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Human breast milk, infant formula, and follow-up milks comparison by electronic tongue

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ABSTRACT

Human breast milk, infant formula, and follow-up milks were tested by a commercial electronic tongue (αAstree, Alpha MOS) with the aim to determine taste diversity, since it has been recently shown that infants exposed to different tastes early in life, develop different food preference at a later age. Human milk (36 samples) were obtained from 13 lactating women, while 12 samples of infant formula and 14 samples of follow-up milk were obtained from the Croatian market and opened prior to analysis. Human breast milk samples showed a much higher diversity than both infant formulae and follow-up milks. These results suggest that breast-fed infants are exposed to a broader sensory experience, while formula fed infants are exposed to less diverse taste. Future studies will probably answer how this influences later food choice, taste preferences, and consequently, risk of obesity and other chronic diseases.

KEYWORDS

electronic tongue, human milk, infant formula, follow-up milk, PCA

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1. INTRODUCTION

Many recent studies have shown that there are inherent differences in taste between human breast milk and infant formula composition, which opens unresolved questions regarding implications of this notion on dietary habits, food choice, and finally health. The aim of this study was to provide contribution to the understanding of this complex relationship.

The complexity of the relationship is due to numerous factors, including variability of human breast milk composition that is influenced by many factors including maternal diet, gender of the baby, lactation stage, and others (Nasser et al., 2010; Fujita et al., 2012).

The pronounced differences in nutrient content and flavour profiles of early milk provide an ideal model system to test the hypothesis that infants exposed to higher levels of certain flavour compounds exhibit elevated taste preferences for them. Variations in breast milk composition have been reported to reflect the mother's diet via several intertwined metabolic pathways that produce indirect effects. However, the literature suggests that some metabolic pathways modulate certain human milk components directly through the dietary intake (Innis, 2014). The exposure effect begins very early in life through food eaten by a breastfeeding mother and may as well influence the infant's acceptance of novel food (Forestell and Mennella, 2007). The epidemiologic studies confirm the Barker hypothesis and provide strong evidence that early nutrition plays an important role in mediating relations between patterns of early growth and subsequent risk of chronic diseases (Tarry-Adkins and Ozanne, 2011).

The process of dietary learning begins in utero, where the foetus may learn from dietary flavour cues transmitted to amniotic fluid (Sullivan and Birch, 1994; Hepper, 1995; Schaal et al., 2000). Prenatal and early postnatal exposure to a variety of flavours enables the young infant to favourably respond to a now familiar flavour, which in turn, facilitates the transition from foetal life through the breastfeeding period and eventually to a varied solid food diet (Mennella et al., 2001).

Many studies focussing on flavour compounds have been conducted by employing an electronic tongue (Hruškar et al., 2010; Kovács et al., 2011), but there is no published literature to the best knowledge of the authors concerning electronic tongue analysis of human breast milk or infant formula and follow-up milks.

Potentiometric sensor arrays have been widely used in food analysis, including evaluation and determination of aroma compounds in dairy products (Winquist et al., 1998; Dias et al., 2009; Hruškar et al., 2009; Pan et al., 2019). Electronic tongues crudely mimic the human gustatory system and the communication with the human brain. In electronic tongue systems, sensors represent nonspecific receptors, while multivariate statistical analysis provides pattern recognition in complex sensor outputs. The most used multivariate analysis technique is Principal Component Analysis (PCA), which is primarily used as a dimensionality reduction tool. Reduction of data dimensionality allows detecting patterns and allows pattern visualisation retaining as much important information present in the original data as possible (Sipos et al., 2012).

The aim of this study was to compare human breast milk, commercially available infant formula, and follow-up milks by an electronic tongue and to contribute to the understanding of potential long-term implications of sensory inequality of human breast milk vs. commercial feeding.

2. MATERIALS AND METHODS

2.1. Human breast milk, infant formula, and follow-up milk samples

Human milk samples were obtained from 13 healthy lactating women at day 16 to day 397 postpartum, aged 29.1 ± 4.86. The samples obtained from different women were numbered from "1" to "13", and labels A, B, and C were added conveying sampling days. Milk samples from each donor (200 mL per sample) were collected within 4 h after meal on three independent days within a single week. After collection, milk samples were transferred to the laboratory for immediate HPLC and electronic tongue analyses. In total, 36 human breast milk samples were collected and analysed.

Infant formula (12 samples) and follow-up milks (14 samples) were obtained on the Croatian market and opened prior to analysis. The infant formula and follow-up milks were prepared with deionised water according to the manufacturer's instructions. All samples were labelled by letters according to the manufacturer ("N", "H", "No", "A", "Hu", "B") and by letter or number denoting infant formula ("P" intended for premature infants, "1" intended for infants aged one to six months, "2" intended for infants aged six to 12 months, or follow-up milk labelled "3" for children aged one to three years).

2.2. HPLC analysis of human breast milk, infant formula, and follow-up milk samples

Lactose content was determined in all investigated samples. HPLC analyses were performed using a Shimadzu Class-VP HPLC system (Shimadzu, Japan) equipped with the RID-10A (Shimadzu, Tokyo, Japan) detector. The separation was performed with a Shodex Asahipak NH2P-5O-4E column (250 × 4.6 mm, 5 μm). Analysis was performed in triplicate by a modified HPLC method by Ferreira et al. (1998).

2.3. pH analysis of human breast milk, infant formula, and follow-up milk samples

The pH measurements of the human breast milk, infant formula, and follow-up milk samples were performed in triplicate with a Mettler-Toledo pH/Ion S220 m.

2.4. E-tongue measurements

The αAstree liquid and taste analyser was purchased from Alpha M.O.S., France. The electronic tongue uses a 16-position 730 Sample Changer and 759 Swing Head for sampling, both from Metrohm Ltd., an interface electronic module, and a sensor kit developed by Alpha M.O.S., France, a reference Ag/AgCl electrode, and a mechanical stirrer both from Metrohm Ltd. The electronic tongue comprises of seven non-specific, cross-selective potentiometric sensors (named JB, BA, BB, CA, GA, HA, and ZZ), which are chemically modified field effect transistors (CHEMFETs). The αAstree electronic tongue was connected to a computer built according to instructions (Alpha MOS manual) with the Astree II software (Alpha M.O.S., Version 3.0.1., 2003) installed. The Astree II software automatically gathers and stores the sensors output data.

Human breast milk, infant formula, and follow-up milks were analysed by the electronic tongue. During the experiment, 36 samples (three sampling days per donor) of human breast milk, 12 samples of infant formula, and 14 samples of follow-up milk were analysed. Human breast milk samples were analysed individually on the day the sample was collected. The analysis

parameters set by the electronic tongue were identical to those of infant formula and follow-up milk samples. Sample analysis was carried out at 25 °C. For each analysis sequence of infant formula or follow up milk samples, a maximum of seven samples were randomly inserted into the 16 position autosampler, and each sample was analysed for 300 s in 1 s intervals. Three analysis cycles for each sample were performed during every analysis sequence giving three measurements for each sample. Hydrochloric acid diluted in deionised water (0.01 mol l^{-1}) was analysed as a reference sample after each sample measurement to follow and later correct the drift of the sensors in time. Additionally, conditioning of the potentiometric sensor array was performed with a freshly prepared infant formula sample prior to each analysis session. The conditioning of the sensor array was performed until the response of each sensor changed less than 2 mV in 100 s. The sensors were rinsed with deionised water for 30 s after each measurement.

2.5. Data analysis

The average sensor outputs of the last 10 s of sample measurements collected by the Astree II software (Alpha M.O.S.) were imported to Microsoft Excel (Microsoft Excel 2017) and centered (Daszykowski et al., 2007). The sensor drift correction was performed using data obtained by the analysis of the reference sample (hydrochloric acid, 0.01 mol l^{-1}). After the analysis of sensor responses, only data acquired by sensors JB, BB, CA, and ZZ were taken for downstream analysis. The outputs of sensors BA, GA, and HA were found to be unchanging over time and between samples due to an unknown interference rendering the responses unusable for further data analysis. The mean value of three measurements per sample was calculated and subjected to evaluation with PCA. PCA was employed as a statistical method for the evaluation of the data in order to recognise patterns in the sensor outputs, which could be associated with taste differences in human breast milk, infant formula, and follow-up milks. Firstly, a PCA was performed with all samples included to investigate possible differences between human breast milk on one hand and infant formulae and follow-up milks on the other. Afterwards, separate PCAs were performed on human breast milk samples as well as infant formula and follow-up milk samples to investigate possible differences within the aforementioned sample groups. Pearson's correlations were calculated between sensor responses and the pH and lactose content of the samples, and correlations with a P-value ≤ 0.05 were considered statistically significant. Additionally, sensor loadings were calculated for each PCA. All data analyses were carried out using Statistica 13 (Tibco Inc., 2019).

3. RESULTS AND DISCUSSION

3.1. Comparison of human breast milk with infant formula and follow-up milks with the electronic tongue

Human milk and infant formula differ in taste, flavour, and palatability as suggested by previous studies (Emmett and Rogers, 1997; Mennella et al., 2009; Hausner et al., 2010; Tarry-Adkins and Ozanne, 2011). Fig. 1 presents the PCA score plot of human breast milk on one hand and infant formula and follow-up milks on the other obtained by the electronic tongue. The first three components represent 98.4% of the detected variance (Fig. 1). The samples are divided on the first

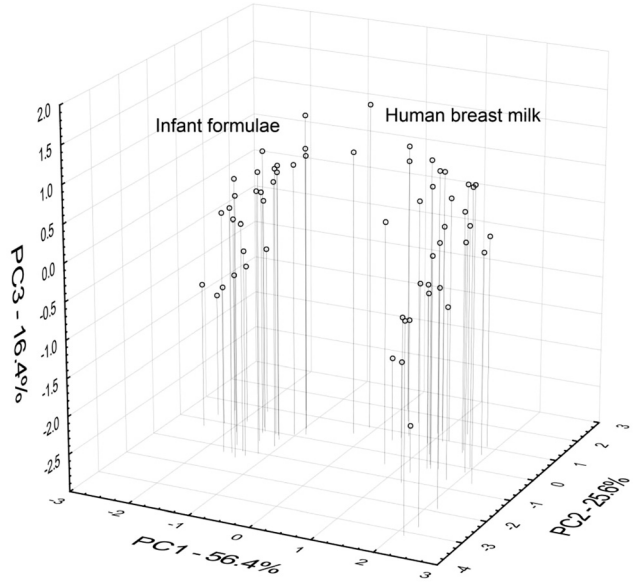

Fig. 1. PCA plot of human breast milk and infant formula and follow-up milk samples obtained by the electronic tongue

principal component into two groups. The first group is human breast milk samples, while the second group is infant formula and follow-up milk samples (Fig. 1). The electronic tongue derived plot confirms that the greatest difference between the analysed samples comes from the sample origin, i.e. whether the samples are human breast milk or infant formula and follow-up milk.

A correlation analysis was performed (Table 1) between the sensor responses and the sample pH and lactose content. The sensors CA and ZZ correlate highly with sample pH, while no statistically significant correlation was observed between the sensor responses and lactose content (Table 1). Additionally, Table 2 shows sensor impacts on the first three principal components. According to Table 2, sensors ZZ and CA have the highest impact on the first

Table 1. Correlation between sensor responses and the pH and lactose content of the sample

	Human breast milk and infant formula samples combined		Human breast milk samples		Infant formula and follow-up milk samples	
Sensor	Lactose content (g/100 kcal)	pH	Lactose content (g/100 kcal)	pH	Lactose content (g/100 kcal)	pH
BB	0.14	0.00	0.41*	0.37*	−0.22	−0.05
CA	0.33	0.79*	0.31	0.44*	0.53*	0.38*
JB	0.28	0.33	0.33	−0.01	0.06	−0.13
ZZ	0.26	0.83*	0.26	0.67*	0.08	0.02

*P-value ≤0.05.

Table 2. Sensor and variable loadings on the first three principal components

Sensor	Human breast milk and infant formula samples combined			Human breast milk samples			Infant formula and follow-up milk samples		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
BB	0.06	−0.98	−0.20	−0.80	−0.30	−0.48	−0.82	0.32	−0.22
CA	−0.94	0.03	−0.30	−0.88	0.23	−0.02	0.52	−0.68	−0.43
JB	−0.68	−0.24	0.70	−0.17	−0.95	0.25	−0.40	−0.69	0.59
ZZ	−0.96	0.08	−0.21	−0.83	0.23	0.43	−0.71	−0.47	−0.39

component, meaning these sensor responses were responsible for sample placement along the first principal component.

Another interesting aspect of this comparison comes from the second and third principal components. That is, human breast milk samples show noticeably higher diversity than the quite uniform infant formula samples. According to Table 2, sensors BB and JB are mainly responsible for sample placement on the second and third principal components, respectively. The wider placement of the human breast milk samples on the PCA scatterplot indicates that the composition of human breast milk is more complex and varied than that of the infant formula or follow-up milk samples. Although the sensor JB correlates with both lactose content and pH, the correlations are low, and therefore, the variation of the placement cannot be accurately described by only these chemical parameters. Hausner et al. (2009) came to a similar conclusion by flavour profiling of the human breast milk and infant formula. According to their own investigation, human breast milk contained a high variety of terpenes both from day to day from a single donor and between donors, which accounted to a more divergent taste exposure to the breast-fed infant. By comparison, the flavour profiles of infant formula had very little variation (Forestell and Mennella, 2007).

3.2. Human breast milk samples

The PCA plot of the human breast milk samples analysed by the electronic tongue is presented in Fig. 2. The first three principal components account for 93.1% of the variance between the samples. The samples are showing a subtle tendency to group according to the sample donor, i.e. samples from the same lactating woman tend to group together.

Hausner et al. (2010) obtained similar results, where flavour profiles of human breast milk varied both intra-subject and inter-subject, confirming the obvious similarities and differences between the analysed samples. The placement of human breast milk samples on the first principal component was influenced by sensors CA, ZZ, and JB, while on the second principal component was mostly influenced by sensor JB (Table 2). The correlation between the sensor responses, pH, and lactose content is shown in Table 1. As can be seen from Table 2, sensors that influenced the sample placement on the first principal component (sensors ZZ, CA, and BB) correlated with the samples' pH, and sensor BB also correlated with lactose content.

3.3. Infant formula and follow-up milk samples

Fig. 3 presents the PCA plot of the analysed infant formula samples with 89.5% of the variance explained. Although infant formula samples are quite uniform in their profile, some grouping

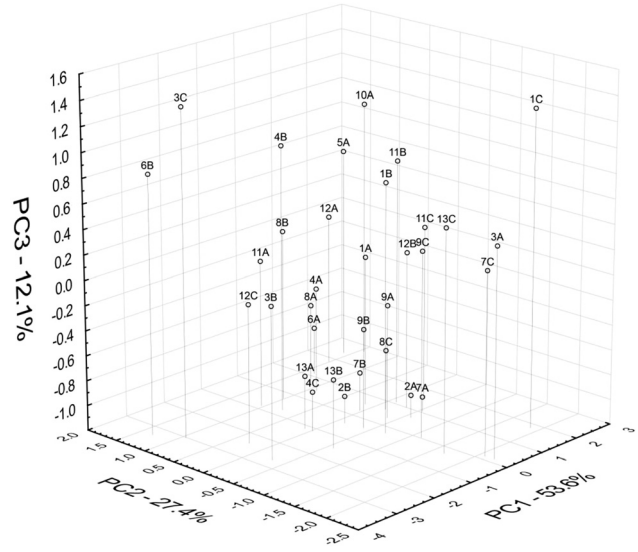

Fig. 2. PCA plot of human breast milk samples obtained by the electronic tongue

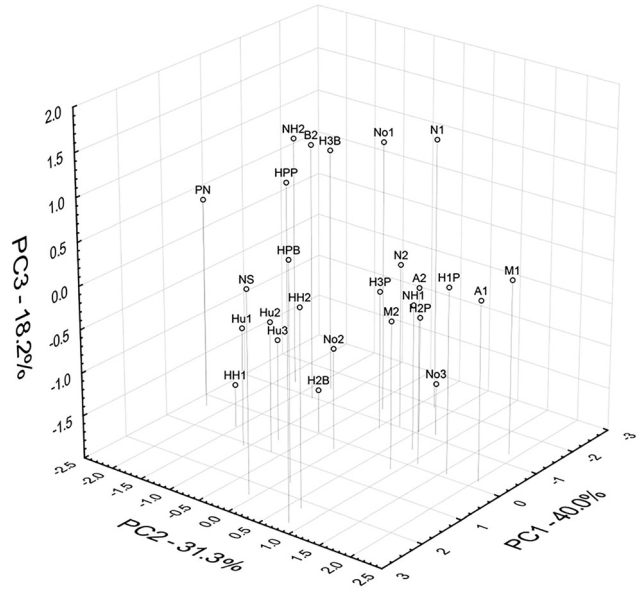

Fig. 3. PCA plot of infant formula and follow-up milk samples obtained by the electronic tongue

tendencies occur, as in the case of infant formula intended for premature infants (samples PN, HPB, NS, HPP) (Fig. 3). On the other hand, infant formula samples intended for infants aged one to six months (labelled "1"), six to 12 months (labelled "2"), and follow-up milks (labelled "3") were not separated on the PCA score plot (Fig. 3).

4. CONCLUSIONS

The aim of this study was to investigate human breast milk taste by an electronic tongue among lactating women. Additionally, evaluation of commercially available infant formula and follow-up milks was carried out. Human breast milk samples showed higher diversity as seen by the electronic tongue than either infant formulae or follow-up milks. Human breast milk showed high variability both within samples from a single donor and also between donors, but a slight grouping of human breast milk samples from a single donor was observed. The obtained results suggest that breast-fed infants are exposed to a broader sensory experience, while formula fed infants are exposed to a less diverse taste. Future studies will probably answer how this influences later food choice, taste preferences, and consequently, risk of obesity and other chronic diseases.

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