equilibrium part of the curve (50–70 s) was selected.

In Fig. 2, a two-dimensional PCA aroma-map shows the results when blank and processed beef samples aroma profiles from different storage time are compared. In this map, separation between beef and blank samples is evident. A QF value of 18.34 was obtained between clusters. Also, this map points out that the methodology used is suitable for cooked beef analysis since the water vapour presence did not affect the measurement (Visser & Taylor, 1998).

3.2. Classification of VCT beef samples according to WOF odour standard samples

The results presented in Fig. 3 show that processed beef samples storage for St = 0 to St = 20 days clustered together and samples for St = 34 and St = 45 assembled in a different group. Average QF values inside these two clusters were 2.39 and 2.02, respectively, and between each other was 5.26.

WOF odour standard samples were presented to the E-nose to determine the similarities of the aroma patterns between these samples and those corresponding to different storage times. PCA analysis showed a match between WOF odour standard and VCT beef samples with 34 days or more of refrigerated storage. For ANN recognition of those samples, a recognition file was used with two descriptors. These descriptors were Class I for processed beef profiles corresponded to St = 0 to St = 20 and Class II for St = 34 to St = 45.

To validate the performance of the neural network a cross-validation method was used where raw data were separated in two groups: one set for training the ANN

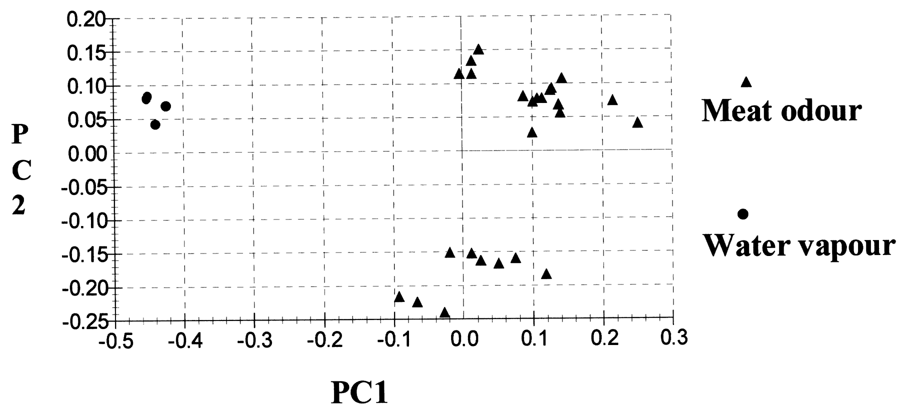

Fig. 2. Principal component (PC) analysis of aroma profiles of blank samples and VCT beef samples assessed by an electronic nose.

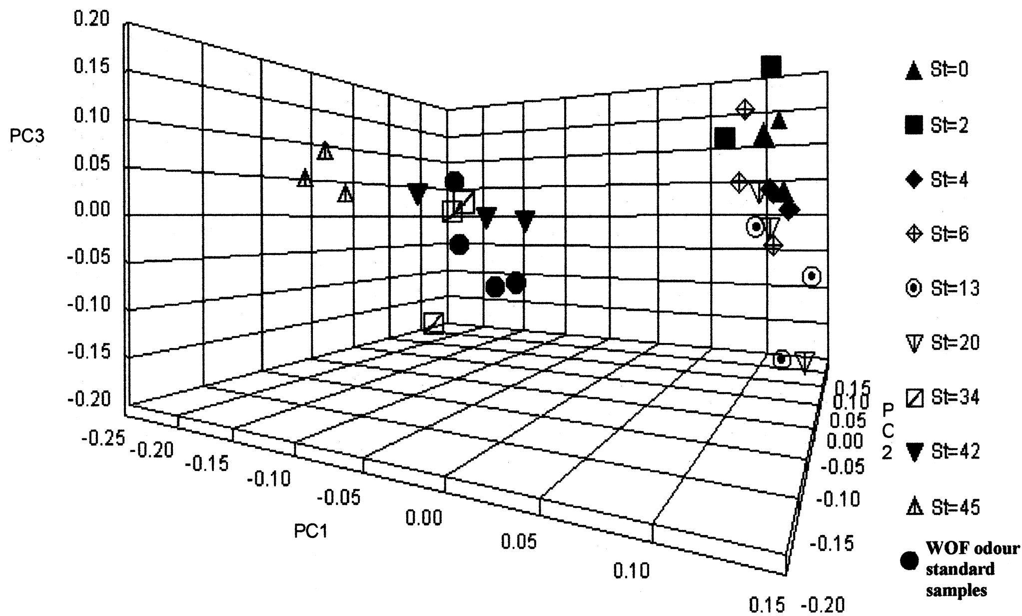

Fig. 3. Principal component (PC) analysis of aroma profiles of VCT processed beef samples, stored at refrigerated conditions up to 45 days, and WOF odour standard samples. Comparison of aroma profiles assessed by an electronic nose.

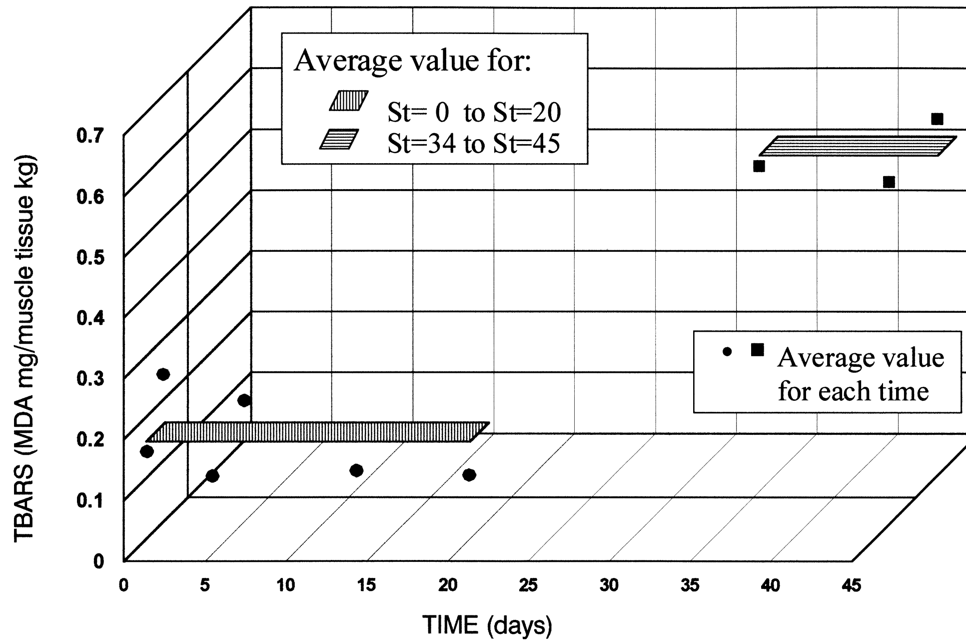

Fig. 4. Evolution of TBARS number of VCT processed beef samples during refrigerated storage.

and the other as test samples. The recognition confidence obtained for all test samples, using recommended learning parameters, was above the 70% (Stitt, Gaumind, Frazier & Hanson, 1998). Under these conditions, WOF odour standard samples were classified as Class II with a recognition confidence above 75%.

TBARS results are shown in Fig. 4. The values remain low, around a mean value of 0.1604±0.0860 MDA mg/kg muscle tissue, from the beginning of the assay up to 20 days of storage (St = 0, 2, 4, 6, 13 and 20). For these sampling days the analysis of variance showed no significant differences between values ($p > 0.05$). However, TBARS levels for samples with 34 to 45 days of storage were significantly higher (Duncan's new multiple range test) than those stored for fewer days, with a mean group value of 0.5250±0.1900 MDA mg/kg muscle tissue. No significant differences were observed in TBARS values within this group ($p > 0.05$). Thus, TBARS values indicate an increment in lipid oxidation after 20 days of chill storage, which has been associated with the development of WOF (Stapelfeldt, Bjorn, Skorg, Skibsted & Bertelsen, 1992). This behaviour was in accordance with the E-nose classification of WOF odour standard samples in the current study.

Present results are in agreement with previous investigation data (Grigioni, Margaría, Gallinger, Sánchez & Pensel, 1999), where sample sensory characteristics were analysed by a trained panel. In that case, Duncan multiple range test grouped ($p < 0.05$) the judges response score for the aroma intensity into two groups: one for samples with 20 or less days of storage (mean sensory score 5.1) and the other for samples with 34 to 45 days of storage (mean sensory score 3.5). The decrease in cooked beef odour intensity was concurrent with off-odours development. According to the lexicon used to WOF odour description (Love, 1988), the predominant off-odours were associated with WOF.

According to Vaudagna et al. (2000) the off-flavours encountered in the samples could not be associated with the development of microbial spoilage. Under the thermal treatment conditions described above, the total viable count (TVC) mean values were lower than the detection limit (Log_{10} 1.93 CFU/cm^2) for samples stored up to 13 days. At the end of the storage period, TVC was 1.09×10^3 CFU/cm^2. Based on these results, the product preserves its microbiological acceptability during the whole period of storage.

4. Conclusions

The E-nose analyses, using the dynamic stripping technique, can successfully differentiate the aroma alteration of VCT processed beef meat during the storage period.

This method of analysis can also correctly classify samples with a specific aroma. Samples with more than 20 days of storage were recognised as presenting WOF odour when compared to WOF odour standard samples. The recognition confidence obtained by the ANN is expected to increase when a higher number of samples are used to training the network.

E-nose sample classification was in agreement with TBA results, that showed an increment of TBARS

values for those samples at levels commonly associated with oxidised flavour.

This new technology provides a non-destructive method to analyse VCT meat products in a few minutes, making it a valuable complement to traditional techniques used in food odour research.

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