PAPER

USE OF ELECTRONIC NOSE TO DISCRIMINATE MEATS FROM BULLS FED DIET WITH OR WITHOUT FLAXSEED INCLUSION AND SUBJECTED TO DIFFERENT AGING PERIODS

M. MOSCHINI*[a], S. SIGOLO[a], A. BRUGIAPAGLIA[b], M. RENNA[b], C. LUSSIANA[b] and A. PRANDINI[a]

[a]Department of Animal Science, Food and Nutrition (DIANA), Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122 Piacenza, Italy

[b]Department of Agricultural, Forest and Food Sciences (DISAFA), Università di Torino, Largo Paolo Braccini 2, 10095 Grugliasco, Torino, Italy

*Corresponding author: Tel.: +39 0523599192; Fax: +39 0523599259

E-mail address: maurizio.moschini@unicatt.it

ABSTRACT

A metal-oxide sensors array electronic nose (e-nose) was used to discriminate beef loins (*Longissimus thoracis*) obtained from Piemontese bulls fed without or with flaxseed and subjected to 3 different aging periods (2, 7, 10 days) at 4°C. At 7 days of aging, samples were also assessed for flavor intensity by panelists. A comparison between e-nose and panel assessments was performed subjecting a 7 days e-nose reading on cooked meat to partial least square regression for flavor prediction.

The e-nose could not discriminate populations in meat samples, however it could represent a valuable tool in supporting flavor scoring from sensory evaluation.

Keywords: aging, beef meat, electronic nose, flaxseed, sensory evaluation

1. INTRODUCTION

Red meat has been addressed has having high content of saturated fatty acids (SFA). Several studies have shown a positive relationship between dietary SFA and the onset and development of several widespread human pathologies, such as cardiovascular diseases and various forms of cancer (BOADA *et al*., 2016). In response to consumers demand, in recent years different feeding strategies aiming at reducing SFA and contemporarily at increasing polyunsaturated fatty acids (PUFA), particularly omega-3 fatty acids, in ruminant-derived food products have been developed (SHINGFIELD *et al*., 2013). Omega-3 PUFA bring numerous beneficial effects on human health as they favor normal embryogenesis and brain development, and protect against cancer, cardiovascular and neurodegenerative diseases (CALDER, 2013).

Flaxseed, one of the richest natural sources of α-linolenic acid (C18:3 n-3), has been shown to be an effective feed ingredient in increasing the content of omega-3 PUFA in beef (JUÁREZ *et al*., 2011). However, an increase of highly unsaturated fats may pose to alterations of meat flavor, mainly because of the derived greater susceptibility to oxidative breakdown (JUÁREZ *et al*., 2012). Moreover, when the proportion of C18:3 n-3 approaches 3% of muscle fatty acids, flavor liking scores assessed by human panelists can be significantly altered, even in case of only slight decreases of lipid oxidative stability (Wood *et al*., 2008).

Therefore, when applying feeding strategies to increase the omega-3 PUFA content of meat, the associated investigation of meat flavor is determinant. Flavor is a very complex attribute of meat palatability; it chemically acts on taste and smell receptors, and plays a key role in acceptability by consumers (KHAN *et al*., 2015). Meat flavor has traditionally been evaluated either by trained assessors or by head-space gas chromatography or mass spectrometry, these methods being time-consuming, labor-intensive and costly, particularly for routine quality control application. The development of objective automated non-destructive techniques that can easily and rapidly characterize meat flavor is an impelling need for the meat industry (NARSAIAH and JHA, 2012). Chemical sensor systems (i.e., electronic noses) are technologies for the at- or on-line discrimination of populations according to volatile compounds. These systems involve various types of electronic chemical gas sensors and with partial specificity which, combined to suitable statistical methods, allow for pattern recognition of simple or complex families of volatile chemical compounds (GHASEMI-VARNAMKHASTI *et al*., 2009). Over the last twenty years, several studies have been carried out using the electronic nose (e-nose), as a rapid and non-destructive method, to assess meat quality (GHASEMI-VARNAMKHASTI *et al*., 2009; Hong *et al*., 2012; LOUTFI *et al*., 2015). Some studies also showed the potential of e-nose to aid or replace olfactory sensory analysis of meat performed by trained panelists (MILDNER-SZKUDLARZ *et al*., 2007), but limited literature is currently available correlating e-nose response to flavor intensity assessed by sensory panels (LOUTFI *et al*., 2015).

The aims of this study were: (i) to evaluate whether the e-nose could be used to discriminate meat beef loins (*Longissimus thoracis* muscle; LM) samples obtained from bulls fed diets without or with flaxseed and aged for 2, 7 or 10 days, and (ii) to compare, at 7 days of aging, e-nose reading and sensory panel evaluation in the assessment of meat flavor intensity.

2. MATERIALS AND METHODS

2.1. Animals, dietary treatments and sampling procedures

Animal care and experimental procedures were carried out in compliance with European Union legislation on the protection of animals used for scientific purposes (EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION, 2010).
Eighteen male calves of the Piemontese breed (4.5 ± 0.59 months old; mean ± sd) were purchased from a local dealer and randomly allotted into two pens (9 animals/pen). Animals had free access to fresh water and were fed for 172 days the same base diet (adaptation period) consisting of a commercial concentrate for fattening cattle, ryegrass hay, corn meal, distillers dried grains, dried beet pulp and soybean meal. The adaptation period was followed by a treatment period of 135 days during which pens were randomly assigned to two treatment diets: control or flaxseed diet. The amount of ground flaxseed (dry matter (DM): 917 g/kg; ether extract: 360 g/kg DM; α-linolenic acid: 200 g/kg DM) in the flaxseed diet was set at 100 g/kg DM. All diets (Table 1) were formulated according to NRC (NRC, 2000) to fulfill the nutritional requirements of Piemontese young bulls.
At the end of the treatment period animals were slaughtered. A portion of the LM between the 8th and the 10th thoracic vertebra from the right side of the carcass was taken 24 h after slaughter and transferred under refrigerated conditions to the lab. Then, the LM was cut into three 2 cm thick steaks. Four equal subsamples were obtained from the first steak; each subsample was sealed in a commercial food grade polymer bag and kept for 2, 7 (two subsamples) or 10 days (d) in a controlled environment at 4°C, away from direct light, until e-nose headspace analysis. One subsample was aged for 7d at 4°C, then vacuum-packed and stored at -80°C until sensory evaluation by panelists.

2.2. Analysis of feed

AOAC International (2000; 2003) procedures were used to determine DM, ash, crude protein (CP), ether extract (EE) in flaxseed and diets. Feed chemical composition was expressed as g/kg DM.
Feed fatty acid (FA) composition was assessed as described by RENNA *et al.* (2014). Feed FA results are reported as g/kg of total detected FA.
The proximate and the main FA compositions of the diets are reported in Table 1.

2.3. E-nose procedure

A PEN 3 Portable Electronic Nose (Airsense Analytics GmbH, Schwerin, Germany) equipped with an array of 10 metal-oxide sensors (Table 2) and a pattern recognition software for data recording and processing (WinMuster, v. 1.6.2.13) was used.
The meat subsamples were subjected to e-nose reading either as raw (2, 7 and 10d) or cooked (7d). The meat was cooked in a flask in a water-bath at 70°C for 30 minutes. Before starting the e-nose assay a calibration procedure was carried out to account for variations in relative humidity of the air, temperature and possible drift of sensors over time. The air filtered through an active carbon filter was used as zero gas. At the end of the calibration procedure the sensors responses were recorded (G0). Then, about 50 grams of raw or cooked meat were cut into 2 cm^3 particles and put into a 250 mL flask equipped with teflon/silicon septum cup and let it stand for 30 minutes at 25°C to allow for a uniform distribution of gasses in the flask headspace before the e-nose analysis. Upon analysis, a needle connected to the e-nose was used to perforate the septum of the flask containing the meat sample and air of the headspace was absorbed into the air detection chamber

with a flow rate of 400 mL/min. Before each sample reading, the detection chamber was flushed for 330 s with reference air (air filtered through an active carbon filter) for sensors recovery. Then, upon flowing of headspace sample air the sensors' responses (G) were recorded once per second and for 60 s. The 60 s measurement interval was selected to allow sensors to reach a stable signal value. The sensor response to the substances in the headspace was defined by the conductance ratio G/G0. A G/G0 threshold value of 6 was set for the sensor number 2 in the array (W5S) through an automatic dilution system to protect the sensor array from overloading.

Table 1. Ingredients, proximate composition and main fatty acid profile of the experimental diets fed in the treatment period.

	Control	Flaxseed
Ingredients (g kg^{-1} DM)		
Concentrate A	333	258
Corn meal	254	294
Dried sugar beet pulp	160	102
Ryegrass hay	121	151
Barley meal	78	61
Ground flaxseed	0	100
Soybean meal	35	14
Concentrate B	22	23
Proximate composition (g kg^{-1} DM)		
DM (%)	86	87
CP	173	170
EE	38	75
Ash	66	60
Net Energy (MJ kg^{-1}DM)	7.92	8.19
Main fatty acid composition (g kg^{-1} of TFA)		
C16:0	173.20	109.20
C18:0	22.60	24.90
C18:1 n-9	260.65	240.95
C18:2 n-6	492.50	319.15
C18:3 n-3	31.15	292.80

Abbreviations: DM, dry matter; CP, crude protein; EE, ether extract; TFA, total fatty acids.
Concentrate A: corn, wheat middlings, sunflower meal, roasted dehulled soybean meal, wheat bran, roasted soybean meal, vitamins and minerals; Concentrate B: corn germ meal, wheat middlings, wheat bran, corn, vitamins and minerals.

Table 2. Sensitivity and selectivity of the sensors in the portable electronic nose device (PEN 3 Portable Electronic Nose, Airsense Analytics GmbH, Schwerin, Germany).

Number in array	Sensor	General description	Reference
1	W1C aromatic	Aromatic compounds	Toluene, 10 ppm
2	W5S broad range	Broad range sensitivity react on nitrogen oxides and ozone, very sensitive with negative signal	NO_2, 1 ppm
3	W3C aromatic	Ammonia, used as sensor for aromatic compounds	Benzene, 10 ppm
4	W6S hydrogen	Mainly hydrogen, selectively (breath gases)	H_2, 100 ppb
5	W5C aromatic-aliphatic	Alkanes, aromatic compounds, less polar compounds	Propane, 1 ppm
6	W1S broad methane	Sensitive to methane (environment) ca. 10ppm, broad range, similar to W2S	CH_4, 100 ppm
7	W1W sulphur organic	Reacts on sulphur compounds (H_2S 0,1ppm) otherwise sensitive to many terpenes and sulphur organic compounds, which are important for smell (limonene, pyrazine)	H_2S, 1 ppm
8	W2S broad alcohol	Detects alcohol's, partially aromatic compounds, broad range	CO, 100 ppm
9	W2W sulphur-chlorine	Aromatic compounds, sulfur organic compounds	H_2S, 1 ppm
10	W3S methane-aliphatic	Reacts on high concentrations >100ppm sometimes very selective (methane)	CH_4, 100 ppm

2.4. Sensory evaluation

The steaks were placed in a refrigerator to thaw for 24 h at 4°C, then cooked without salt or spice addition in a double plate grill, preheated at 250°C, until the final internal temperature reached 70°C, which was monitored by individual thermocouples inserted into the geometric center of each steak (American Meat Science Association (AMSA), 1995). Upon reaching 70°C, the steaks were trimmed of external connective tissue, cut into 1.3 x 1.3 x 2 cm samples, wrapped in a foil pouch and labeled with three-digit random numbers. A sensory quantitative affective test based on intensity scale (MEILGAARD *et al*., 2006) was performed by 39 males and 24 females consumers, ranging in age from 21 to 60 years old. Panelists recruited for testing the samples, previously involved in surveys on beef preference/acceptance tests, were regular consumers of beef and had not diet restriction or allergies.
Samples from each treatment were randomly served one at a time to each panelist. Five sessions with approximately 12 panelists per session were carried out in individual booths in a sensory testing laboratory under artificial white lighting. Four samples (two per dietary treatment), each served 5 minutes apart, were offered to each consumer per session, for a total of 252 assessments over the five sessions. Panelists evaluated beef flavor intensity using an unstructured scale, consisting of a 15 cm long horizontal line, with anchor points labeled with the expression "extremely bland" (0 cm) and "extremely intense" (15 cm) (MEILGAARD *et al.,* 2006). The panelists expressed each evaluation by making a vertical line across the horizontal line at the point best reflecting their perception of the magnitude of flavor. The panelists were asked to rinse their mouth with still water served at room temperature during the one minute break imposed between consecutive samples.

2.5. Statistical analysis

All analyses were performed using SAS (Statistical Analytical System (SAS), 2003). Significance was declared at $P \leq 0.05$.
The e-nose measurement produced 60 readings for each sensor for a total of 600 readings for each sample. However, multiple readings from a sensor are correlated each other. Therefore, among the sixty available readings from each sensor only one (59^{th}) and in a plateau condition was considered for subsequent analysis, for a total of 10 values/sample.
The Mahalanobis Distance (MD) was used to calculate similarity between samples within classes (i.e. aging and diet).
The clustering of samples was investigated based on the sensors activation patterns. At first, variables (W1C, W5S, W3C, W6S, W5C, W1S, W1W, W2S, W2W, W3S) were subjected to the Stepdisc procedure and all of them were suited for entering the discriminant analysis. Then, the dataset was subjected to the Factor procedure for Principal Component Analysis (PCA) and the Candisc procedure for Canonical Discriminant Analysis (CDA).
Sensory evaluation data were subjected to the GLM procedure. The model included the diet as fixed effect whereas the panelist and diet x panelist interaction entered the model as random effects (NAES *et al.,* 2011).
The relationship between e-nose data, either on raw or cooked samples, and sensory scores assessed by panelists was analyzed by partial least square regression (PLS) for flavor prediction.

3. RESULTS AND DISCUSSION

3.1. E-nose analysis

When exposed to the headspace gas the sensor array produces a particular pattern in which each curve represents a different transient sensor response (Fig. 1). The x-axis represents the time of reading and the y-axis the sensors' ratio of conductance. For some sensors, the conductivity grows rapidly and then decreases to a stable condition whereas for some others the change in resistance, and therefore the G/G0 ratio is minimal or below the unity. Only one point toward the end of sample measurement was considered for each sensor and their mean responses within classification groups (i.e. diet and aging) are shown in Fig. 2. The polar plot suggests some sensors are more relevant than others in terms of signal response between control and flaxseed within the day of aging. Sensors W1C, W3C, W5C, W1W, W2W and W3S differentiate between diets at 7d of aging (P<0.05). Detailed information about sensors characteristics are not available in literature (SMYTH and COZZOLINO, 2013), as well as there are no reports that interrelate the response of a sensor to a particular chemical component. Nevertheless, association between sensors and groups or families of substances are outlined (Table 2) and from this we can address strongest reactive sensors at 7d (except broad range sensors: W1S, W5S) into three groups: sensors reactive to aromatic compounds (W1C, W3C and W5C; in which samples from flaxseed fed animals were in average 12% lower than control), sensors reactive to sulfur compounds (W1W, W2W; in which samples from flaxseed fed animals were in average 37% lower than control) and sensor W3S reactive to high concentration of methane in which samples from flaxseed fed animals were in average 10% higher than control.

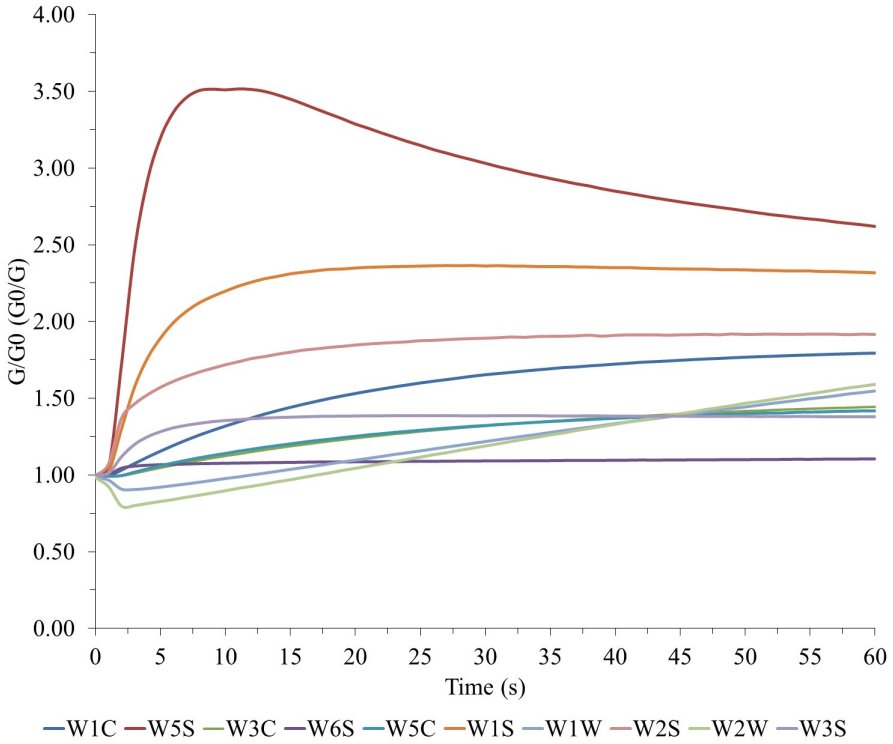

Figure 1. Example of electronic nose reading. The sensor gas response is expressed as G/G0 or G0/G (for sensors showing a negative behavior in presence of chemical compounds; W1C, W3C, W5C), where G and G0 represent the resistance of the sensor in sample gas and in zero gas air, respectively.

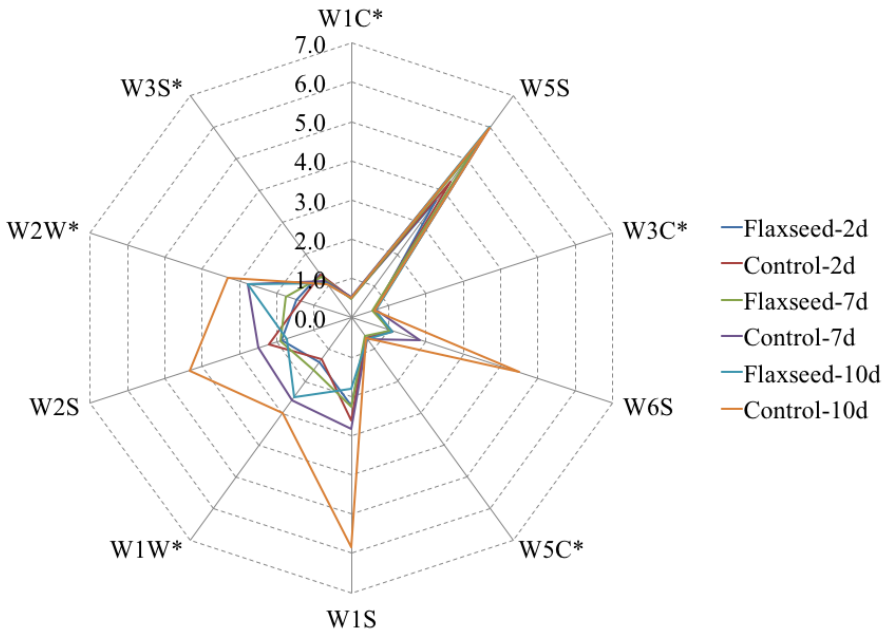

Figure 2. Polar plot of the average responses of sensors when exposed to raw meat samples at different aging (2, 7 or 10 days) and from animals fed control or flaxseed diet (n = 9). The gas response is expressed as the G/G0 ratio, where G and G0 represent the resistance of the sensor in sample gas and in zero gas air, respectively. Values with superscript (*) and within the 7 days aging differentiate for $P<0.05$

The MD classifies the observation into the nearest population by calculating the distance between the unit vector and the centroid for population. The MD takes into account the correlation of the data within the cluster, it is unit less and it measures how many deviations is the value from the cluster centroid. The MD enlarged with the increase of aging (Table 3).

Table 3. Mahalanobis distance of sensors (between class means).

	Control			Flaxseed		
Day	**2**	**7**	**10**	**2**	**7**	**10**
2	0	9.176	14.909	0	4.914	12.117
7	9.176	0	7.344	4.914	0	8.714
10	14.909	7.344	0	12.117	8.714	0

As previously reported (HONG *et al.*, 2012) also in the present work the e-nose seems capable of suggesting divergences between samples kept for different length of time at 4°C. The increments of the distances were different among samples from animals being fed different diets. The distances in flaxseed were 54% and 81% compared with control, respectively at 7d and 10d. Even though less pronounced (HONG *et al.*, 2012), we could speculate that in our condition the large MD in the control group might suggest an early

start of the aging related modification leading to different responses of the e-nose sensor array. Nevertheless, after an initial latency time, at 10d the MD of flaxseed seemed similar to the control.
Correlation patterns with sensors were obtained with PCA to help in the discrimination process among meat samples. The stepdisc procedure suggested all sensors could be included in the discriminant analysis (SAS, 2003) and a 0.66 value for the Kaiser measure of sampling adequacy exceeded the threshold value of 0.60 (STEVENS, 2009), therefore supporting the data set as suitable for the PCA analysis (CERNY and KAISER, 1977).
The PCA is a variable reduction method yielding linear combination of original variables (principal components, PC). The maximum number of PC equals the number of considered variable (i.e. number of sensors). The latent constructs were obtained with the PRIN method of the proc Factor procedure, with Varimax rotation, and retained in accordance to the eigenvalue-one criterion (STEVENS, 2009). Then, variables loading vectors and PC scores were obtained.
Following a Varimax orthogonal rotation, three factors were extracted explaining 95.2% of the total variability of data (Table 4). By giving a magnitude of at least 0.4 as indicator of a salient variable-factor relationship, the sensors W5S, W1W, W2W and W3S loaded on PC1 (32.89%), sensors W1C, W3C and W5C loaded on PC2 (31.39%) whereas sensors W6S, W1S and W2S loaded on PC3 (30.95%). The orthogonal factor rotation simplifies the interpretation of extracted factors and from that we could suggest PC1 as related mainly to the proteolysis activity, PC2 mainly addressing processes leading to aromatic compounds formation, whereas PC3 included the broad range sensors and a sensor reactive to hydrogen. To identify pattern of correlation among sensors responses, score coefficients for each variable were obtained and principal component scores for each sample were calculated. Fig. 3 shows a two-dimensional plot of the analysis score of meat samples with PC1 and PC2. The control at 7d and 10d and the flaxseed at 10d tended to cluster in the positive quarter for the considered PCs.

Table 4. Loading vectors of sensors on Varimax rotated extracted PC and proportion of explained variance.

	PC1	PC2	PC3
W1C	0.02	0.96*	0.03
W5S	0.90*	-0.28	0.08
W3C	0.11	0.99*	-0.04
W6S	0.32	0.02	0.94*
W5C	0.17	0.97*	-0.08
W1S	0.16	-0.07	0.98*
W1W	0.84*	0.31	0.36
W2S	0.22	-0.06	0.97*
W2W	0.87*	0.29	0.36
W3S	-0.90*	-0.19	-0.20
Proportion	32.89	31.39	30.95

PC = Principal Component, *Variables loaded on extracted components (i.e. loading vectors higher than 0.40).

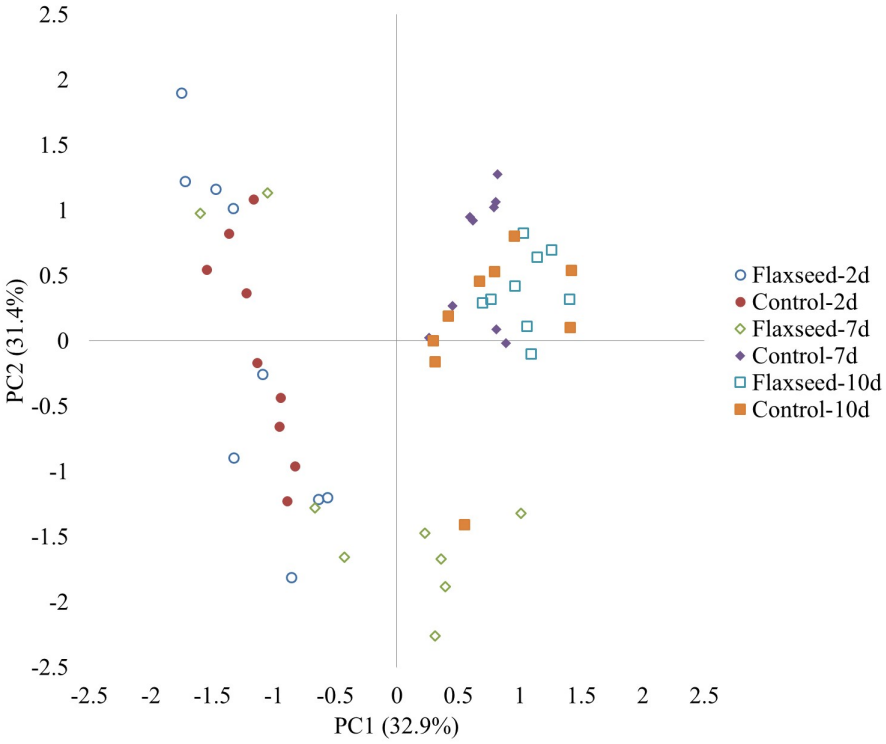

Figure 3. Score plot of principal component analysis (PC) of raw meat samples at different aging (2, 7 or 10 days).

As in PCA, also the CDA performs a dimension-reduction through linear combination of quantitative variables and helps to discriminate differences among classes. When performing the CDA the R squares indicated all sensors showed a significant (P<0.05) difference between the classification groups for all canonical variables (Can). The raw canonical coefficients of the first canonical variable showed that classes differ on the linear combination of the sensors selective for aromatic compounds (Table 5). The plotting of the first two canonical variables (Fig. 4) revealed Can1 has more discriminatory power and it is capable of discriminating meat samples into four groups: flaxseed at 2d, control at 2d and flaxseed at 7d, control at 7d and flaxseed at 10d, control at 10d. However, the better result in samples discrimination compared to PCA was inherent to the algorithm used for group separation since the CDA is a supervised learning method relying on group labels.

3.2. Sensory analysis

Sensory analysis was performed after 7d of aging when usually beef is offered for sale as retail cuts. The amount of fat in meat was similar between samples coming from differently fed animals (5.2 *vs.* 4.6 g kg^{-1}, respectively for control or flaxseed; data not shown). The flavor intensity was higher (P<0.05) in meat samples from flaxseed-fed animal compared with meat samples from control-fed animals (7.84 and 6.74, respectively). Feeding flaxseed doubled the intramuscular content of total n-3 PUFA (from 21.1 to 46.7 g/kg of total detected FA – data not shown) and the proportion of C18:3 n-3 in LM from flaxseed-fed bulls reached 3.0% while in control-fed bulls remained lower than 0.9% (data not shown); such modifications of the fatty acid profile of meat might explain the higher flavor intensity scored by panelists.

Table 5. Raw canonical coefficients for canonical variables.

	Can1	Can2	Can3	Can4	Can5
W1C	64.874	75.721	24.026	-118.107	1.820
W5S	0.798	-0.783	1.410	-0.636	-1.294
W3C	-71.757	-81.159	-137.678	96.442	-125.821
W6S	-5.924	-0.211	2.979	5.177	-5.727
W5C	57.716	20.960	150.921	-7.927	115.196
W1S	3.565	-3.336	-7.029	-6.538	6.833
W1W	-4.616	-5.562	13.041	3.326	0.050
W2S	3.976	6.841	7.977	4.485	-3.202
W2W	7.278	6.173	-15.159	-4.777	-0.785
W3S	-21.962	13.150	7.314	2.614	-25.212
Proportion, %	85.65	6.04	4.49	2.32	1.50
R^2	0.98	0.75	0.69	0.53	0.42

Can = Canonical variable.

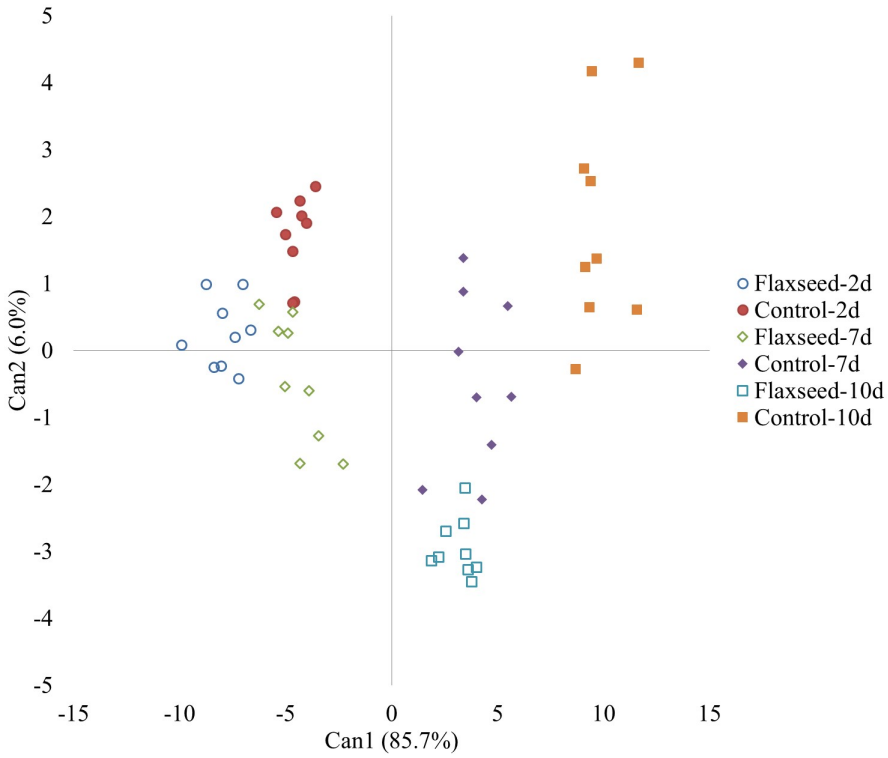

Figure 4. Score plot of canonical discriminant analysis (Can) of raw meat samples at different aging (2, 7 or 10 days).

Several studies have shown that animal diet can strongly influence the fatty acid composition of meat (PONNAMPALAM *et al.*, 2001; WOOD *et al.*, 2003; BAS *et al.*, 2007; VAHMANI *et al.*, 2015). The variation of fatty acid compositions has profound effects on meat quality, because fatty acid composition determines the firmness/oiliness of adipose

tissue and the oxidative stability of muscle, which in turn affects flavor and muscle color. High PUFA levels may produce alterations in meat flavor due to their susceptibility to oxidation and the production of unpleasant volatile components during cooking (WOOD *et al.*, 1999). Even if increases in overall liking scores were reported (VATANSEVER *et al.*, 2000), most studies have shown decreases in panelist preferences for meat from animals fed diets high in unsaturated fatty acids (CAMPO *et al.*, 2006), sometimes due to the related increase of oxidation products (YANG *et al.*, 2002).

3.3. Relationship between e-nose data and sensory scores

The 7d e-nose data on cooked meat were analyzed by PLS to investigate the relationship between sensors readings and flavor scores. Meat samples used in the two assays were cocked with different methods, water bath for e-nose and in a double plate grill for sensory evaluation. While the cocking methods could lead to different textural attributes of meat, the flavor is however not affected (CHOI *et al.*, 2016). The water-bath method selected for the e-nose assay was to minimize Maillard products and their reaction with volatile compounds (AASLYNG and MEINERT, 2017) and effects of high grilling temperatures on variability of volatile compounds and therefore on pattern observed during the e-nose assay. The e-nose sensor responses (predictor variable) were used to predict the flavor score (dependent variable) from sensory evaluation. Since the restricted number of samples, an independent data set for validation was not possible, therefore a one at a time cross-validation method was used to choose the number of extracted factors minimizing the predicted residual sum of squares (PRESS) and the van der Voet's test (VAN DER VOET, 1994) was used to select the fewest number of factors (i.e. with residual PRESS not statistically different than the minimum PRESS) (Table 6). The contribution of each sensor in fitting the PLS model was based on the Variable Importance for Projection (VIP) statistic of Wold (WOLD, 1994) with a minimum threshold value of 0.8. The VIP shows the contribution of sensors in fitting the PLS model for both sensors and flavor (Table 7). A small (in absolute value) coefficient of center and scaled parameter and a small VIP (i.e. <0.8) suggest low importance of the predictor in the PLS model. The parameter estimate in original scale represents the coefficients of each predictor in the PLS model. The predicted results by PLS *vs* observed results from sensory evaluation are shown in Fig. 5. The retained factors in PLS explained 99.6% and 82.5% of the variance of independent (sensors) and dependent (flavor) variables, suggesting in our condition the e-nose could represent a tool supporting the sensory evaluation by panelists.

4. CONCLUSIONS

The approach used in data evaluation could not clearly indicate the e-nose as capable of discriminating populations in meat samples from differently fed animals and with different days of aging. Within the sensor array used, sensors having major importance in discriminating power were the ones reacting to aromatic compounds, followed by sensors that could be related to proteolysis reactions. Differences among samples were observed at the 7d of aging. In our condition, when performing PLS regression the e-nose proved to be a valuable tool supporting the sensory evaluation. Additional efforts are needed to better understand the relationship between sensor activation and flavor intensity toward the identification of the substances acting in flavor intensity.

Table 6. Steps of the partial least square (PLS) method with cross-validation for the 7d cooked meat samples.

PLS Factors	Cross-validation	
	Root mean PRESS	Comparison Significance
0	1.059	0.034
1	1.035	0.047
2	0.997	0.072
3	0.869	0.095
4	0.912	0.079
5	0.911	0.04
6	0.956	0.036
7	0.792	0.101
8	0.931	0.011
9	0.659	0.064
10	0.613	1
Minimum root mean PRESS		0.613
Minimizing number of factors		10
Smallest number of factors with P>0.1		7

Retained factors	Percent Variation Accounted for			
	Independent variable		Dependent variables	
	Current	Total	Current	Total
1	32.338	32.338	48.745	48.745
2	35.152	67.490	7.229	55.974
3	26.017	93.507	2.970	58.944
4	3.608	97.115	11.002	69.946
5	1.896	99.011	2.042	71.987
6	0.514	99.525	4.166	76.153
7	0.066	99.591	6.364	82.519

Table 7. Variable importance for projection (VIP) and regression coefficients values for sensors in the prediction of flavor from e-nose reading on 7d cooked meat.

Variable	VIP	Centered and Scale Parameter estimates	Parameter estimates in original scale
Intercept	-	0	31.891
W1C	0.748	-0.247	-8.208
W5S	0.878	0.620	0.305
W3C	0.722	2.223	48.322
W6S	1.411	3.075	5.115
W5C	0.878	-3.922	-76.491
W1S	1.404	-1.693	-2.259
W1W	0.660	0.056	0.138
W2S	1.337	-1.624	-4.393
W2W	0.645	0.104	0.246
W3S	0.881	0.488	3.451

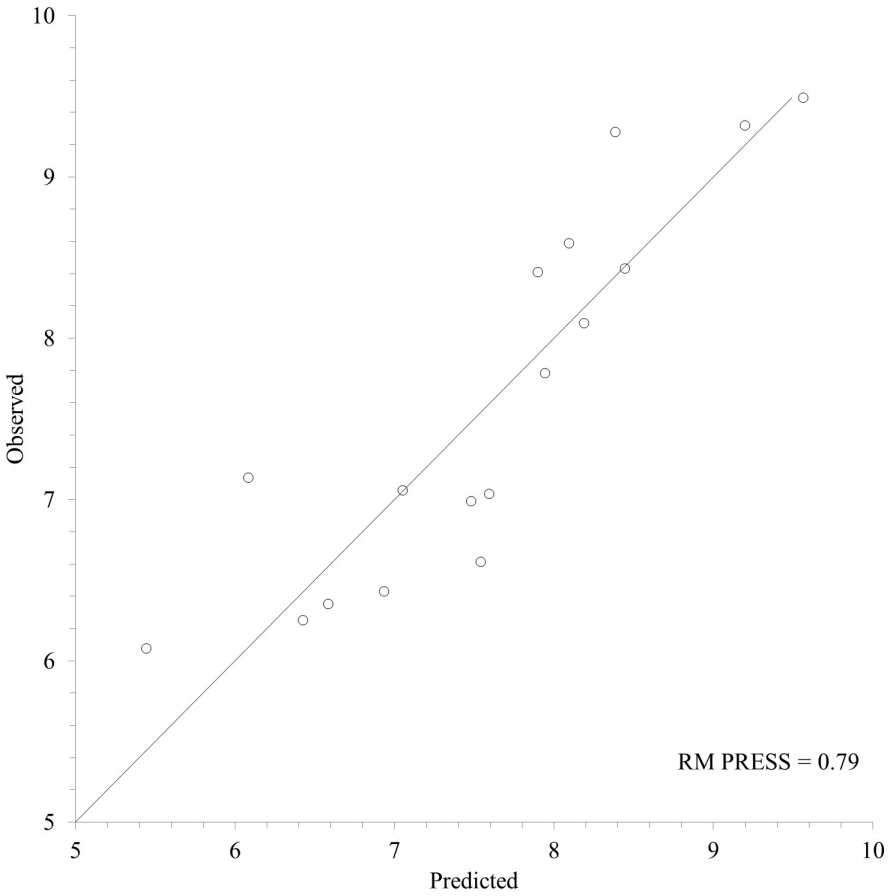

Figure 5. Observed and predicted flavor intensity scores (scale: 1 = poor to 15 = intense) evaluated by partial least squares regression with sensor responses as predictor matrix. RM PRESS = root mean square of predicted residual sum of squares.

ACKNOWLEDGEMENTS

The Authors gratefully acknowledge Dr. Claudio Pini and the staff of the AN.FO.RA. farm (Fontanellato, PR) for technical assistance in diet formulation and care of animals.

REFERENCES

Aaslyng M.D. and Meinert L.2017. Meat flavor in pork and beef – From animal to meal. Meat Sci. In press.

American Meat Science Association (AMSA) 1995. Research guidelines for cookery, sensory evaluation and instrumental tenderness measurements of fresh meat. National Live Stock and Meat Board, Chicago, IL, USA.

AOAC International. 2000. Official methods of analysis. 17th ed. Gaithersburg, MD, USA: Association of Official Analytical Chemists.

AOAC International. 2003. Official methods of analysis. 17th Ed., 2nd revision. Gaithersburg, MD, USA: Association of Official Analytical Chemists.

Bas P., Berthelot E., Pottier E., Normand J. 2007. Effect of level of linseed on fatty acid composition of muscles and adipose tissues of lambs with emphasis on trans fatty acids. Meat Sci. 77:678-688.

Boada L.D., Henríquez-Hernández L.A. and Luzardo O.P. 2016. The impact of red and processed meat consumption on cancer and other health outcomes: Epidemiological evidences. Food Chem. Toxicol. 92:236-244.

Calder, P.C. 2013. Nutritional benefits of omega-3 fatty acids In, Jacobsen C., Skall Nielsen N., Frisenfeldt Horn A. and Moltke Sorensen A.D. (eds.) *Food Enrichment with Omega-3 Fatty Acids.* Cambridge, GB, Woodhead Publishing pp. 3-26. (Woodhead Publishing Series in Food Science, Technology and Nutrition, 252).

Campo M.M., Nute G.R., Hughes S.I., Enser M., Wood J.D. and Richardson R.I. 2006. Flavour perception of oxidation in beef. Meat Sci. 72:303-311.

Cerny B.A. and Kaiser H.F. 1977. A Study Of A Measure Of Sampling Adequacy For Factor-Analytic Correlation Matrices. Multivar. Behav. Res. 12(1):43-47.

Choi Y.S., Hwang K.E., Jeong T.J., Kim Y.B., Jeon K.H., Kim E.M., Sung J.M., Kim H.W. and Kim C.J. 2016. Comparative Study on the Effects of Boiling, Steaming, Grilling, Microwaving and Superheated Steaming on Quality Characteristics of Marinated Chicken Steak. Korean J. Food Sci. Anim. Resour. 36(1):1-7.

European Parliament and the Council of the European Union 2010. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Available from: http://eur-lex.europa.eu/legal-content/IT/TXT/?uri=OJ:L:2010:276:TOC

Ghasemi-Varnamkhasti M., Mohtasebi S.S., Siadat M. and Balasubramanian S. 2009. Meat Quality Assessment by Electronic Nose (Machine Olfaction Technology). Sensors 9(8):6058-6083.

Hong X., Wang J. and Hai Z. 2012. Discrimination and prediction of multiple beef freshness indexes based on electronic nose. Sens. Actuators B Chem. 161(1):381-389.

Juárez M., Dugan M.E.R., Aalhus J.L., Aldai N., Basarab J.A., Baron V.S. and McAllister T.A. 2011. Effects of vitamin E and flaxseed on rumen-derived fatty acid intermediates in beef intramuscular fat. Meat Sci. 88:434-440.

Juárez M., Dugan M.E.R., Aldai N., Basarab J.A., Baron V.S., McAllister T.A. and Aalhus J.L. 2012. Beef quality attributes as affected by increasing the intramuscular levels of vitamin E and omega-3 fatty acids. Meat Sci. 90:764-769.

Khan M.I., Jo C. and Tariq M.R. 2015. Meat flavor precursors and factors influencing flavor precursors-A systematic review. Meat Sci. 110:278-284.

Loutfi A., Coradeschi S., Mani G.K., Shankar P. and Rayappan J.B.B. 2015. Electronic noses for food quality: A review. J. Food Eng. 144:103-111.

Meilgaard M.C., Carr B.T. and Civille G.V. 2006. Sensory evaluation techniques. CRC press.

Mildner-Szkudlarz S., Zawirska-Wojtasiak R., Korczak J. and Jelen H.H. 2007. A comparison of human and electronic nose responses to flavour of various food products of different degree of lipids oxidation. Pol. J. Food Nutr. Sci. 57(2):195-202.

Naes T., Brockhoff P. and Tomic O. 2011. Statistics for sensory and consumer science. John Wiley & Sons.

Narsaiah K. and Jha S.N. 2012. Nondestructive methods for quality evaluation of livestock products. J. Food Sci. Technol. 49(3):342-348.

NRC, 2000. Nutrient requirements of beef cattle. 7th Ed. Update 2000. Washington, USA: The National Academies Press.

Ponnampalam E.N., Trout G.R., Sinclair A.J., Egan A.R., Leury B.J. 2001. Comparison of the color stability and lipid oxidative stability of fresh and vacuum packaged lamb muscle containing elevated omega-3 and omega-6 fatty acid levels from dietary manipulation. Meat Sci. 58:151-161.

Renna M., Gasmi-Boubaker A., Lussiana C., Battaglini L.M., Belfayez K. and Fortina R. 2014. Fatty acid composition of the seed oils of selected Vicia L. taxa from Tunisia. Ital J Anim Sci 13(2):308-16.

SAS (Statistical Analytical System) 2003. SAS/SAT guide for personal computers, version 9.13. SAS Institute Inc., Cary, NC, USA.

Shingfield K.J., Bonnet M. and Scollan N.D. 2013. Recent developments in altering the fatty acid composition of ruminant-derived foods. Animal. 7(s1):132-162.

Smyth H. and Cozzolino D. 2013. Instrumental Methods (Spectroscopy, Electronic Nose, and Tongue) As Tools To Predict Taste and Aroma in Beverages: Advantages and Limitations. Chem. Rev. 113(3):1429-1440.

Stevens J.P. 2009. Applied Multivariate Statistics for the Social Sciences, Fifth Edition. Routledge.

Vahmani P., Mapiye C., Prieto N., Rolland D.C., McAllister T.A., Aalhus J.L., Dugan M.E.R. 2015. The scope for manipulating the polyunsaturated fatty acid content of beef: a review. J Anim Sci Biotechnol, 6(1):29-41.

Vatansever L., Kurt E., Enser M. and Nute G.R. 2000. Shelf life and eating quality of beef from cattle of different breeds given diets differing in n-3 polyunsaturated fatty acid composition. Anim. Sci. 71(3):471-482.

Van der Voet H. 1994. Comparing the predictive accuracy of models using a simple randomization test. Chemom. Intell. Lab. Syst. 25(2):313-323.

Wold S. 1994. PLS for multivariate linear modeling. Chemom. Methods Mol. Des. 2:195.

Wood J.D., Enser M., Fisher A.V., Nute G.R., Richardson R.I., Sheard P.R. 1999. Manipulating meat quality and composition. Proceedings of the Nutrition Society. 58:363-370.

Wood J.D., Richardson R.I., Nute G.R., Fisher A.V., Campo M.M., Kasapidou E., Sheard P.R., Enser M. 2003. Effects of fatty acids on meat quality: a review. Meat Sci. 66:21-32.

Wood J.D., Enser M., Fisher A.V., Nute G.R., Sheard P.R., Richardson R.I., Hughes S.I. and Whittington F.M. 2008. Fat deposition, fatty acid composition and meat quality: A review. Meat Sci. 78:343-358.

Yang A., Brewster M.J., Beilken S.L., Lanari M.C., Taylor D.G. and Tume R. K. 2002. Warmed-Over Flavor and Lipid Stability of Beef: Effects of Prior Nutrition. J. Food Sci. 67(9):3309-3313.

Paper Received June 24, 2017 Accepted February 12, 2018