ELECTRONIC NOSE AND SPME-GAS CHROMATOGRAPHY FOR THE STUDY OF THE ODOR PROFILE OF WHOLE MILK POWDER

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Abstract

The present study describes the strength of the electronic nose and headspace solid-phase microextraction-gas chromatography (HS-SPME-GC) in food odor analysis. These methodologies were successfully applied to odor characterization of whole milk powder manufactured in a factory of the Central Dairy Area of Argentina.

An electronic nose comprised of a set of 28 conducting polymeric sensors was used. Different assays were conducted in order to define the protocol of analysis and examine the discriminative capability of the E-nose. For that purpose, a series of samples were evaluated while selected E-nose parameters undergone incremental changes in a pre-assigned routine, including sample equilibrium time, loop fill time, loop equilibrium time and inject time.

HS-SPME was used to isolate volatiles compounds such as dimethyl sulfide, pentanal, hexanal, and butyric acid, compounds that contribute significantly to describe the odor and flavor acceptance of whole milk powder. Isolated compounds were characterized and quantified by GC with FID detection. Extraction curves, limits of detection, repeatability, and linearity were investigated. Limits of detection ranged between 0.6-400$\mu g K g^{-1}$.

Applying the Spearman correlation method, differences in the lineal relation between sensors and volatile compounds were observed according to the season of powder production.

This study shows that the electronic nose technology can be applied to milk powder odor characterization, complementing analytical techniques like HS-SPME-GC used in food odor research.

Resumen

El presente estudio combina una nariz electrónica y microextracción en fase sólida por espacio de cabeza-cromatografía gaseosa para el análisis del aroma de leche entera en polvo elaborada en la región central de la Cuenca Lechera Argentina.

Diferentes ensayos fueron realizados para definir el protocolo de análisis y la capacidad de discriminación de la nariz electrónica, la cual posee 28 sensores polímeros conductores. Con este fin, distintas muestras fueron analizadas mientras se producían cambios incrementales en parámetros operacionales de la nariz electrónica. Estos parámetros incluyen tiempo de equilibrio de la muestra y tiempo de llenado, equilibrio e inyección del loop. Microextracción en fase sólida del espacio de cabeza se usó para aislar los compuestos volátiles dimetil sulfide, pentanal, hexanal y ácido butírico, los cuales contribuyen al aroma y el sabor de la leche en polvo. Estos compuestos se caracterizaron y cuantificaron por cromatografía gaseosa con detector de ionización de llama. Se determinaron curvas de extracción, repetitibilidad y linealidad. Los límites de detección se encontraron en el rango de 0.6-400μgKg^{-1}.

Aplicando el método de correlación de Spearman se observaron diferencias en la relación lineal entre sensores y compuestos volátiles de acuerdo a la estación climática de elaboración de la leche en polvo.

Este estudio muestra que la nariz electrónica puede ser aplicada para caracterizar el aroma de leche en polvo, en combinación con técnicas analíticas tales como microextracción en fase sólida por espacio de cabeza-cromatografía gaseosa.

Introduction

In the analysis of food odor, Electronic nose (E-nose) was proved to be a powerful method to analyze agricultural product, grains, sheep meat, ground and processed beef, etc. However, the number of studies dedicated to dairy products is still limited, probably due to the complexity of their matrixes [1,2]. The E-nose system consists of a multi-sensor array able to monitor the interaction with different groups of molecules. The signal response of each sensor comprises a pattern ("fingerprint") that identifies the odor profile of the sample. E-nose recognizes odor patterns but not specific compounds, in much the same way as the mammalian olfactory system. These patterns are compiled into a data base for further analysis using statistical routines like pattern recognition methods, cluster analysis and artificial neural networks. E-nose offers some advantages such as simple operation procedures and rapid analysis suitable for on-line monitoring. However, it has limitations, regarding the chemical information of the food system, to completely analyze odor.

Analytical methods as gas chromatography (GC), and their combination with mass spectrometry (MS), have been developed for food analysis. Solid phase microextraction (SPME) [3,4] is a sample preparation technique that uses a fused-silica fiber coated with an appropriate stationary phase. Analyte in the sample is directly extracted and concentrated to the fiber coating. This method saves preparation time, solvent purchase and disposal costs, and improve detection limits [4]. It has been successfully used in combination with GC and GS-MS for the extraction of volatile and semi-volatile organic compounds from environmental, biological and food samples [4,5].

The aim of the present work was to develop an electronic nose method and a SPME–GC procedure for qualitative and quantitative determination of volatile compounds present in manufactured whole milk powder.

Experimental procedure

Milk Samples. Commercially processed spray-dried whole milk was obtained from a factory of the Central Dairy Area of Argentina. Samples were collected over a period of one year corresponded to 3 batches in Autumn, southern hemisphere, (March, April and

May), 6 in Winter (June, July and August), 5 in Spring (September, October and November) and 5 in Summer (December, January and February). High-heat powders (WPNI 0.72mg/g) were manufactured from standardized milk, with a fat to solids-not-fat ratio of 0.375, the heat treatment used being of 90-93°C during 3 minutes. Samples of 800g polyethylene packages in cardboard boxes were stored at –20 ±1ºC immediately after collection and kept until analysed.

Electronic nose and GC devices. An AromaScan A32S (OSMETECH PLC, Electra House, Electra Way, Crewe, UK) electronic nose with a detector array of 28 conducting polymeric sensors and Shimadzu series 14B gas-liquid chromatograph, equipped with a flame ionization detector were used.

Materials. Dimethyl sulfide, pentanal, hexanal and butyric acid (Fluka), SPME fiber (Supelco, Bellefonte, PA, USA) were purchased from SIGMA-Aldrich of Argentina S.A.

Statistical analysis. The relationships between volatile compounds and sensors' signals were analyzed using the Spearman correlations coefficients (SPSS® Advanced Statistics 12.0, SPSS Inc, Chicago, IL, USA).

Results and discussion

Electronic Nose Protocol

Different operational parameters influence the performance of E-nose systems such as flow profile and nature of the carrier gas and reactions kinetics of volatiles compounds among others. Then, in order to define the protocol of analysis and examine the discriminative capability of the E-nose, a series of samples were evaluated while selected E-nose parameters undergone incremental changes in a pre-assigned routine. This procedure allows to vary one parameter at a time, including sample equilibrium time, loop fill time, loop equilibrium time and inject time.

In headspace analysis, the concentration of the analyte in the gas phase must be maximized to reach an adequate sensitivity. The sensitivity depends on the nature of the samples, including both the analyte and the matrix, and the temperature. Different sample preparation conditions were tested: powder, milk powder reconstituted to 13% total solids by distilled water at 40°C and reconstituted milk powder with salt addition. To assess the discriminative ability of the E-nose, high-heat commercial powders produced under different heat thermal processes and samples with undesirable odor characteristics were evaluated. Light-oxidized samples were prepared according to Claassen et al. [6].

During data acquisition, different sampling time periods can be selected. Data can be analyzed whether an equilibrium of the sensor responses is reached or not. In order to select the sampling period to collect data, two lapses of time at 30-40 s (steep stage of the curve) and 50-70 s (plateau part) were considered.

As a result, the E-nose methodology was optimized under the following conditions. An aliquot of 3g of whole milk powder was placed in a 22ml vial and sealed. The acquisition method was set with an initial reference period of 27s (time T), a sample period of 60s, a wash time of 10s with 2% butanol-water solution and a reference interval of 120s. Nitrogen (oxygen free) was used as a carrier gas with a reference humidity of 50% (relative humidity

at 30°C). The employed multisampler method was the Stopped Flow technique with a loop-fill time of 25 s and an equilibration time of 20 s. Prior to data acquisition, samples were held at 45 ±1°C for 10 min to stabilize headspace conditions. This temperature was selected in order to reproduce sample conditions during SPME-GC analysis. For data analysis, a period from 50 s to 70 s was used. The experimental conditions developed ensured that a correct baseline was established allowing the recovery up of sensors between samples. Analysis were carried out in triplicate.

HS (Head Space)-SPME-GC-FID analysis

Sample preparation. Whole milk powder was reconstituted to 13% total solids by distilled water at 40°C and it was stirred during 4min. Then, 10 mL of the reconstituted milk with 10μL of internal standard solution (50mgKg^{-1} 4-methyl-2-petanone) and a microstirring bar were placed in a 27mL glass GC vial and capped.

HS-SPME development

Fiber Choice Fiber coated with 75μm carboxen-polydimethylsiloxane (CAR-PDMS) was chosen for the analysis in accordance with Marsili et al., 1999 [6].

Fiber conditions. Solutions of standards were prepared containing 5000 mgKg^{-1} of each compound in whole reconstituted milk powder. Several concentration solutions were obtained by further dilutions with reconstituted whole milk powder. The CAR-PDMS fiber was held in the headspace of a standard mixtures at 45±1°C under magnetic stirring for 15, 30 and 45 minutes. After exposed, the fiber was immediately desorbed in the gas chromatograph injector for 5min at 280°C. Each measurement was repeated three times. The results showed that chromatogram peak areas of the studied compounds increased as a function of the extraction period, indicating that the equilibrium had not been reached within 45min. Even though SPME has a maximum sensitivity at the equilibrium point, full equilibrium is not necessary for accurate and precise analysis. The reason is based on the linear relationship between the amount of the adsorbed analyte and its initial concentration in the sample [7]. Butyric acid had a low extraction efficiency. Adding soluble salts to the samples improves the extraction efficiency due to the salting-out effect [7]. Although these effect could improve butyric acid extraction, no salt was added to reproduce E-nose methodology. Taking into account the considerations of Kataoka et al. [7] and that chromatographic analysis had taken 48.38 min., an extraction time of 45min. was chosen. This ensured sufficiently low limits of detection without extending the total analysis time.

Limit of detection, repeatibility and calibration curves. Generally, sensitivity depends on the analyzed compound, i.e. nonpolar compounds are easily detected at ppb levels, while it is sometimes difficult to detect polar compounds at ppm levels [8]. The limit of detection (LOD) was estimated as the analyte concentration producing a signal five times larger than noise. LOD values were 2.4μgKg^{-1} for dimethyl sulfide, 0.7μgKg^{-1} for pentanal, 0.6μgKg^{-1} for hexanal and 400μgKg^{-1} for butyric acid. Repeatability (mean of five repetitions) was expressed as the relative standard deviation for peak area at the 50μgKg^{-1} standard concentration for pentanal, hexanal, heptanal, octanal and at 1000μgKg^{-1} for butyric acid. These values fell below 10% for pentanal and hexanal and above 15% for dimethyl sulfide and butyric acid.

The high sensitivity of SPME fibers leads to a linear range which is usually under 1ppm [8]. Calibration curves have been prepared in the range of 10 to 50µgKg^{-1} for dimethyl sulfide, pentanal, hexanal and in the range of 500 to 2500µgKg^{-1} for butyric acid. Linearity of these curves were characterized by r^2 values of 0.997 for dimethyl sulfide, 0.995 for pentanal, 0.997 for hexanal and 0.966 for butyric acid.

GC-FID analysis

Nitrogen was used as carrier gas at 1ml min^{-1}. Separation of the compounds was performed on a 30m x 0.25mm id x 0.25µm FFAP capillary column (J & W Scientific, Folson, CA). The following column temperature-programming sequence was used: an initial of 40°C was maintained for 2min before being increased to 180°C at 4°Cmin^{-1}, then raised at 40°Cmin^{-1} to 235°C and held for 10min. The FID temperature was set at a 280°C. The injector was operated in the splitless mode for 2min. Compounds were identified by comparing retention times with those of standard compounds. Quantitative determinations were carried out by the method of internal standards. Analysis of the samples were carried out in triplicate.

Milk powder odor profile

Milk odor profiles are caused by a complex mixture of volatile compounds that can be more or less manifested in different types of milk depending on the presence of pro-oxidants, antioxidants, storage conditions, etc. Since in this assay milk powder samples were evaluated without storage, the observed behavior of the volatile compounds could be associated to raw material characteristics [9].

The methodologies developed for E-nose and HS-SPME-GC were successfully applied in odor characterization of whole milk powder. Both, electronic nose and volatile compounds data, allowed the discrimination of the odor profiles according to the manufacture season [9].

Spearman correlation was applied to volatile compounds and E-nose data. Coefficient values are presented in brackets at a significant level of 0.05. Hexanal correlates significantly with S1 (0.6) and S2 (0.5) in Winter, with S10 (-0.6), S13 (-0.5), S14 (-0.6), S24 (-0.5) and S28 (-0.6) in Spring, and with S13 (0.7) in Summer. Pentanal correlates significantly with S9 (-0.7), S11 (0.8), S15 (0.7), S27 (-0.7) and S28 (-0.7) in Autumn and with S15 (-0.5) in Summer. Dimethyl sulfide correlates significantly with S2 (-0.7), S10 (0.8), S12 (0.7), S15 (-0.8), S16 (-0.8), S23 (-0.9), S27 (0.8) and S28 (0.8) in Autumn, with S15 (0.6) in Spring and with S9(-0.7), S11 (-0.5) and S12 (0.5) in Summer. Butiryc acid correlates with S11 (0.7) and S14 (0.7) in Autumn, with S5 (-0.5), S16 (0.), S19 (-0.5) in Winter, with S4 (0.7), S5 (0.5), S11 (-0.7), S14 (-0.7) and S19 (-0.7) in Summer.

Correlation results indicate differences in the lineal relation between volatile compounds and sensors as a function of the season of milk powder production. Either hexanal or pentanal are good indicators of oxidative processes of whole milk powder. This seasonal variation should be taken into account when an E-nose system is applied to milk odor characterization for early detection of oxidative processes compounds. A similar behavior was observed with dimethyl sulfide, a volatile compound related to microbiological deterioration.

Conclusion

This study shows that E-nose technology can be applied to characterize milk powder odor, complemented by analytical techniques like HS-SPME-GC. A seasonal effect in odor characteristics, due to raw materials used in powder production, should influence the E-nose performance. Both, the design of E-nose systems and the used of statistical procedures should be tuned to the complexity of these matrixes for food applications.

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