Controlling Maillard Reactions in the Heating Process of Blockmilk Using an Electronic Nose

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An electronic nose has been used to classify blockmilk products subjected to various heating processes based on their volatile composition. Multivariate analyses of electronic nose and GC/MS data are highly comparable with respect to relative changes in aroma profile going from raw to final product. Predictive properties of various neural networks based on the raw sensor output were moderate to good.

Keywords: *Electronic nose; blockmilk; Maillard reactions; sensory analysis; neural network analysis*

INTRODUCTION

The control of aroma development in industrial food processing is extremely important. However, processes such as heating, drying, fermenting, blending, etc. are usually controlled by measuring descriptive aroma derivatives. These are usually single parameter measurements such as color, pH, or concentrations of certain chemicals or biomolecules that can be measured chromatographically or spectrophotometrically. When it comes to the assessment of flavor quality going from raw material to final product, sensory panels and GC−MS are currently used. With the maturation of gas sensor technology, a new alternative method becomes available to assess flavor quality. This enables process control based on the full spectrum of an aroma. Since a series of gas sensors effectively mimics mammalian olfactory sensing, the term 'electronic nose' is used for such devices (Pearce, 1997). Although many potential applications especially in food processing (Pearce et al., 1993; Tomlinson et al., 1995; Börjesson et al., 1996; Eklöv et al., 1998; Maul et al., 1998; Arnold and Senter, 1998) and other life sciences (Jonsson et al., 1997; Gibson et al., 1997; Ping et al., 1997) have been reported, the only current commercial application of electronic aroma sensing, to our knowledge, is in the detection of off-flavors in cooked pork meat, the so-called boar taint (AnnorFrempong et al., 1997a,b).

Blockmilk is an important half product in the food industry. It is obtained by heating and drying/concentrating mixtures of milk and sugar to ca. 98% dry matter and is mainly used in the production of chocolate. Many important flavor components are formed during the heating steps via Maillard reactions (Hodge, 1967; Scarpelino and Soukop, 1993). Effective process control systems must therefore eliminate under- and overprocessing, since this results in undesirably flavored blockmilk. This paper describes the use of an electronic nose to distinguish between intermediate products from a crucial heating step in the blockmilk process. These results are set against the results of other analytical data such as GC−MS and sensory analysis. The full GC−MS data of blockmilk flavor will be published elsewhere (Muresan, 1999).

Current commercial electronic noses consist of an array of sensors, the sensing mechanism based on various sensor techniques. In the present case, the sensors are coated with thin layers of conducting polymers. Upon exposure of the sensor head to organic vapors, the electrical resistance of the polymers changes. Since all sensors have been prepared under slightly varying conditions, they all give different time-dependent response patterns toward volatile compounds. Therefore, each aroma or mixture of volatiles in general gives a unique sensor response pattern or "fingerprint". Various techniques can be used to extract useful information from the response patterns, among which statistical multivariate methods and artificial neural networks are the most important. Since these procedures can be automated easily, the electronic nose offers an aroma analysis with high reproducibility, speed, and objectiveness.

MATERIALS AND METHODS

Blockmilk Samples. Eight blockmilk samples at various stages of processing were obtained from Coberco Isoco, Zwolle, The Netherlands, and stored at −50 °C. Part of the production process is drawn schematically in Figure 1. K represents the starting material (pasteurized milk and sugar); L and M samples are taken after two consecutive concentrating steps; N−Q samples are the intermediate products of an increasingly severe heating step; and X is the final product, which is obtained by drying of Q.

Electronic Nose. A commercially available electronic nose with 12 conducting polymer-coated sensors (Neotronics eNOSE 4048, U.K.) was used, without controlling the relative humidity (Visser and Taylor, 1998). It was equipped with an autosampler (Tekmar Precept II, The Netherlands), customized for dynamic headspace transfer to the electronic nose. To avoid cross-contamination due to the high persistence of blockmilk aroma, the entire tubing system was flushed with dry nitrogen for 10 min between dynamic sampling. Blockmilk samples for the electronic nose were prepared by thoroughly mixing 60.0 g of blockmilk with an equal amount of MilliQ water (R > 18 MΩ) immediately before performing the experiment. The

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10.1021/jf981327a CCC: $18.00
Published on Web 10/14/1999

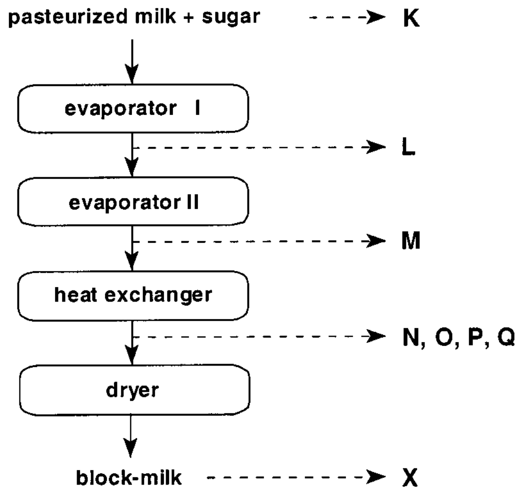

Figure 1. Schematic representation of the blockmilk manufacturing process and the corresponding sampling (dashed arrows).

aroma profiles of various intermediate blockmilk products were analyzed by following the sensor response of all 12 conducting polymeric sensors for 4 min. The maximum sensor response was usually obtained in 2 min for most sensors. The response values after 3 min were used in statistical processing.

GC−MS Analyses. Dynamic headspace isolation of volatile compounds was performed in triplet according to Luning (1994) using Tenax TA as the adsorbent. To 10 g of the sample, 10 g of MilliQ water ($R > 18$ MΩ) was added and stirred during 2 h at 35 °C, while the sample was flushed with 30 mL/min purified nitrogen. For each triplet experiment, a system control sample was made by stirring 10 g of MilliQ water under the same headspace conditions. Volatiles were desorbed from Tenax tubes (Tekmar 6016 desorber/autosampler, Interscience, The Netherlands) and via internal trapping at −100 °C injected in a capillary column (CP-52-CB, 50 m, 0.32 mm i.d., $d_f = 1.2$ μm). The gas chromatograph (Fisons 8533, Interscience) was equipped with an FID. The GC−MS (Carlo Erba, Mega 3600, QMD 1000, Interscience) was equipped with a thermal desorption unit (Tekmar 5010, Interscience). For both devices, thermal desorption conditions (5 min at 250 °C) and GC column conditions (10 min isotherm at 40 °C, followed by 3 °C/min to 190 °C, 10 °C/min to 250 °C, and finally 5 min isotherm at 250 °C) were identical. Electron impact mass spectral analysis was carried out at 70 eV.

Sensory Analysis. The sensory analysis was carried out using a trained panel of 23 members using Quantitative Descriptive Analysis. The samples were scored for nine flavor attributes and five taste attributes.

Data Analysis. The sensor response curves were recorded and processed using Neotronics software. The neural network analyses were performed using a two-layer back-propagation network with the sensor responses at saturation as input (Bishop, 1995) using Neotronics software. Subsequent principal component analyses (PCA) and multivariate techniques such as multiple discriminant analysis (MDA) were performed using Unistat 4.0 (Unistat Ltd, U.K.) and The Unscrambler 6.1 (Camo AS, Norway). The GC analysis input data are constituted by the differences (dissimilarities) of intensities of volatile constituents (i.e., aroma profile) among samples. In this respect a weighted Euclidean distance as a dissimilarity index, S, calibrated in the [0,1] range, was used (Gordon, 1981). For two chromatograms A and B, $S(\mathrm{AB}) = 0$ indicates a perfect match, while $S(\mathrm{AB}) = 1$ indicates no similarity. The distance matrix based on the dissimilarity coefficients was analyzed by multidimensional scaling (MDS, SPSS), since this statistical method was specifically conceived to handle dissimilarities (Schiffman and Beekert, 1986). MDS analyzes the dissimilarity data in a way that displays the structure of the distance-like data as a geometrical picture.

Table 1. Process Parameters of Blockmilk Samples

sample	treatment	dm (wt %)[a]
K	pasteurized milk + added sugar	20.5
L	high temp pasteurization + first-stage evaporation	57.9
M	second-stage evaporation	62.8
N	mild caramelization	63.8
O	medium caramelization	64.3
P	intense caramelization	62.4
Q	severe caramelization	61.9
X	drying the severely caramelized product	97.8

[a] Percentage dry matter content.

Table 2. Distance Matrix between Analyzed Samples Using a Weighted Euclidean Dissimilarity Index Based on GC−MS Data

sample	K	L	M	N	O	P	Q	X
K	0.00	**0.38**	0.38	0.48	0.65	0.70	0.81	0.78
L		0.00	**0.32**	0.46	0.62	0.68	0.84	0.72
M			0.00	**0.42**	**0.63**	**0.69**	**0.82**	0.73
N				0.00	0.48	0.58	0.77	0.72
O					0.00	0.32	0.69	0.65
P						0.00	0.63	0.61
Q							0.00	**0.72**
X								0.00

RESULTS AND DISCUSSION

Electronic Nose. The blockmilk samples are derived from various drying and concentrating steps. Consequently, the dry matter content increases during processing (Table 1). Since the concentration of the flavor components in the headspace is related to the dry matter content, the dependence of the sensor response to dry matter content or rather the amount of water was established.

When samples of K were diluted with a factor 1, 2.5, and 5, keeping the headspace volume constant, the sensor response curves were found to be identical for all three samples. Thus, it is assumed that, even with variations in dry matter content in the prepared samples (10.3% for K to 48.9% for X after dilution), headspace contents as measured by the electronic nose are in all cases related to aroma compounds and not simply related to the amount of water.

Multivariate Analyses. The classification of samples K−X by the electronic nose has been evaluated by correlating the MDA plots to quantitative aroma profiles obtained from dynamic headspace GC.

The distances or proximities matrix between the analyzed samples is presented in Table 2. The bold values correspond to the process presented in Figure 1. An inspection of the S indices reveal a close similarity between K, L, and M samples [$S(\mathrm{KL}) = 0.38$, $S(\mathrm{LM}) = 0.32$]. The N−Q samples collected after the heating step are different as compared to the initial samples (K−M) and are also different from one another. During this step, considerable changes in the aroma profile occurred as visually evidenced by dramatic color and viscosity changes as well. The final product X obtained from the sample Q seems to be different from the other samples ($S > 0.7$).

When the table of dissimilarity values was subjected to MDS (Figure 2), a plot similar to the MDA plot (Figure 3) of all electronic nose data is obtained. The graphical similarity between the MDA plot of electronic nose data (Figure 3) and the multidimensional scaling plot of the distance matrix between the samples based on quantitative GC data (Figure 2) indicates that the

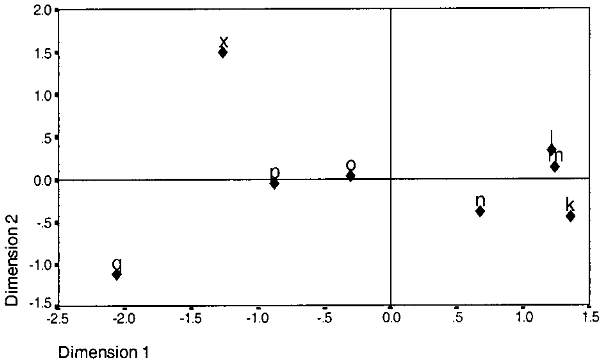

Figure 2. First two dimensions of multidimensional scaling of GC dissimilarity distances between samples from blockmilk manufacturing process.

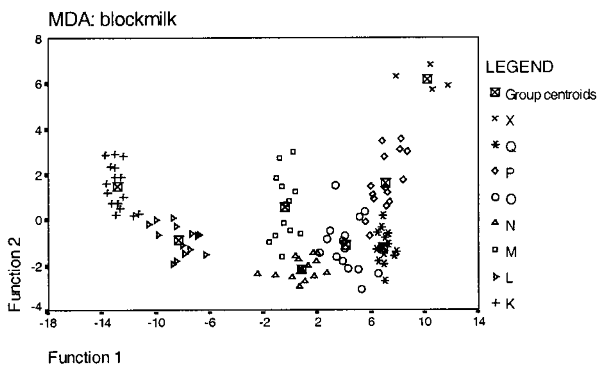

Figure 3. MDA plot of all blockmilk samples K−X.

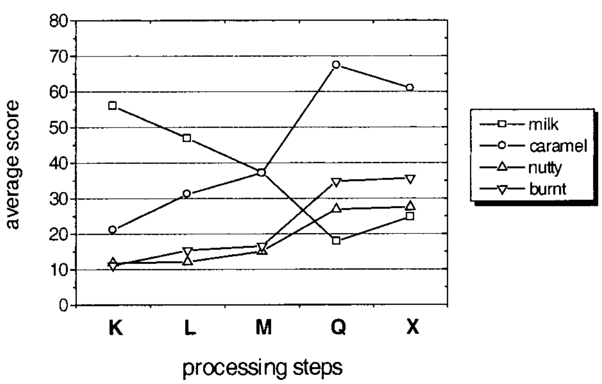

Figure 4. Sensory analysis of blockmilk products.

electronic nose is largely able to determine differences in aroma profiles in a qualitative manner.

This is further evidenced by sensorial data. No major changes in aroma profile were observed for the initial processing steps. Perceived milk flavor decreased while the scores for the other descriptors remained more or less constant. Many volatiles are generated during the heating step due to the Maillard reactions. This is consistent with the increase seen in perceived caramel, nutty, and burnt flavor. During the final step (the drying), volatiles decreased in their intensity, but the sensory aroma profile is not essentially changed (Figure 4). When the sensor response data for all blockmilk samples and the sensorial scores are subjected to PCA analysis, the model shows that the sensors in fact respond to volatiles associated with the descriptors caramel, nutty, burnt, and chocolate (Figure 5). These volatiles are typically Maillard reaction products that are formed during the caramelization step.

Table 3. Predictive Scores and Confidence Levels of ANN-1

sample	predictive score (%)[a]	confidence level (%)[b]
K	62.5	96.6
L	100	98.9
M	85.7	95.1

[a] Predictive score is defined as the percentage of correct classifications of the testing samples, with confidence >90%. [b] Confidence level is defined as the average confidence of all correctly classified samples.

Neural Network Prediction. Multiple discriminant analysis is a supervised statistical processing tool and requires input of additional data, which allows the statistical algorithm to classify samples in their correct class. However, for predictive purposes, this is highly unsuited. To allow recognition (i.e., correct classification) of unknown samples relative to previously measured reference data, artificial neural networks (ANNs) can be created. In a self-learning routine, a computer program becomes able to recognize unclassified samples as belonging to a certain class based on the raw data of the sensor output in a supervised manner. Sixteen independent response curves for each blockmilk product K−Q were used to train two neural networks. The first neural network (ANN-1) used 8 out of the 16 measurements as training data, with the remaining 8 measurements as test data for the first three blockmilk products (K−M). The second (ANN-2) was trained using only N−Q data since these are the actual products of the heating process in which the electronic nose could be applied, also with 8 out of the 16 measurements used as training data.

Neural network ANN-1, trained using only K−M samples, is perhaps not very interesting from a processing point of view but nonetheless gives good predictive scores and confidence levels (Table 3). This is especially pleasing since the aroma profiles of K−M are very similar according to GC−MS and sensory analysis (see above) and are not separated very well using multivari-

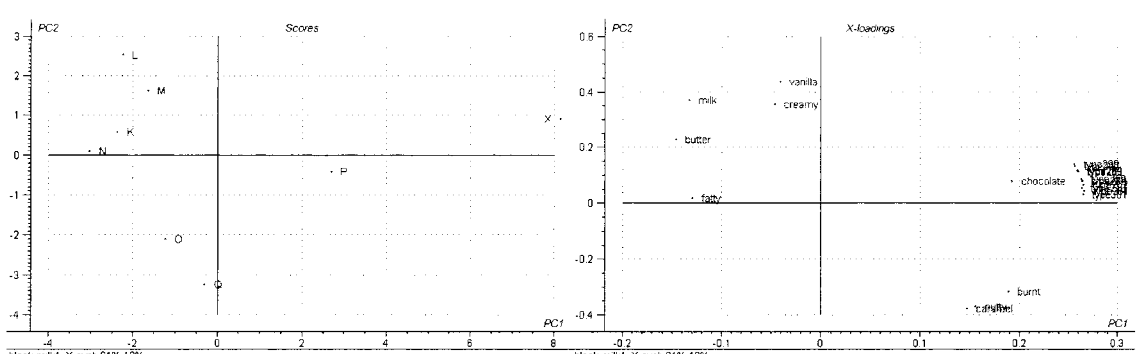

Figure 5. PCA model of blockmilk samples based on sensorial scores and sensor responses.

Table 4. Predictive Scores and Confidence Levels of ANN-2

sample	predictive score (%)[a]	confidence level (%)
N	75.0	99.8
O	42.9	93.4
P	100	99.6
Q	85.7	87.0

[a] See Table 3 for definition of terms.

ate analyses. Hence, this neural network is well able to discriminate between these half products with almost identical aroma profiles.

Various neural networks were trained specifically for the heating step and contained only N–Q data. The results vary slightly depending on which 8 of the 16 measurements are used for training purposes. This heterogenity presumably arises from small differences in sample preparation, headspace development, and sensor drift. The results from a typical neural network (ANN-2) are collected in Table 4. Neural network ANN-2 gave overall a poorer result as compared to ANN-1 both for predictive scores and confidence levels, even though the cluster separation of the N–Q data in MDA is similar to that of the K–M data (Figure 3). Especially the predictive scores for sample O, of which some of the test data could not be classified using ANN-2, is rather low with 42.9%.

CONCLUSIONS

A commercially available electronic nose works quite well in determining the relative differences between blockmilk samples from various process steps. This procedure is much faster and cheaper than tedious GC–MS determinations, and although less quantitative, the electronic nose results correlate well with both GC–MS data and sensorial scores. Therefore, using an electronic nose for aroma analysis in food or the food processing industry is very promising. Prediction or classification of “unknown” samples using artificial neural networks was found to give encouraging results.

ACKNOWLEDGMENT

The interest and support of Coberco Isoco, Zwolle, The Netherlands, are gratefully acknowledged.

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Received for review December 4, 1998. Revised manuscript received July 7, 1999. Accepted July 30, 1999.

JF981327A