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# Machine Olfaction to Evaluate the Stability of the Odor Profile of Pancakes Enriched with Docosahexaenoic Acid and Anthocyanins

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## Abstract

Increasing consumer awareness on good health has drawn the attention to health promoting natural dietary substances. However, since the organoleptic profile of foods highly influences the consumers' preference, and it is often decisive in the purchase, it is important to objectively describe and evaluate the effect of the applied bioactive ingredients on aroma. In this study, pancakes enriched with docosahexaenoic acid and anthocyanins were tested with electronic sensor array technology against control products. Samples were analyzed with an Alpha MOS FOX4000 electronic nose (EN) after 20 to 297 days of frozen storage at  $-20^{\circ}\text{C}$ . Multivariate analysis of the acquired EN data showed a strong relation between the number of days that samples were stored and the odor describing sensor signals of enriched samples ( $R^2 = 0.59$ ), but the observed relation was broken in the case of control (not enriched) samples ( $R^2 = 0.08$ ). When a supervised classification of enriched and control samples was done, the ratio of correctly identified samples in cross-validation was 95.1% at short-term storage ( $< 140$  days), while the hit rate dropped to 80.4% at prolonged storage ( $> 140$  days). This signified the existing but less intensive odor differences. The electronic nose technology was proven to be applicable in the characterization of one type of bioactive-enriched foods, while it was also useful in the monitoring of odor alterations during storage.

**Keywords** Bioactive · Smell · Aroma · Preference · Electronic nose · Multivariate data

## Introduction

The projected incidence of chronic diseases and the potential of diet in reducing this risk have gained significant respect (Hooper and Cassidy 2006). The increasing consumer awareness towards foods drew the attention to identifying health promoting natural dietary substances (Siró et al. 2008) and to producing functional foods that benefit human health beyond the effect of nutrients alone (Ferguson 2009). Bioactives are natural components of plants and animal foods possessing a variety of physiological functions promoting human health and well-being, and contributing to reduced risk of diet-related diseases. Normally, they are present at very low concentrations in foods (Kris-Etherton et al. 2002), and to supply them in the diet through fortified foods offers a better way to meet the metabolic needs as compared to oral dietary enrichment (Maki et al. 2003).

However, bioactives may have off odor or poor oxidative stability that can generate further unpalatable volatiles (Hu et al. 2015). The organoleptic profile of foods highly influences the consumers' preference (Sørensen et al. 2003)

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and it is often decisive in the purchase (Plutowska and Wardencki 2007). Accordingly, the characterization of olfactory quality as a method for quality assurance is of great interest to the food industry (Mielle 1996). In traditional analytics, the identification and quantification of aroma components are mostly based on GC-MS methods following separation steps, although the relationship between the chromatographic data and the perception of the global aroma of a product is not easily described (Nagy et al. 2014). On the other hand, the same odor stimuli result divergent perceptions in the brain of different individuals — influenced by habits, previous experiences, preconceptions, preferences, health status, age, genetics, etc. (Hasin-Brumshtein et al. 2009; Keller et al. 2007; Peris and Escuder-Gilabert 2009). Thus, the results of human sensory panels often applied for characterization and qualification of a food product hold significant uncertainty.

Electronic noses (EN) mimic the sense of smell through detecting and distinguishing odors precisely in complex samples and at low cost, making the technology useful for diverse applications, involving food qualification (Peris and Escuder-Gilabert 2009). Since the first development of EN (Persaud and Dodd 1982), various versions of technology were released using different sensors and joint multivariate data analyses procedures (Mielle 1996; Röck et al. 2008).

The objective of this study as a subsidiary study of the European project PATHWAY-27 (<http://pathway27.eu>) set out to tackle the diverse scientific understanding of the role and mechanisms of bioactive compounds of food matrices in influencing human health, was the evaluation of the effect of docosahexaenoic acid (DHA) and anthocyanins (AC) enrichment on the odor profile of pancakes, and to highlight aroma variations caused by storage, using machine olfaction technology. Thus, testing the applicability of the electronic nose technology in the quality assessment of one type of bioactive-enriched foods was targeted.

## Materials and Methods

### Samples

Bioactive-enriched pancakes (BEP) were produced by the ADEXGO Kft. (Balatonfüred, Hungary) using OVO-DHA® (Applications Sante des Lipides, France) and Eminol® (ABRO BIOTEC SL, Spain) as sources of DHA and AC, respectively. Details on bioactive sources are reported in Karakaya et al. (2016). Control pancakes (CP) containing no DHA or AC were produced by the ADEXGO Kft. according to the same basic recipe (components: wheat flour, egg, salt, milk, water, and sunflower oil) and processing as used for BEP (Bub et al. 2019).

The amount of OVO-DHA® used for enrichment was set to obtain a 250 mg DHA concentration in each serving (100 g) of the ready-to-eat pancakes, which is close to the daily adequate intake (AI) dose for adults set by EFSA (EFSA 2010). The amount of Eminol® used for enrichment was set to obtain a 320 mg AC concentration in each serving (100 g), which is reported to have beneficial effects on LDL and HDL cholesterol (Yu et al. 2009). Energy and macronutrient content of pancake is reported in (Bub et al. 2019).

The ready-to-eat pancakes were stored frozen (−20 °C) in vacuum sealed trays (100 g per tray) until the preceding day of the electronic nose (EN) measurement, when samples were thawed in refrigerator (+4 °C). Before EN measurements, packaging was opened, and the pancake was chopped into fine pieces (ca. 2 mm). One gram of each chopped sample was loaded into glass vials and sealed with PTFE-silicon septa. The pancake samples ( $n = 86$ ) originated from 28 production days in a 9-month interval, and the days of storage (i.e., days between production and EN measurement) ranged from 20 to 297 days.

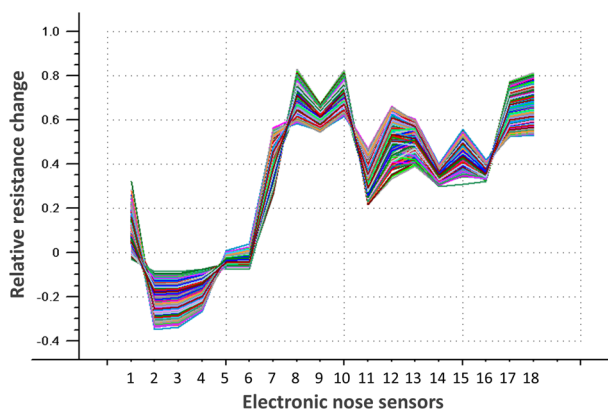
### Electronic Nose Measurement

An αFox4000 (Alpha M.O.S, Toulouse, France) type electronic nose with 18 metal oxide semiconductor (MOS) sensors was used for measuring the volatile compounds in the headspace of 20-mL sealed vials containing 1 g of individual pancake samples. The 18 MOS sensors were labeled by the manufacturer: 1: LY/LG, 2: LY/G, 3: LY/AA, 4: LY/GH, 5: LY/gCTI, 6: LY/gCT, 7: T30/1, 8: P10/1, 9: P10/2, 10: P40/1, 11: T70/2, 12: PA2, 13: P30/1, 14: P40/2, 15: P30/2, 16: T40/2, 17: T40/1, 18: TA2. P initials refer to plate type sensors, T initials refer to tube type sensors, both based on tin dioxide sensitive layer. LY sensors are plate type, with chromium titanium oxide and tungsten oxide sensitive layer. An Alpha M.O.S HS100 auto sampler was used for sampling the headspace (injected volume: 1000 µL), and synthetic air was used as a permanent air flow (150 mL/min). The acquisition time and time between subsequent analyses were 120 s and 1080 s, respectively. Incubation of sample before acquisition was performed at 40 °C, with an equilibration time of 300 s.

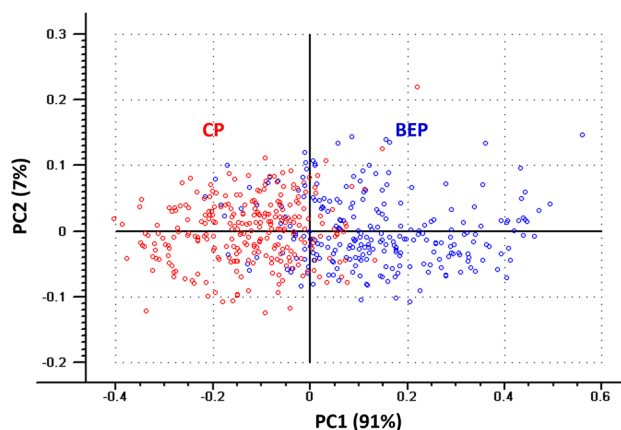
The EN measurements were performed on 9 days in a 3-week period. Experiment was designed in a way that the effect of the EN sensor drift (aging of MOS sensors during the 3-week period) was minimized, and the effect of storage time (20–297 days) was boosted: samples with various numbers of days spent in storage were analyzed during each EN measurement. Ten samples with different storage periods were selected for every EN measurement day, and six subsamples of each sample were sniffed. The samples and subsamples were introduced to sniffing in a random order. In total, 540 EN measurements were done.

### Multivariate Data Analysis

The EN instrument measured the relative resistance changes ( $\Delta R/R_0$ ) of each MOS sensor after the injection of the volatiles of the headspace. The recorded 18-dimensional data of EN measurements were analyzed with multivariate methods. Principal component analysis (PCA) as a non-supervised classification method was used for the description of the multidimensional patterns of the dataset (Cowe and McNicol 1985). The possibility of group identification based on the odor properties was investigated with discriminant analysis (DA). The supervised classification of DA used the first 5 principal components of the previously run PCA as input data against the class variables (Bázár et al. 2015). Partial least squares regression (PLSR) was used to generate calibration model on the EN data and storage days (Næs et al. 2017). The separation of BEP and CP groups along a certain PC of a PCA was quantified with the separation index (SI), where the distance of the group centroids was divided by the sum of the standard deviations (SD) of the groups. Where SI is equal to 1, the SD of the groups just covers the distance of the centroids. An SI below 1 indicates the overlapping, while an SI above 1 indicates the separation of groups — the higher the SI the better the separation. PCA models were run with leverage correction. DA and PLSR models were tested with nine-fold cross-validations, when EN data of each of the 9 measurement days were left out of the model construction at once and used for validation, iteratively. Thus, all samples were used both in the models (eight times) and in the cross-validations (once), and at a certain fold of the cross-validations, one ninth of the total samples were used for testing the models. Since the measurement days caused the largest variation in the EN data due to the drift of the MOS sensors, and there were no samples repeatedly measured on more than one day, this type of cross-validation

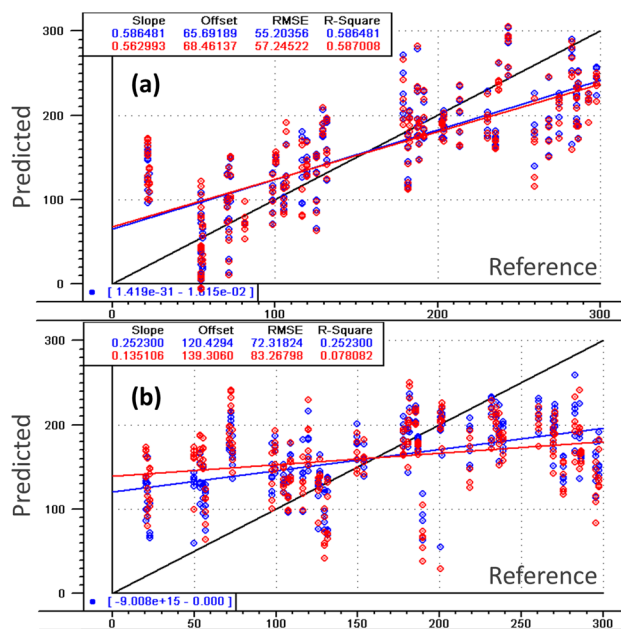


**Fig. 1** The relative resistance changes ( $n = 540$ ) measured on the 18 MOS sensors of the electronic nose after the injection of the headspace of treated (BEP) and control (CP) pancake samples

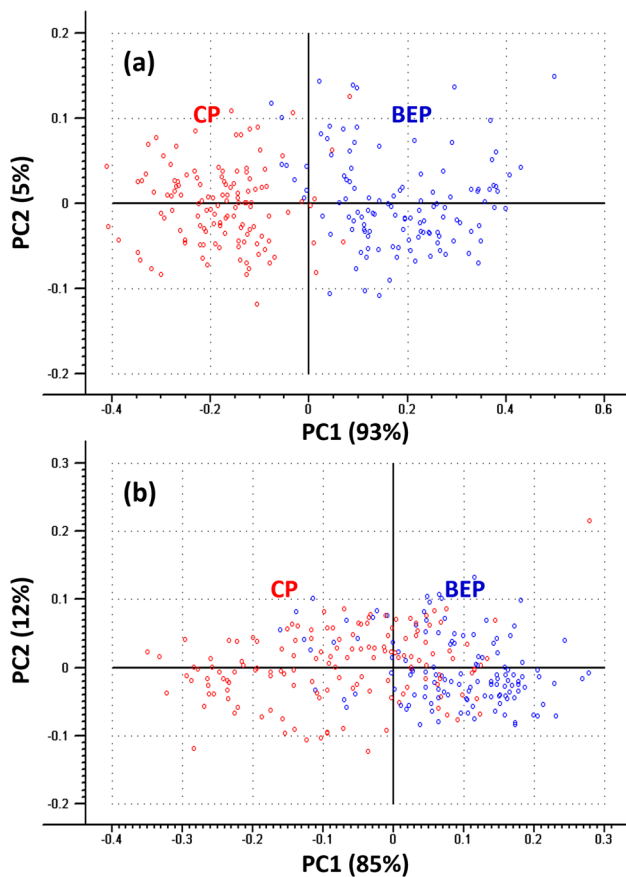


**Fig. 2** PCA score plot showing the separation of the DHA + AC supplemented (BEP) and control (CP) groups of pancakes based on their multidimensional odor profile measured with an electronic nose. The ratio of the variance explained by the individual principal components is indicated on the axes

was adequate for all qualitative and quantitative models. The sole purpose of modeling was to describe the EN data-based multidimensional properties (differences and similarities) of the sample groups, and none of the models was planned to be used for further predictions. Accordingly, no further sophistication of cross-validation was exercised.



**Fig. 3** Results of the PLSR calibrations (blue) and cross-validations (red) on the odor profile and storage time. **a** The odor profile of DHA + AC-supplemented BEP samples show good relation with the time of storage at  $-20\text{ }^{\circ}\text{C}$ . There is a strong relation in the first part and a slighter relation in the second part of the storage. **b** There is no relation described between the odor profile of the control (CP) samples and their storage time



**Fig. 4** PCA score plots indicating the separation of the bioactive-enriched (BEP) and control (CP) groups of pancakes when **a** short-term storage (20–140 days) or **b** long-term storage (140–297 days) was applied at  $-20\text{ }^{\circ}\text{C}$ . The ratio of the variance explained by the individual principal components is indicated on the axes

The performance of DA models was evaluated with the ratio of successfully classified samples (hit rate, %) in the cross-validation. The PLSR models were rated by the coefficient of determination during cross-validation ( $R^2_{CV}$ ). To decrease irrelevant sensor deviations, multiplicative signal

correction (MSC) was applied on the EN data (Martens and Stark 1991). The Unscrambler 9.7 (CAMO Software AS., Oslo, Norway) software and the IBM SPSS for Windows v26.0 (IBM Corp., Budapest, Hungary) were applied for multivariate data analyses.

## Results and Discussion

The EN signals of the 18 MOS sensors measured during the 540 sniffing processes are plotted in Fig. 1.

By using all the measured data of the odor profiles, the BEP and CP products were separated slightly in the PCA score plot (Fig. 2), i.e., SI along PC1 was equal to 1. The general PCA score plot also showed that BEP group had larger variability compared with the CP group ( $SD = 0.15$  and  $0.11$ , respectively). This recognition suggested that the time dependent changes of the sensor signals could hold useful information.

PLSR models were fitted on the multivariate EN data ( $X$  variables) and the days of storage ( $Y$  variable), for the BEP and CP samples, separately (Fig. 3). The PLSR results indicated that the storage had a larger effect on the BEP products since acceptable PLSR model was built on the days of storage if the samples of this group were applied ( $R^2_{CV} = 0.59$ ). It was possible to predict the time of storage based on the odor profile variation of the BEP samples, indicating the time-dependent changes of the volatile compounds. However, this relation was broken in the case of the CP samples ( $R^2_{CV} = 0.08$ ).

Based on the PLSR results, new PCA was performed in two rounds. First, odor patterns of samples analyzed after less than 140 days of storage were involved in the PCA. As indicated in the score plot of Fig. 4a, the CP and BEP groups show similar variability and very good separation (SI along PC1 = 1.68). The score plot of Fig. 4b shows the result of the second PCA model when odor signals of samples with more than 140 days of storage were involved. Generally, CP

**Table 1** Results of the discriminant analyses showing the hit rates of the bioactive-enriched (BEP) and control (CP) pancake samples during training and cross-validation of the classification models. The average hit rate of 95.1% dropped to 80.4% when storage lasted longer than 140 days

Storage time	Model testing	Treatment	Predicted group membership	
			BEP <sup>1</sup>	CP <sup>2</sup>
Short-term storage (< 140 d)	Training	BEP	95.8	4.2
		CP	5.6	94.4
	Cross-validation	BEP	95.8	4.2
		CP	5.6	94.4
Long-term storage (> 140 d)	Training	BEP	82.4	17.6
		CP	20	80
	Cross-validation	BEP	82.4	17.6
		CP	21.4	78.6

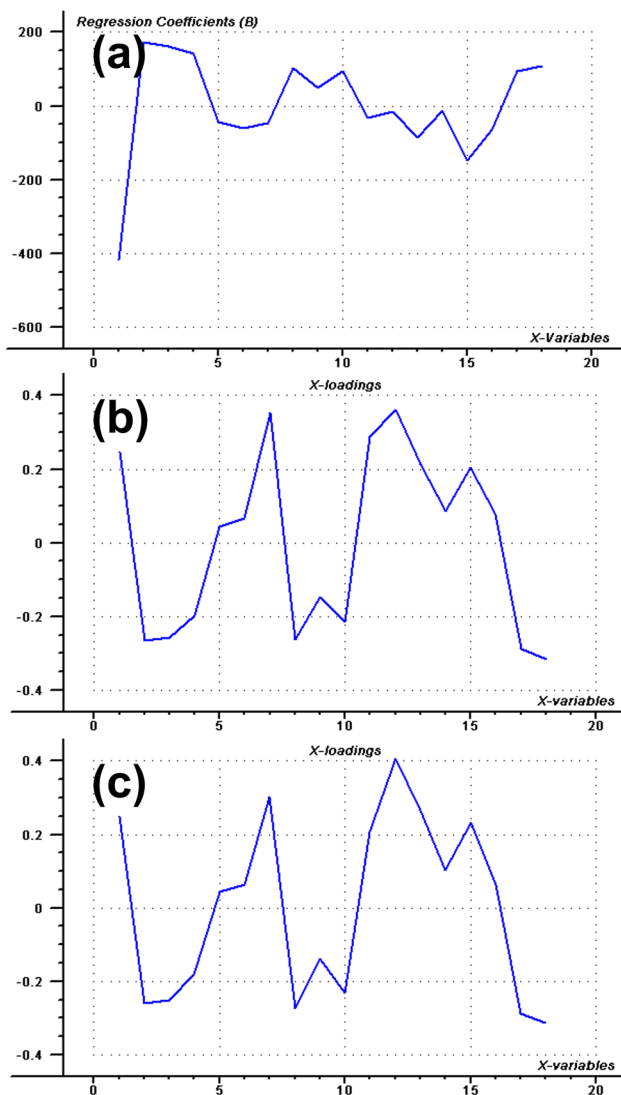
<sup>1</sup>Bioactive-enriched pancake; <sup>2</sup>control pancake



and BEP groups show more similar odor profile after long storage (SI along PC1 = 0.77).

According to these results, it can be assumed that the odor profile of the BEP group was not consistent during the storage that lasted for almost 300 days. The CP group showed a lower level of variance in odor during the whole period, and there was no time-related pattern identified. After prolonged storage, the initially detected strong odor difference between BEP and CP products decreased; the PCA score plot showed less separation.

The first five principal components of the above mentioned two PCA models were stored and used as input



**Fig. 5** **a** The regression coefficients of the PLSR calibration on storage time using the odor profile of the BEP products (Fig. 3a), indicating the power of the 18 MOS sensors (*X*-variables) within the calibration. **b** Loadings for the 18 MOS sensors (*X*-variables) when PCA is performed with pancakes of short-term storage (20–140 days) or **c** long-term storage (140–297 days) at  $-20^{\circ}\text{C}$  (Fig. 4)

variables in DA classifications. Table 1 shows the results of the training and cross-validation for samples stored at  $-20^{\circ}\text{C}$  for less or more than 140 days, respectively. The average hit rate (i.e., ratio of correctly classified samples in cross-validation) was 95.1% in the case of short-term storage, while the hit rate dropped to 80.4% in the case of long-term storage.

## Discussion

The multivariate analysis of the instrumentally recorded odor patterns showed prominent differences between control and DHA + AC-enriched pancake samples. Both unsupervised PCA and supervised DA models confirmed that the initially detected difference in the odor of the enriched and control samples decreased during frozen storage (at  $-20^{\circ}\text{C}$  for 297 days). This could be due in part to AC degradation and conversion to insoluble polymeric brown pigments (Karakaya et al. 2016). The odor profile of the enriched group was less consistent during the long-term storage compared with that of the control group, indicating the time-dependent changes of the volatile compounds when DHA + AC treatment was administered. However, volatiles of DHA + AC treatment remained after long-term (over 140 days) storage, since discrimination based on the measured odor profile was still possible with over 80% hit rate. This is consistent with a previous study showed that 21 days of storage at room temperature caused negligible losses in AC content of bakery products (Karakaya et al. 2016).

Both qualitative and quantitative results showed that the typical odor of the BEP products enriched with DHA and AC weakened and became more similar to the neutral odor of the CP products during the period of 20 to 297 days of frozen storage. Similarly to these results, Karakaya et al. (Karakaya et al. 2016) reported the instability and decreased bioaccessibility of combined DHA and AC enrichment in bakery products while stored at room temperature for 21 days.

The evaluation of the regression coefficient vector (Fig. 5a) showed that sensors 1, 2, 3, 4, and 15 had the largest influence on the PLSR calibration fitted on the storage duration of the BEP products (shown in Fig. 3a). The loadings of the PCAs of samples with less or more than 140 days of storage (shown in Fig. 4) were very similar to one another (Fig. 5b and c). Loading graphs of PC1 indicate that sensors 1, 2, 3, 4, 7, 8, 10, 11, 12, 13, 15, 17, and 18 had the most prominent variation, and these are also responsible for the separation of the scores of the two groups.

## Conclusions

These results suggest that electronic nose technology as a rapid and affordable tool for objective smell fingerprinting is useful in the characterization of the investigated food type and shall be tested in the monitoring of the aroma stability of other bioactive-enriched foods. In the present study, chemical analysis was not applied to confirm the presence and change of the mentioned bioactive ingredients; thus, conclusions can be drawn only based on the odor profiles instead of the biological value of the products. Based on the described methods and results of this study, MOS sensors can be selected for checking the quality changes of bioactive enriched pancakes during frozen storage and to identify these value-added products against the regular ones.

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**Author Contributions** Conceptualization: GB, CPV, DD, FC, AB, and TT; methodology: GB; measurement: GB and CsP; data analysis: GB, CsP, and HGY; writing — original draft preparation: GB and HGY; writing — review and editing: GB, HH, ÉCs, HGY, CPV, DD, FC, AB, and TT; visualization: GB; supervision: HH, ÉCs, and TT; project administration: HH and ÉCs; funding acquisition: TT. All authors have read and agreed to the published version of the manuscript.

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**Data availability** Data matrix including the reference and odor variables is available at 10.6084/m9.figshare.14099309.

**Code availability** Not applicable.

## Declarations

**Ethical Approval** This article does not contain any studies with human participants or animals performed by any of the authors.

**Consent for Publication** Authors agreed with the content and further publication of this manuscript

**Conflicts of Interest** George Bazar declares that he has no conflict of interest. Hajnalka Hingyi declares that he has no conflict of interest. Éva Csavajda declares that she has no conflict of interest. Csaba Palkó declares that he has no conflict of interest. Haruna Gado Yakubu declares that he has no conflict of interest. Carlos Pineda-Vadillo declares that he has no conflict of interest. Didier Dupont declares that he has no conflict of interest. Francesco Capozzi declares that he has no conflict of interest. Alessandra Bordonni declares that she has no conflict of interest. Tamás Tóth declares that he has no conflict of interest.

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