Investigating venison sausages by means of human sensory panel and electronic nose

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ABSTRACT

Three types of venison sausages produced of red-deer meat were investigated in three different ripening stages. Human sensory panel was appointed to evaluate customer preference of the sausages. Natural samples containing no pepper received the lowest marks, while samples with sweet and hot pepper were appreciated very similarly. Electronic nose technique was applied to identify the different types and ripening stages by the volatile compounds. It was possible to classify the samples according to the spicing protocol, with a high accuracy (ratio of successfully classified samples in cross-validation was over 90%). Human preference and electronic sensor signal datasets were combined and calibration equation was developed in order to predict consumer response by means of electronic nose. Odor preference and overall impression, as the most relevant attributes of consumer responses can be predicted with a high accuracy and precision ($R^2>0.9$).
(Keywords: food, meat, qualification, red-deer, ripening, sensor array)

INTRODUCTION

Venison is a frequently used raw material for restaurants and home cooking. The wild deer population in Europe is low, however, the breeding of the species within farm condition is showing an increasing tendency. From kitchen technological point of view the venison haunch roasts and the tenderloin are easy to cook in contrast with the shoulder and other tougher cuts. The latters are processed into hamburger and sausage in the Unites States and reserves for goulashes and ragouts in Europe where the venison is rare and quite expensive. It has to be mentioned that nowadays a clear tendency can be seen towards the processed – ready to eat – products which are appropriate for home consumption.

The characteristic texture and taste of game differs from poultry and farmyard animals as in general its meat is darker, tougher and has a stronger taste. According the result of *Rodbotten et al.* (2004) the game could be separated from the meat samples of the examined 15 species including poultry, pork and beef by sensory profiling. The sensory quality of venison was studied by *Wiklund et al.* (2003) and *Hutchison et al.* (2010) in red deer. In a preliminary study of *Soriano et al.* (2006) dry sausage made from deer or wild boar meat were compared based on their physicochemical characteristics and free fatty acid composition.

Relatively low number of publication can be found in the literature focusing on the headspace volatile compounds composition of red-deer meat or product. Analyzing smoked dried meats of beef, horse, goat and venison *Hierro et al.* (2004) emphasized the rule of lipid oxidation, amino acid degradation and carbohydrate fermentation in volatile generation.

Physicochemical measurements are traditionally used in the food industry for qualification of products, without aroma component characterization. The identification and quantification of these components is now mostly based on GC-MS methods following separation steps. It has to be mentioned that the relationship between the chromatographic data and the perception of the global aroma of a product is not easily described. However it is known that the same odor stimuli result divergent perceptions in the brain of different individuals. Consequently, the basic shortcoming of the human sensory panel is the low repeatability and reproducibility connected to the sensory susceptibility of panelists (*Plutowska and Wardencki,* 2007).

To overcome this burden, since the first development of electronic nose (EN) (*Persaud and Dodd,* 1982) various versions of ENs were released using different sensors e.g. metal oxide gas sensors (MOS). The signals of sensory arrays produce the so-called fingerprint of the given flavor which is evaluated with chemometric methods. The EN system is exceptionally sensitive towards the volatile compounds of food materials as reviewed by *Peris and Gilabert* (2009). In order to classify samples the EN combines the response profiles of various sensors which react to the different type of volatile and dissolved compounds. However, analytical methods such as GC-MS are still needed to describe the compositional differences of the discriminated samples.

ENs were used to monitor sausage fermentation detecting the changes of volatile compounds emission (*Eklov et al.,* 1998). According to the results both the sensory panel and the EN were applicable to detect differences between groups of different fermentation stages.

The aim of this study was to evaluate the effect of different spicing protocol on the sensory properties of sausages produced from the same raw materials, and to test the applicability of EN system on describing aroma profile of venison products.

MATERIALS AND METHODS

Sausage samples
Samples were delivered by a slaughter house producing sausages with traditional Hungarian flavor, using red-deer (*Cervus elaphus*) meat, pork and lard, as raw materials. Three different types were prepared: natural sausage (N) without pepper, delicacy sausage (D) with sweet pepper (paprika - *Capsicum annuum var. longum*), hot sausage (H) with hot-pepper – all other compounds were applied with the same concentration. Sausages were ripened after production according to the formula of the product. Samples were collected one week before standard ripening time (T1), at optimum time (T2), and one week after (T3).

Electronic-nose measurement
An αFox 4000 (ALPHA MOS, Toulouse, France) type EN was used for headspace analysis of samples. The equipment consists of three sensor chambers with six MOS inside of each (LY2/AA, LY2/G, LY2/gCT, LY2/gCTl, LY2/Gh, LY2/LG, P10/1, P10/2, P30/1,P30/2, P40/1, P40/2, PA2,T30/1, T40/2, T70/2, T40/1, TA2). The operation temperature of the chambers was 65 °C. The adsorption of volatile compounds

onto the surface of MOS generated a change in the electrical resistance. The extent of change varied with the type of compound and its concentration in the headspace (HS). The response (R) obtained can be formulated as follows: $R = f\{S_g, H(P)\}$, where S_g corresponds to the sensitivity and selectivity of the gas sensor, and $H(P)$ corresponds to the sample headspace generated. The equipment was operated and raw data were collected with AlphaSoft 12.3 software (ALPHA MOS, Toulouse, France).

EN investigations were carried out on fresh samples on all sampling days. There is no possibility to merge and use the signals of the individual sampling days in one dataset, because of the drift of the sensors. Thus, repeated measurement was executed at the end of the trial (T4), using all of the previous samples (T1, T2 and T3) at the same time. Samples of former weeks were stored in vacuum bags on 4 °C. Thus, altogether four EN measurements were obtained. During EN measurements at T1, T2 and T3 dates, 20 individual samples were taken from each type of sausages (N, D, H) and were sniffed once (n = 3 x 20 per sampling dates). During the last EN measurement (T4) all three samples of all three types were involved, and 7 individual samples of each were sniffed (n = 3 x 3 x 7). According to the applied static HS technique, the samples were placed into vials of 20 ml and were sealed hermetically with silicon septa. After the equilibrium has been established between the matrix and gaseous phase, an ALPHA MOS HS 100 auto-sampler was used for sampling the HS.

Atmospheric compressed air was transformed into hydrocarbon and CO_2 free, dry carrier gas (Parker Balston TOC-1250 Total Organic Carbon gas generator, Parker Hannifin Manufacturing Ltd., Maidstone, Kent, UK) and was used as a continuous air-flow. The acquisition time and time between subsequent analyses were 120 and 1200 seconds, respectively. During the phase of EN method development, the use of the following parameters produced the most acceptable signal intensity values and were used in the further investigations in order to get adequate result: sample quantity 3 g, respectively, sample temperature 50 °C, equilibration time 120 s, injection volume 1500 µl, injection intensity 1500 µl/s, air-flow rate 150 ml/min. Samples were placed on the holder tray of auto-sampler and were analyzed in random order.

Human sensory panel test
Ten non-trained panelists were involved in human sensory panel test on T1, T2 and T3 sampling days, simultaneously with EN measurements. Sausages were provided according to the row of the presumed aroma intensity, i.e. group N was served first, then D and H.

Sixteen questions were asked requiring judgment on the following attributes: color preference, redness, texture preference, particle size, fatness, hardness, masticability, flavor preference, game meat flavor, flavor intensity, taste preference, hotness, game meat taste, taste intensity and overall impression. Sensory panel test was organized and executed according to *Williams* (1979) applying self-adjusting scale. In the section of combined evaluation based on human sensory and EN dataset, only the attributes relating to aromatic properties were used during data analysis.

Data analysis
Our investigations were focused on describing basic differences between aromatic properties of the types and to monitor the changes of the product attributes during ripening.

The multisensor arrays of EN were interfaced with computers which collected the sensor signals via RS-232 ports. The raw EN sensor values were saved in the form of

relative resistance changes $((R_0-R)/R_0$, where R_0 is the resistance at t=0 (baseline resistance), and R is the resistance at the response peak). To avoid the problem of multi-colinearity that originates from the cross sensitivity of the sensors, multivariate statistical procedures must be applied to analyze EN datasets. In our study, the classification of samples was performed by means of Principal Component Analysis (PCA) and Discriminant Factor Analysis (DFA) of AlphaSoft 12.3 program. PCA as an unsupervised multivariate statistical method using uncorrelated linear combinations of the original variables reveals the internal structure of the dataset in a way which best explains the variance. On the other hand, DFA as a supervised multivariate method creates an equation which minimizes the possibility of misclassifying cases into their respective groups (*Hines et al.,* 2003).

To avoid overfitting of DFA, leave-one-out cross-validation (CV) was performed that is an iterative method, where each and every samples are once left from the creation of the model and identified as if they were unknown, thus, an impression on the performance of the classifying method is obtained (STONE, 1974). Quality of classification methods was evaluated numerically. In case of PCA, discrimination index (DI) was calculated, that indicates the ratio of the sum of surfaces of all groups to the total surface bordered by the groups. If DI is high, groups are compact and centroids are far from each other. Event of overlapping groups results negative DI, which shows the percent of intersection surface to the total surface. DFA was evaluated by means of percent of correctly classified samples during CV. If the recognition value occurred to be more than 90%, the validity of the model was accepted.

Panel Check v1.4.0 freeware statistical software was applied to evaluate results of human sensory panel. After checking the outliers, the impact of the panelist individuals (assessor effect) and the type of sausage (product effect) on the investigated attributes was rated by means of two-way ANOVA. Consensus test was carried out and result was presented on a bi-plot figure where attributes and investigated groups were positioned based on the principal components generated from the sensory dataset.

Partial least squares (PLS) regression of AlphaSoft 12.3 software was applied to analyze the relationship between signals of sensor array system and human sensory panel responses. Sensory panel scores of individuals were averaged within each group of sausages. These average data were imported into the dataset of EN. Calibration equations were developed in order to predict human sensory characteristics by the EN sensor signals. Accuracy and precision of the quantitative models were tested with cross-validation. Results were evaluated by the determination coefficient (R^2), standard error of calibration (SEC) and standard error of cross-validation (SECV).

RESULTS AND DISCUSSION

Electronic nose

At the first attempt, the classification of the sausage classes was done within each sampling day. *Figure 1* shows the result of PCA in T1 sampling. It can be seen that the three different products form individual groups (N, D, H) based on the principal components generated from the response of the EN sensor array system. The isolation of the three groups is much better in DFA where the chemometric process takes into account not only the EN sensor signals but also the grouping protocol determined by the user. The ratio of successfully classified samples during DFA cross-validation was 98%, 93% and 95% in T1, T2 and T3 sampling.

Figure 1.

Classification of the sample groups by means of PCA (a) and DFA (b) method, based on the EN dataset of T1 sampling
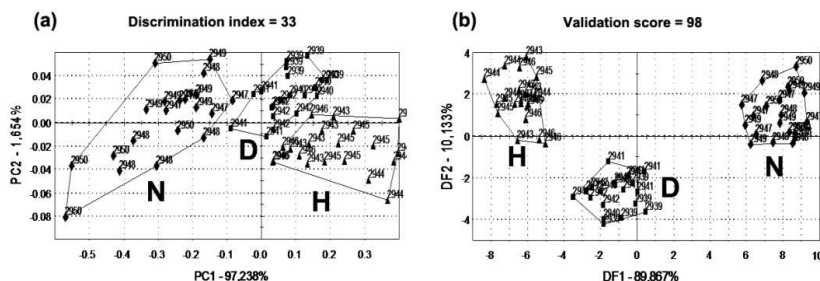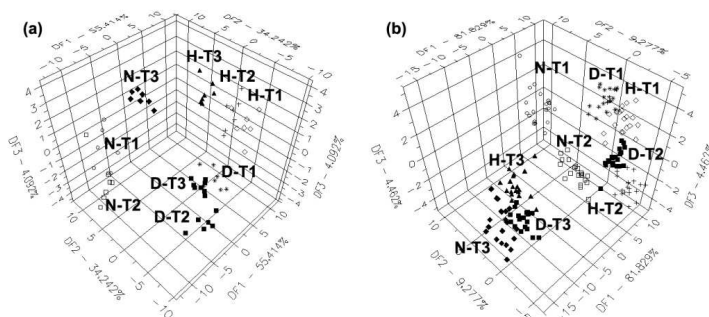

Figure 2 shows the reason of application of T4 sampling. In *Figure 2a,* one can see the groups of the different spicing protocols, and within these groups the ripening sub-groups can also be seen.

Figure 2.

3D visualization of DFA results based on a sensor signal dataset obtained (a) in T4 sampling, or (b) in the original ripening time (T1, T2, T3)


This result was gained from the dataset of T4 sampling, when all samples of the three weeks were sniffed at the same time. *Figure 2b,* however, was calculated when a merged dataset was used, when EN sensor signals of the three weeks were collected into one file and handled together. It is easy to detect that the main grouping variable here is the day of sampling and the differently spiced sausages can be detected within the sampling days. This shows that the EN system had noticeable level of drift, that this had a dominant impact on the classification procedure – this is what we definitely do not want to see during investigation of the sausage samples. The total explained variance covered by the first three discriminant factors was 89.6% or 95.4% in case of the first or the second calculation method.

Results of the PCA and DFA classification models based on the dataset obtained in T4 sampling can be seen in Figure 3. Changes induced by ripening can be observed

easily when analyzing the PCA graph. Groups of the different weeks align alongside the first PC, which describes more than 95% of the total variance.

Figure 3.

Classification of the sample groups by means of PCA (a) and DFA (b) method, based on the EN dataset of T4 sampling, when both sausage types and ripening terms are taken into account
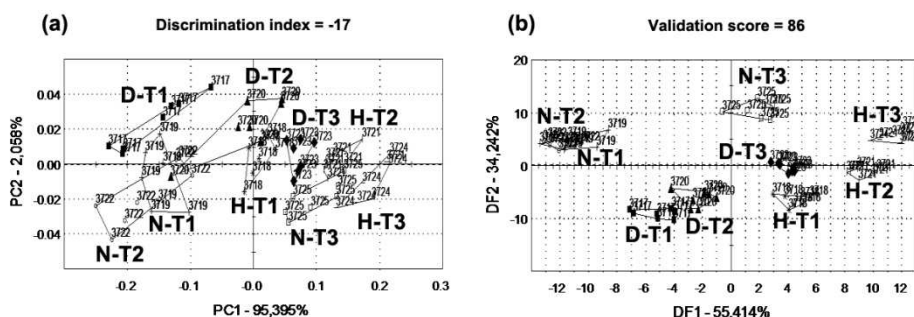

This means that the duration of ripening had the biggest impact on the smell parameters of the products. DFA plot indicates the same, since DF1 has the 55.4% of explained variance and groups also orientate alongside the first factor. It is also visible in *Figure 3b* that samples of T1 and T2 samplings are much more similar to each other than to the samples of T3. This might be caused by the longer vacuum-bag storage of the prior samples, and only few days extra storage of T3 samples. The biggest difference between samples of T1&2 and T3 can be seen in case of the N sausages, which had the lowest amount of added spices, namely red-pepper. It can be expected that human sensory panel will find the highest differences between the ripening terms in case of N samples.

The same PCA diagram is shown in *Figure 4a* as presented in *Figure 3a*, the only difference is that the duration of ripening is irrelevant in this analysis.

Figure 4.

Classification of the sample groups by means of PCA (a) and DFA (b) method, based on the EN dataset of T4 sampling, when only sausage types are taken into account
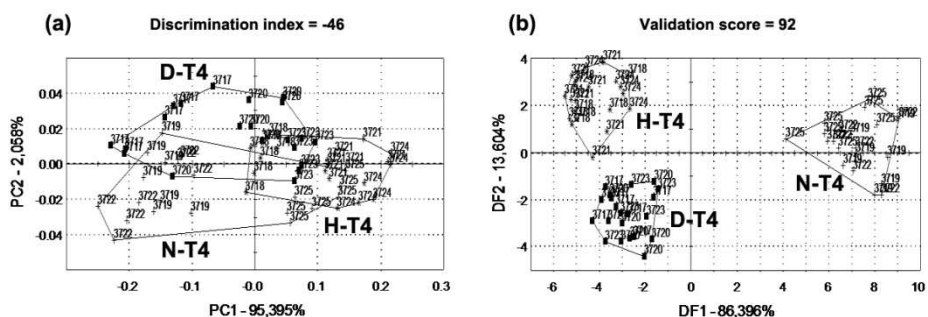
All N samples originated from the three sampling days were ordered into one single group, and this was applied for the D and H groups, as well. PCA groups are still overlapping, just as they were in the former analysis, but DFA shows a completely different picture (*Figure 4b*). Samples of each type of sausages are within the compact groups that are isolated nicely in the 2D plane described by DF1 and DF2. These first two discriminant factors cover the total variance of the dataset.

There is no further classification detectable within the groups of types, so the classification system was orientated only on type and not on the ripening. The high amount of successfully classified samples during cross-validation (92% - 58 hits, 5 misclassification) shows the accuracy of the model.

Human sensory panel

It was realized at the beginning of data evaluation that game meat flavor and taste cannot be taken into account in further analyses since the human panel was not trained for these sensory attributes, thus ratings were indefinite. The first step of the sensory data evaluation was the detection of inconsistent panelists. After exclusion of one panelist recommended by consensus test of PanelCheck software, the following results were achieved.

At T1 sampling, assessor effect was found to be non-significant regarding all investigated attributes, since product effect was significant in case of taste intensity, hotness, texture preference, color preference and redness. At T2 sampling, also taste preference and overall impression was significantly influenced by the type of the product.

The most significant differences between groups were found in T3 sampling, one week after the standard ripening period. Besides masticability, all other attributes were found to be significantly affected by the product type, while assessor effect remained non-significant (*Figure 5*). This proves that sensory scores were given based on the properties of the products and not on to the temporary impressions of the panelists.

Figure 5.

Assessor effect and product effect on sensory attributes during T3 sampling
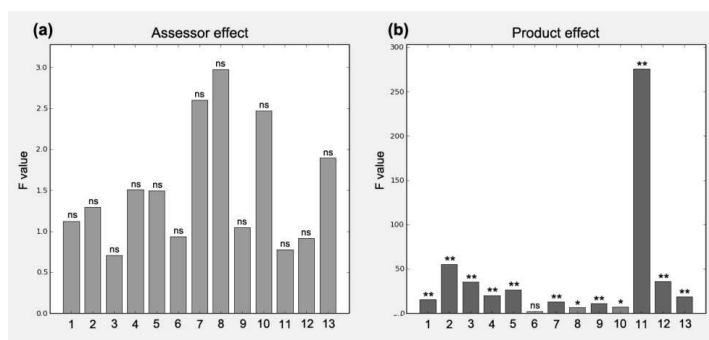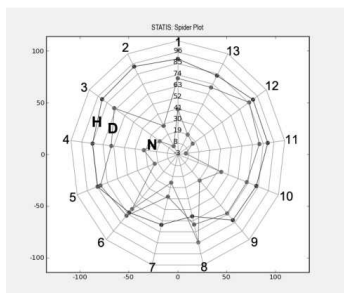

Taste intensity (1), hotness (2), taste preference (3), flavor intensity (4), flavor preference (5), masticability (6), hardness (7), fatness (8), particle size (9), texture preference (10), redness (11), color preference (12), overall impression (13)

There were clear differences detected between the three sausage groups regarding sensory parameters. *Figure 6* shows the profile diagram of the venison sausages in T2 sampling time. Typical Hungarian sausages contain pepper and – as it was shown in this trial as well – consumers desire the color and taste of this spice. Results for all quality parameters of group N were worse than those of the other two groups. Even fatness was rated with higher value, however the total fat contact of the groups did not differ. The same tendency was found in all sampling dates, but differences among groups were more expressed when ripening was longer.

Figure 6.

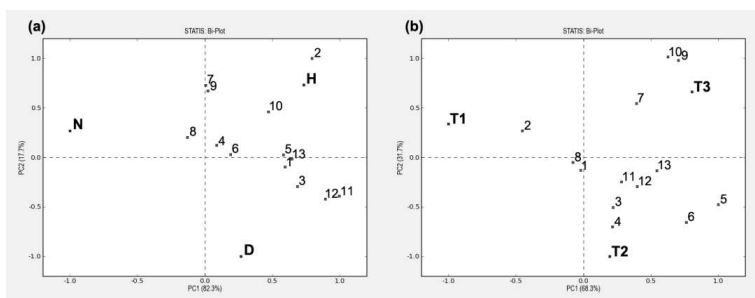**Profile diagram of the three venison sausage types at T2 sampling**

Taste intensity (1), hotness (2), taste preference (3), flavor intensity (4), flavor preference (5), masticability (6), hardness (7), fatness (8), particle size (9), texture preference (10), redness (11), color preference (12), overall impression (13)

Figure 7a shows the result of the bi-plot analysis when the three sausages are represented in the quality plane defined by the investigated sensory properties. Results related to different sampling times have only slight differences that show the consistent rating attitude of the panelists.

Figure 7.

Results of bi-plot analyses of (a) the three sausage types at T2 sampling, and (b) the three samplings of group D

Taste intensity (1), hotness (2), taste preference (3), flavor intensity (4), flavor preference (5), masticability (6), hardness (7), fatness (8), particle size (9), texture preference (10), redness (11), color preference (12), overall impression (13)

Group N is located the furthest from the positive properties, while it is close to hardness and fatness, properties not preferred by consumers. According to these results, the best product is group H since it is the closest to the most of the preferable characteristics. *Figure 7b* shows also a result achieved with bi-plot analysis, where the three samplings of group D are represented in the quality plane of sensory properties. In case of this sausage type the optimal ripening time seems to be T2, the standard ripening interval. Results obtained for group N showed the accentuated tendency that panelists deem samples with longer ripening much fattier than those having shorter ripening. Preference of group H improved through the whole investigation since T3 samples were the closest to the attributes describing consumer satisfaction.

Combined evaluation

Human sensory panel scores were merged with EN dataset and complex evaluation was done in order to describe the regression between human and electronic responses regarding the same sausage samples. Calibration was developed for only those attributes which can be related to odor properties of samples. Thus, odor intensity, odor preference, overall impression and fatness were evaluated – the latter attribute was involved into the analysis as most of the odorant molecules are bound to fat, so fat content has close relationship to odor properties. *Table 1* contains the results of calibrations and cross-validations for the investigated attributes in case of each sampling days.

Table 1.

Results of EN based PLS regression on human sensory attributes of the sausage samples investigated in different ripening stages

	Mean	T1 (n=60)			T2 (n=60)			T3 (n=60)			T4 (n=63)		
		R^2	SEC	SECV	R^2	SEC	SECV	R^2	SEC	SECV	R^2	SEC	SECV
Odor intensity	57±14	0.78	1.56	1.63	0.86	1.01	1.03	0.91	5.12	5.22	0.64	7.80	8.16
Odor preference	61±19	0.97	2.03	3.19	0.97	1.09	1.54	0.91	5.00	7.39	0.78	5.76	7.86
Overall impression	59±19	0.96	1.64	3.26	0.88	2.86	4.77	0.91	3.98	7.21	0.82	4.29	7.86
Fatness	70±8	0.97	0.29	0.71	0.84	1.12	2.46	0.90	1.39	3.07	0.89	1.06	2.51

R^2: determination coefficient; SEC: standard error of calibration; SECV: standard error of cross-validation

In order to rate the level of SEC and SECV, also the mean of each sensory attribute is involved in *Table 1*. *Figure 8* visualizes the relationship between the predicted and reference values of odor preference concerning the three sausages of T1 sampling.

Figure 8.

**EN based PLS calibration on odor preference of sausages investigated
during T1 sampling**
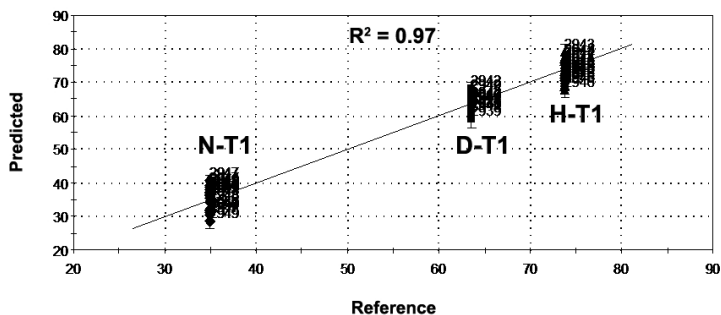
It is notable that odor preference and overall impression, as the most relevant attributes of consumer responses can be predicted with a high accuracy and precision by means of EN technology. Calibration on sensory attributes was less accurate in case of T4 sampling. This can be explained by the fact, that T1 and T2 samples were stored in vacuum bags for two and one weeks before T4 measurement that was executed one day after T3 sampling, thus, the odor properties of the mentioned sausages might have been changed a bit. However, human reference values of these T4 EN measurements were obtained also from the original panel tests of the T1, T2 and T3 samples. This means that samples sniffed by human panelists and samples sniffed by EN were not identical in case of T4 sampling – this might have caused the slight decrease of accuracy and precision. In case of T1, T2 and T3 sampling EN and human sensory tests were done simultaneously.

CONCLUSION

Ripening of venison sausage is an important part of production. Electronic nose technique as used in this trial is a useful tool to identify the different stages of ripening of the investigated sausage types. Also the type of sausage can be detected with the applied method during a single sampling. Results of panel tests show that consumers do not prefer samples without pepper, typical spice of Hungarian sausages. Ripening time seems to optimal if the standard or one week prolonged period was applied. Electronic nose is applicable not only for qualitative, but also a quantitative method to predict consumer preference.

Expressed differences exist within the sensory preferences of consumers from different countries. From this point of view an electronic sensor based method could be a reference for the reliable characterization of food sensory properties.

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