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Title: Discrimination and characterisation of 3 cultivars of Perilla frutescens by means of sensory descriptors and electronic nose and tongue analysis

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Abstract: Perilla frutescens is an annual plant widely used in therapeutics as well as in food preparations in Asian countries. Despite its interesting properties this plant is unknown to Western people.
The aim of the present study was to describe the sensory characteristics of Perilla frutescens by means of sensory evaluation. Instrumental sensory devices (electronic nose and tongue) were used to discriminate different cultivars of this plant. The relationship between the measures obtained through human senses and instrumental analysis was also investigated.
Results evidenced marked differences among cultivars tested. The Korean variety of Perilla was perceived as the least bitter and was associated to a high intensity of Cooling sensation. The crisp green-leaved Perilla was the most odorous sample being described by high intensity of Grassy and Floral odour and, accordingly, by electronic nose sensors, while the crisp red-leaved Perilla was perceived as the least astringent and pungent. Sensory diversity might be explained by the different chemical composition of Perilla chemotypes.

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Title: Discrimination and characterisation of 3 cultivars of *Perilla frutescens* by means of sensory descriptors and electronic nose and tongue analysis.

Food Research International

Dear Editor,

Please find enclosed the revised version of the paper *Discrimination and characterisation of 3 cultivars of Perilla frutescens by means of sensory descriptors and electronic nose and tongue analysis* and the answers to each point raised by the reviewer.

We hope that the corrections made according to reviewer's suggestions will lead to the acceptation of our submission.

With kind regards,

Monica Laureati

*Detailed Response to Reviewers

Comments from Reviewer(s) and/or Editor (black font) and related authors and/or co-authors answers (red font):

Reviewer #1:
The title of the paper is misleading. "Application of sensory evaluation and electronic nose and tongue to describe the sensory properties of Perilla frutescens". Neither the electronic "nose" or "tongue" can describe any sensory attribute. They may be calibrated to relate a specific measurement to a sensory description that has been determined by a panel of human assessors. I would prefer the title to describe the project better. A well-trained panel provided the sensory description of the three varieties of Perilla. Measurements from the instruments were calibrated to that sensory profile and were able to discriminate between the three varieties studied. Perhaps it is more accurate to say that "Electronic Nose and Tongue measurements differentiated three varieties of Perilla frutescens".
We agree with the referee in modifying the title. The title of the revised paper is "*Discrimination and characterisation of 3 cultivars of Perilla frutescens by means of sensory descriptors and electronic nose and tongue analysis*". Along with this, we referred to electronic tongue and nose analysis as measures that may help in discriminating/differentiating the samples and not to its overall characterisation. This adjustment has been made to the title and elsewhere in the paper (abstract: lines 13, 14; introduction: lines 61, 62; discussion: lines 265, 266).

The work itself is interesting and well conducted. The largest problem with the research is the limited sample set. Figure 3 shows the PCA of the three varieties. The 6 replications achieved through the use of "hidden control" and 3 trials are reasonably tight. However, it is still a representation of three products, forming a triangular pattern in the PCA. There is no measure of confidence around any of these points. It would be useful to have more samples representing a wider range of treatment. Then the sensory space may be more robust and the instruments given an opportunity to learn the boundaries between samples.
We agree with the referee that the number of samples was low. Of course we recognise this weakness of our experimental design which is unfortunately unsolvable.
Actually, the evaluation of the sensory properties of this plant was not an easy task. Perilla is not commonly cultivated in Italy and it is very uncommon in all Europe. A systematic agronomical study of this plant in Europe has never been made: some of us is actually undergoing it in collaboration with another group (unpublished results) and the experimental design includes the cultivation of three of the most important cultivars of Perilla which are Crispa (red and green) and the Korean type. The research has been conducted in open field and for many reasons it would not have been an easy task to cultivate more than a few cultivars of Perilla during all growing season. Beside this, we chose to work on the cultivars mostly used for culinary purposes and therefore interesting for the evaluation of sensory characteristics by the consumers and to perform a reasonable high number of replications (6) for each one. In this respect, it should be noted that notwithstanding the reduced number of cultivars used (3) in the present experiment, the replications were good and the 3 Perilla cultivars were very well differentiated in the multidimensional space.
Despite the weakness due to the limited number of samples, we think that this preliminary, explorative study makes an important contribution to the literature since there are no previous systematic investigations on Perilla sensory properties. Moreover, the limitation of our study has been highlighted over the text and emphasis was directed toward the fact that a larger number of samples should be considered to obtain more robust results (see lines 310-314).

Other modifications (not raised by the referee)
→ Several adjustments to English language were made along the text. Since these are simply editing and/or grammar errors they are not indicated in the revised paper.
→ In the revised paper figure 1 and 2 have been inverted (see figure captions). These figures have been newly uploaded.
→ Some references have been added in the discussion section (lines 289-290)
→ Some sentences have been shifted (lines 93-99 of the first version of the paper became lines 81-87 of the revised paper)

Discrimination and characterisation of 3 cultivars of *Perilla frutescens* by means of sensory descriptors and electronic nose and tongue analysis

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Abstract

Perilla frutescens is an annual plant widely used in therapeutics as well as in food preparations in Asian countries. Despite its interesting properties this plant is unknown to Western people.

The aim of the present study was to describe the sensory characteristics of *Perilla frutescens* by means of sensory evaluation. Instrumental sensory devices (electronic nose and tongue) were used to discriminate different cultivars of this plant. The relationship between the measures obtained through human senses and instrumental analysis was also investigated.

Results evidenced marked differences among cultivars tested. The Korean variety of Perilla was perceived as the least bitter and was associated to a high intensity of Cooling sensation. The crisp green-leaved Perilla was the most odorous sample being described by high intensity of Grassy and Floral odour and, accordingly, by electronic nose sensors, while the crisp red-leaved Perilla was perceived as the least astringent and pungent. Sensory diversity might be explained by the different chemical composition of Perilla chemotypes.

Keywords: *Perilla frutescens*; sensory evaluation; electronic nose; electronic tongue; trigeminal sensations

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Introduction

Perilla frutescens (L.) Britt. (Lamiaceae), also known as "wild coleus", "Chinese basil" and "Perilla mint", is an annual short-day plant widely used in therapeutics as well as in food preparations in Asian countries, especially in China, Japan and Korea.

It is classified into *Perilla frutescens* (L.) Britt. Var. *acuta* Kudo forma *viridis* Makino (green Perilla) and *Perilla frutescens* (L.) Britt. Var. *acuta* Kudo (red Perilla). Green- and red-leaved Perilla are also broadly classified as smooth and crisp varieties depending on margin leaf (Ravindran and Shylaja, 2004).

Since Perilla has many medical properties, it has been subject of a large number of studies. Perilla leaves and stems are reported to have anti-microbial (Yamamoto and Ogawa, 2002), anti-HIV (Kawahata et al., 2002; Yamasaki et al., 1998), anti-tumour (Banno et al., 2004; Ueda et al., 2003) and anti-allergic properties (Guo et al., 2007). It is traditionally used in Chinese medicine to treat various diseases including depression, anxiety, cough and to promote intestinal propulsion.

Aside its pharmacological properties, Perilla leaves are strongly aromatic with a mint-like flavour and therefore suitable for food preparations. The leaves are used as spice, cooked as postherbs or fried and combined with fish, rice, vegetables and soups. It is also chopped and mixed with ginger rhizome and then added to stir-fries, tempuras and salads in many Asian countries (Ravindran and Shylaja, 2004). Due to the presence of anthocyanins, the purple variety is also used to impart colour along with flavour to many pickled dishes, the most known is the Japanese "Umeboshi" (Sawabe et al., 2006).

Perilla frutescens contains several components which significantly influence its sensory quality and pharmacological activities. It has been recently found that some compounds of *Perilla frutescens*, namely perillaldehyde (PA) and perillaketone (PK), are active on TRPA1 ion channels and are therefore involved in the perception of chemestetic sensations (Bassoli et al., 2009).

Despite its interesting properties this plant is completely unknown in Western countries. In USA and Europe this plant has been introduced but it is still consumed only by Asian immigrants.

Very little is published about the sensory properties of such a herb. Morinaka et al. (2001) investigating the aroma of several accessions of *Perilla frutescens* found that the leaves were characterised by a greenish odour, a Perilla-like odour, a pickled ume-like odour, a fresh odour and a stimulating odour, the latter probably associated with a pungent sensation perceived through the nasal cavity.

Most of the studies on *Perilla frutescens* aromatic compounds are focused on the investigation of the volatile component by means of GC methods (Choi, 2004; Lin et al., 2002; Morinaka et al., 2002). However, to the knowledge of the present authors no studies exist that systematically investigate the properties of taste, smell and trigeminal sensations of such a plant.

Therefore, the purpose of the present, preliminary study was to describe the sensory characteristics of *Perilla frutescens* by means of sensory evaluation involving a panel of trained assessors. Instrumental sensory devices such as electronic nose and electronic tongue were also used to discriminate different cultivars. The Electronic Nose (EN) is an instrument that should mimic the human olfactory perception and provide an odour print of the sample; it is equipped with an array of non-selective and broad-spectrum chemical sensors useful for the analysis of headspace of liquid or solid samples (Burtlett et al., 1997). The Electronic Tongue (ET) is a liquid analysis device that mimics the taste-sensing mechanism and information processing of gustatory system; it comprises an array of sensors that are specific for liquid and able to classify four basic qualities: sourness, saltiness, bitterness and umami taste (Toko, 2000; Vlasov et al., 2002). The relationship between the measures obtained by human senses and instrumental sensory analysis was also investigated. Finally, chemotype classification was achieved by HPLC analysis of essential oil obtained by leaves steam distillation.

Material and methods

Perilla samples

Three different cultivars of *Perilla frutescens* were investigated in this study: 2 varieties of *Perilla frutescens* (L.) Britt. var. *crispa* (*Tokita red*: crisp red-leaved Perilla; *Tokita green*: crisp green-leaved Perilla), and a smooth green-leaved Korean variety of Perilla. The plants were seeded and grown in open field at the botanical garden of Fondazione Minoprio, Minoprio Vertemate (CO).

The leaves of each variety were used to prepare infusions evaluated both by sensory panel and by means of instrumental sensory analysis. The leaves were harvested over the month of September using adult plants.

The infusions were prepared adding 14.4 ± 0.2 g of whole leaves in 300 mL of mineral still water at boiling point. The choice to prepare infusions rather than to add Perilla leaves to food was due to the fact that water is an ideal, neutral mean to describe and quantify sensory properties without the intervention of other components that may mask or enhance some specific sensory feature of Perilla samples. Furthermore, it is noteworthy that in this study we involved Italian assessors who had no familiarity with the product in question and therefore we decided to use an "easy product" for this preliminary experiment.

Chemotype classification

The three plants were examined for the classification of the chemotype as described in the literature (Ravindran and Shylaja, 2004). Leaves were steam distilled to extract the essential oil and analysed in TLC and HPLC. Thin-layer chromatography was performed on Merck precoated silica gel 60 F254 plates and the spots were visualised by UV at 254 nm. HPLC analyses were recorded with a Varian PROSTAR liquid chromatograph using RP-column Alltima C18, 5μ (Alltech), isocratic conditions for methanol/water 70/30, flow 1 ml/min, 254 nm. Commercial perillaldehyde (PA) (Sigma Aldrich) was used as a standard; for perillaketone (PK) we used an authentic sample previously prepared by synthesis and characterised for its structure by some of us (Bassoli et al, 2009). In isocratic conditions the two compounds have retention times of 9.17 min (PK) and 11.16 min (PA) respectively. *Perilla crispa* samples red and green contained almost exclusively perillaldehyde and were therefore classified as PA type, whereas the smooth green leaves Korean variety contained perillaketone but not perillaldehyde and was classified as PK type.

Sensory evaluation

Subjects

Fourteen assessors (8 females and 6 males; aged between 22 and 50), recruited amongst the students and staff of the University of Milan were involved in the study. All were familiar with sensory evaluation procedures but none of them had eating experience with any sort of food product added with *Perilla frutescens* before the present experiment.

Participants were asked not to smoke, eat or drink anything, except water, for one hour before the tasting sessions. Before starting the sessions, each participant signed a consent form explaining the aim of the experiment. All participants received a fee for their participation.

Procedure

In order to describe the sensory properties of the 3 *Perilla frutescens* varieties the Sensory Profile method was applied (ISO 13299, 2003).

This method consists of a first training phase in order to acquire familiarity with the product and the methodology and a second phase of evaluation of samples sensory properties.

The training phase consisted of four 1-h sessions over a period of 1 month in order to develop terminology to describe the key sensory attributes of infusions of *Perilla frutescens* leaves. First, panellists were asked to write down terms describing appearance, aroma, taste, flavour and trigeminal sensations that, in their opinion, represented at best Perilla infusions. As training progressed, descriptive terms were defined through panel discussion and redundant terms were excluded by panel consensus. Panel discussions also determined the reference standard used to anchor the scale endpoint label.

Overall, 9 sensory attributes covering aroma, taste, flavour and trigeminal sensations were generated. The list of sensory attributes, with their relevant definitions and reference standards is reported in table 1.

Insert table 1 about here

Once the common vocabulary and the reference standards were defined, panellists performed 4 sessions in sensory booths to acquire familiarity with the scale to be used to quantify each sensory descriptor.

After the training phase, judges evaluated three Perilla samples per session over the course of 2 days of evaluation. Each session was performed in triplicate. During the first session each assessor received 1 sample of smooth green-leaved Perilla and 2 samples of crisp red-leaved Perilla (one of these two samples added as hidden control) while during the second session judges received 1 sample of smooth green-leaved Perilla and 2 samples of crisp green-leaved Perilla (one of these two samples added as hidden control) for a total of 6 Perilla infusions evaluated.

The assessors were instructed to rate the intensity of each sensory attribute using a continuous, unstructured 100 mm line scale anchored at both extremes with “minimum intensity” (left of the scale) and “maximum intensity” (right of the scale).

Samples were served in plastic cup coded with 3-digit numbers and evaluated in individual booths under red light at room temperature. In order to balance the effects of serving order and carry-over, presentation orders were systematically varied over assessors and sessions (MacFie et al., 1989). The sessions were held at the sensory laboratory of DISTAM (University of Milan) designed according to ISO guidelines (ISO 8589, 2007). Data were collected using the software program Fizz v2.31g (Biosystemes, Couternon, France).

Electronic nose

Analyses were performed by a commercial EN (model 3320 Applied Sensor Lab Emission Analyser; Applied Sensor Co., Linkoping, Sweden), consisting of three parts: automatic sampling apparatus, detecting unit containing the array of sensors, and the pattern recognition system. The automatic sampling system supported a carousel of 12 loading sites for samples. The sensor array included 22 different sensors, 10 of which were Metal Oxide Semiconductor Field Effect Transistors (MOSFET) and 12 Metal Oxide Semiconductors (MOS) sensors. The MOSFET sensors were divided into two arrays of five sensors each, one array operating at 140°C and the other at 170°C; the MOS, mounted in a separate chamber, were kept at 400-500°C.

Two mL of Perilla infusions were placed in 20 mL Pyrex® vials with silicone caps and then settled into the automatic sampling carousel. After 1 h equilibration at 20 °C ± 1, the measurement started. The Perilla headspace was pumped over the sensor surfaces for 30 s (injection time) at a flow rate of 300 mL min^{-1}, during this time the sensor signals were recorded. After sample analysis the system was purged with filtered air prior to the next sample injection to allow re-establishment of the instrument base line. The total cycle time for each measurement was 5 min. During the measurement period no sensor drift was experienced. Each Perilla sample was evaluated 3 times and the average of the results was used for data analysis.

Electronic tongue

Analyses were performed with the commercially available Taste-Sensing System SA 402B (Intelligent Sensor Technology Co. Ltd, Japan), namely Electronic Tongue (ET), shown in figure 1.

Insert figure 1 about here

The detecting part of the system consists of 7 sensors whose surface is attached with artificial lipid membranes having different response properties to chemical substances on the basis of their taste (Table 2). For the present work a total of 4 detecting sensors and 2 reference electrodes were used, separated into two groups according to membranes charge: hybrid (CT0; CA0) and positive (C00, AE1).

Insert table 2 about here

The measurement principle of the electronic tongue is based on the capability of taste substances to change the potential detecting sensors through electrostatic or hydrophobic interaction with the hydrophilic and hydrophobic groups of the lipid membranes. The response of each sensor, recorded as the difference between the potential detected by the sensor electrode and the potential of the reference electrode, is elaborated by a computer and processed via a pattern recognition system.

Figure 2 shows the measuring process for Perilla infusions.

Insert figure 2 about here

Detecting and reference electrodes were first dipped into the reference solution (30 mM potassium chloride and 0.3mM tartaric acid) and the electric potential measured for each sensor was defined as Vr. Then the electrodes were dipped for 30 s into the sample solution (Perilla infusion). For each sensor the measured potential was defined as Vs. The “relative” sensor outputs were represented by the differences (Vs-Vr) between the potentials of the sample and the reference solution. Electrodes were rinsed with fresh reference solution for 6 s and then dipped into the reference solution again. The new potential of the reference solution was defined as Vr’. The difference (Vr’-Vr) between the potentials of the reference solution before and after sample measurement is the CPA (Change of Membrane Potential caused by Absorption) value and corresponds to the ET “aftertaste”. In this work we have considered the CPA values for COO sensor and AE1 sensor respectively corresponding to aftertaste from bitterness and astringency . Before a new measurement cycle started, electrodes were rinsed for 90 s with a washing solution and then for 180 s with the reference solution. Each Perilla sample was evaluated 3 times and the average of the results was used for data analysis.

Data analysis

Sensory data were analysed by means of 3-way ANOVA considering *Samples* (6), *Judges* (8), *Replications* (3) and their relevant 2-way interactions as factors, and sensory attributes as dependent variables. When the ANOVA showed a significant effect ($p<0.05$), the Least Significant Difference (LSD) was applied as a multiple comparison test using the statistical software STATGRAPHIS PLUS version 5.0 (Manugest KS Inc., Rockville, USA).

Sensory, EN and ET data were standardised (1/standard deviation) and analysed by means of Principal Component Analysis (PCA) using The Unscrambler® 9.8 statistical software (Camo As, Trodheim, Norway).

Results

ANOVA results and mean ratings for the 9 sensory attributes evaluated by the panel for the 6 Perilla samples are reported in table 3.

Insert table 3 about here

As can be observed, all the sensory descriptors except for Cumin aroma and flavour significantly ($p<0.001$) discriminated the Perilla samples tested.

The interactions *Judges* and *Judges by Replicates* were significant for all the sensory attributes, thus suggesting some inconsistencies in the use of scale and terms by panellists. Inconsistencies among panellists are common in sensory evaluation and the magnitude of these inconsistencies may be small compared to the magnitude of the differences between samples (Pagliarini, 2002).

However, *Replicates* and the interaction *Replicates by Judges* were not significant sources of variation, thus confirming reliability of the panel. The interaction *Sample by Replicates* was also not significant for all the attributes with the exception of Cumin flavour indicating that Perilla infusion samples were stable over replicates.

According to multiple comparison test (table 3), the Korean variety of Perilla (smooth green-leaved Perilla) was perceived as significantly less bitter and most refreshing (high intensity of Cooling sensation). The crisp green-leaved Perilla was the most aromatic sample being characterised by high intensity of Grassy odour and flavour and by Floral odour, while the red-leaved Perilla was perceived as significantly less Astringent and Pungent as compared to the other two varieties of Perilla samples. It is noteworthy that the Perilla samples added as hidden control (for instance smooth green-leaved 1 and smooth green-leaved 2) during sensory sessions were correctly evaluated as not significantly different between them for all the sensory descriptors by assessors thus confirming the good degree of panel training.

Multivariate analysis of sensory and EN and ET data

ANOVA results indicated that the mean scores for each Perilla sample given by the panellist for each attribute could be assumed satisfactory estimates of the sensory profile of the samples. As a result, they were averaged across assessors and submitted to PCA along with EN and ET data achieved on the same samples in order to identify possible relationships between sensory descriptors and instrumental measures.

At this stage Cumin odour and flavour were excluded since only sensory descriptors that significantly contributed to Perilla samples discrimination were included in the multivariate analysis. Furthermore, according to a visual analysis of correlation loading plot, all sensory and instrumental variables that contributed to less than the 50% of total explained variance were removed from the analysis.

Figures 3 and 4 report the principal component scores plot and principal component loadings plot, respectively, from sensory and instrumental data for Perilla infusions.

Insert figures 3 and 4 about here

The variance explained by the first two principal components was 79%. In figure 3 (scores plot) Perilla samples appear to be well separated into three groups. Moving left to right along the first component (explained variance 48%), the crisp red-leaved variety (CR) is separated from the smooth green-leaved variety (SG). The second component (explained variance 31%) distinguishes the crisp green-leaved variety (CG) from the rest of the Perilla samples.

From the loadings plot in figure 4, showing the relationship between the variables and how they influence the system, it was possible to notice that the best separation of Perilla samples was achieved by the combination of the sensory and instrumental variables. In particular the EN variables were dominant in the first principal component while the sensory and ET variables were relevant in particular on the second principal component. The comparison of scores and loadings plot showed that, in agreement with ANOVA results, crisp green-leaved Perilla samples (CG) located in the positive axis of the second principal component were associated to high intensities of Grassy odour and flavour and Floral odour and were discriminated also by MOSFET sensors (FE).

The MOS selected sensors (MO) and the two ET selected variables (CA0-sourness; AE1-astringent) were relevant especially in the discrimination of the Korean variety (smooth green-leaved Perilla: SG) situated in the lower-right quadrant, perceived as the least bitter and associated to a high intensity of Cooling sensation. The crisp red-leaved Perilla samples (CR) situated in the lower-left quadrant were less discriminated by both the sensory and instrumental variables. Considering the sensory attributes, the low intensity of Astringent, Pungent, Grassy and Floral odour and also Grassy flavour distinguished this variety from the other two; also from the EN and ET data the crisp red-leaved Perilla samples (CR) seemed to be less odorous and tasty with respect to the other two varieties.

Discussion

In this preliminary study, the sensory properties of 3 different cultivars of *Perilla frutescens* were defined by means of sensory evaluation. Moreover, a good discrimination of the varieties was achieved using electronic sensory devices.

The identification of the characteristics of aroma, taste, flavour and trigeminal sensations of this herb was not an easy task. Indeed, *Perilla frutescens* is considered a traditional food in Asian countries but it is completely unknown in Western countries, thus the definition of a common vocabulary by the panel, although trained, was somewhat difficult. Some descriptors (e.g. Anise, Nut, Almond aromas and flavours) that were generated during training were then omitted in the vocabulary since there was confusion in the use of the terms among assessors. This might be explained by the fact that assessors were “naïve” to the product tested but also that the infusion of Perilla leaves probably was not the ideal mean to enhance its sensory properties.

Nine sensory attributes comprehensive of aroma (Cumin, Grassy and Floral), taste (Bitter), flavour (Cumin and Grassy) and trigeminal aspects (Astringent, Pungent and Cooling) were generated by the panel.

The fact that all the varieties were characterised by trigeminal sensations seems to confirm the outcome on in vitro assays obtained by Bassoli et al. (2009) who evidenced that PA and PK isolated from fresh and freeze-dried Perilla leaves are able to activate the cloned TRPA1 receptor. The study of TRP active compounds contained in vegetables is very interesting. The attention of researchers to the compounds responsible of trigeminal sensations (e.g. capsaicin in chilli pepper, menthol in mint, isothiocianates in mustard or horseradish) has at least two reasons. In the first place, from a sensory point of view, the somatosensory contribution of such stimuli to the overall perception of food is somewhat important in determining food choice and preference. In the second place, TRP active compounds are interesting from a pharmacological and nutritional point of view. Indeed, beside trigeminal perception, they seem to be involved in other biological mechanisms, such as satiety regulation and pain perception. It has been suggested that these compounds may reduce sensitivity to pain with repeated exposure to the stimulus (Finnerup et al., 2005) and enhance metabolism (Mahmmoud, 2008). Therefore, a reasonable assumption is that a diet rich in TRP active compounds may lead to an increase of pain threshold through a slow but systematic desensitisation of pain sensors and to body weight decrease (Bassoli et al., 2009).

The fact that PA and PK are contained in Perilla varieties is a further spur to study this plant and to consider it as a valuable ingredient also in Western food preparations.

Results also showed a remarkable difference from a sensory point of view of the 3 cultivars of Perilla. The crisp green-leaved variety was the more aromatic (stronger aroma and flavour intensities) whereas the smooth green-leaved variety was the most refreshing and pungent. This result is confirmed also by the instrumental sensory devices, since the crisp green-leaved variety is better explained by the EN sensors, and the smooth green-leaved variety refreshing, astringent and pungent is characterised also by the liquid sensors of the ET. The diversity among the varieties may be explained by a difference in molecular composition. Actually, it is clearly established (Ravindran and Shylaja, 2004) that several Perilla chemically distinct varieties (*i.e.* chemovarieties) exist and are classified on the basis of the predominant chemical component. We found that in our samples the difference between crisp and smooth varieties can be well explained since they own to different chemotypes: in fact, crisp (red and green) are PA type and smooth is PK type. The chemotype seems not to explain completely the sensory difference between the red and the green crisp Perilla samples: in this case it is likely that other minor aromatic components are present in different amounts and can modify significatively the overall taste and flavour of the two plants. A complete analysis of these minor components is ongoing.

Even if this study is preliminary and more samples are required for a better discrimination and classification of Perilla samples, it points out the advantages of combining together gas and liquid sensors, i.e. EN and ET, for distinguishing among the three varieties of Perilla. Furthermore this study shows the possibility of establishing a relation between the output from the instrumental sensory devices and the human sensory assessment of taste and flavour.

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References

Banno, N., Akihisa, T., Tokuda, H., Yasukawa, K., Higashihara, H., Ukiya, M., Watanabe, K., Rimura, Y., Hasegawa, J., & Nishino, H. (2004). Triterpene acids from the leaves of Perilla frutescens and their anti-inflammatory and antitumor-promoting effects. *Bioscience Biotechnology and Biochemistry, 68* (1), 85-90.

Bassoli, A., Borgonovo, G., Caimi, S., Scaglioni, L., Morini, G., Moriello Schiano, A., Di Marzo, V., & De Petrocellis, L. (2009). Taste-guided identification of high potency TRPA1 agonists from Perilla frutescens. *Bioorganic and Medicinal Chemistry, 17*, 1636-1639.

Burtlett, P. N., Elliott, J. M., & Gardner, J. W. (1997). Electronic noses and their application in the food industry. *Food Technology, 51*, 44-48.

Choi, H. (2004). Characteristic odor components of Perilla frutescens var. Acuta kudo oil by aroma extract dilution analysis. *Food Science and Biotechnology, 13* (3), 279-284.

Finnerup, N.B., Otto, M., McQuay, H.J., Jensen, T.S., Sindrup, S.H. (2005). Algorithm for neuropathic pain treatment: An evidence based proposal. *Pain, 118,* 289-305.

Guo, R., Pittler, M. H., & Ernst, E. (2007). Herbal medicines for the treatment of allergic rhinitis: a systematic review. *Annals of Allergy Asthma and Immunology, 99* (6), 483-595.

Kawahata, T., Otake, T., Mori, H., Kojima, Y., Oishi, I., Oka, S., Fukumori, Y., & Sano, K. (2002). A novel substance purified from Perilla frutescens Britton inhibits an early stage of HIV-1 replication without blocking viral adsorption. *Antiviral Chemistry & Chemotherapy, 13* (5), 283-88.

ISO 13299 (2003). Sensory Analysis – Methodology – General guidance to establish a sensory profile. International Organization for Standardization, Geneva, Switzerland.

ISO 8589 (2007). Sensory analysis – General guidance for the design of test rooms. International Organization for Standardization, Geneva, Switzerland.

Lin, R., Tian, J., Huang, G., Li, T., & Li, F. (2002). Analysis of menthol in three traditional Chinese medicinal herbs and their compound formulation by GC-MS. *Biomedical Chromatography, 16* (3), 229-233.

Macfie, H. J. H., Bratchell, N., Greenhoff, K., & Vallis, L. V. (1989). Designs to balance the effect of order of presentation and first-order carry-over effects in hall tests. *Journal of Sensory Studies*, *4* (2), 129-148.

Mahmmoud, Y.A. (2008). Capsaicin Stimulates uncoupled ATP hydrolysis by the sarcoplasmic reticulum calcium pump. *The Journal of Biological Chemistry*, *283*(31), 21418–21426.

Morinaka, Y., Fukuda, N., & Takayanagi, K. (2001). Evaluation of perilla (Perilla frutescens) aroma - odor and their descriptors of perilla leaves. *Journal of the Japanese Society for Horticultural Science, 70* (5), 607-615.

Morinaka, Y., Fukuda, N., & Takayanagi, K. (2002). Evaluation of perilla (Perilla frutescens) aroma - statistical comparison between analytical and sensory data of perilla leaf aroma. *Journal of the Japanese Society for Horticultural Science, 71* (3), 424-433.

Pagliarini E. (2002). *Valutazione sensoriale: Aspetti teorici, pratici e metodologici.* Milano: Hoepli.

Ravindran, P. N., & Shylaja, M. (2004). Perilla. In K. V. Peter, *Handbook of herbs and spices*, (pp. 482-494). Cambridge: Woodhead Publishing Ltd.

Sawabe, A., Satake, T., Aizawa, R., Sakatani, K., Nishimoto, K., Ozeki, C., Hamada, Y., & Komemushi S. (2006). Toward use of the leaves of Perilla frutescens (L.) Britton var. Acuta Kudo (red perilla) with Japanese dietary pickled plum (Umeboshi). *Journal of Oleo Science, 55* (8), 413-422.

Toko, K. (2000). Taste sensor. *Sensors and Actuators B-Chemical, 64*, 205-215.

Ueda, H., Yamazaki, C., & Yamazaki, M. (2003). Inhibitory effect of Perilla leaf extract and luteolin on mouse skin tumor promotion. *Biological and Pharmaceutical Bulletin, 26* (4), 560-63.

Vlasov, Y., Legin, A., & Rudnitskaya, A. (2002). Electronic tongues and their analytical application. *Analytical and Bioanalytical Chemistry, 373*, 136-146.

Yamamoto, H., & Ogawa, T. (2002). Antimicrobial activity of perilla seed polyphenols against oral pathogenic bacteria. *Bioscience Biotechnology and Biochemistry, 66* (4), 921-924.

Yamasaki K., Nakano M., Kawahata T., Mori H., Otake T., Ueba N., Oishi I., Inami R., Yamane M., Nakamura M., Murata H., & Nakanishi T. (1998). Anti-HIV-1 activity of herbs in Labiatae. *Biological and Pharmaceutical Bulletin, 21* (8), 829-833.

Figure captions

Figure 1. Taste Sensing System SA 402B. (a) lipid membrane electrode; (b) reference electrode. The inner of all sensors is filled up with 3.3M KCl and sat.AgCl solution. Ag/AgCl wire is immersed with the solution.

Figure 2. Measurement procedure by Electronic Tongue

Figure 3. Principal component scores plot from the sensory analysis and electronic nose and tongue evaluation of the 6 Perilla infusions (CR=crisp red leaved Perilla; CG=crisp green leaved Perilla; SG=smooth green leaved Perilla; for each Perilla variety "hc" refers to the sample evaluated as hidden control; for each Perilla variety numbers refer to replications)

Figure 4. Principal component loadings plot from the sensory analysis and electronic nose and tongue evaluation of the 6 Perilla infusions

Table 1

Table 1. List of the 9 sensory descriptors of Perilla samples with their relevant definitions and reference standards

Sensory descriptor	Definition	Reference standard
Aroma		
Cumin	Characteristic cumin odour perceived by means of the sense of smell	6 g of crushed cumin seeds (Ducros s.r.l) in infusion for 3 min in 300 mL of hot still mineral water (served at room temperature)
Grassy	Characteristic grass/hay odour perceived by means of the sense of smell	Water solution of Cis-3-hexenol (80 ppm)
Floral	Characteristic odour of flowers/ almond/hazelnut perceived by means of the sense of smell	Water solution of phenylethyl alcohol (40 ppm) in acoholic solution at 0.1%
Taste		
Bitter	One of the basic tastes, caused by water solution of bitter compounds perceived in the oral cavity	Water solution of caffein (0.8 g/L)
Flavour		
Cumin	Characteristic cumin odour perceived in the mouth during swallowing	6 g of crushed cumin seeds (Ducros s.r.l) in infusion for 3 min in 300 mL of hot still mineral water (served at room temperature)
Grassy	Characteristic grass/hay odour perceived in the mouth during swallowing	Water solution of Cis-3-hexenol (80 ppm)
Trigeminal sensation		
Astringent	Sensation of dryness and puckering caused by tannins and perceived in the oral cavity	2 Java green tea bags (Twinings S.p.A.) in infusion for 3 min. in 400 mL of hot still mineral water (served at room temperature)
Pungent	Sensation of tingling perceived in the oral cavity	6 g of crushed cumin seeds (Ducros s.r.l) in infusion for 3 min in 300 mL of hot still mineral water (served at room temperature)
Cooling	Sensation of coolness caused by menthol perceived in the oral cavity	8 g of mint fresh leaves in infusion for 3 min. in 300 mL of hot still mineral water (served at room temperature)

Table 2

Table 2. List and characteristics of electronic tongue detecting sensors.

Attribute	Name of detecting electrodes	Characteristics (Taste information)
Blend Membrane	AAE	Umami taste and umami richness
	CT0	Saltiness
	CA0	Sourness
Positively charged Membrane	C00	Bitterness and acidic bitterness
	AE1	Astringency
Negatively charged Membrane	AC0	Bitterness
	AN0	Bitterness

Table 3

Table 3. Sensory evaluation results from three-way ANOVA and least significant difference (LSD) test on 6 Perilla samples (S=Samples; J=Judges; R=Replicates). Means with the same letter by row are not significantly different (p<0.05).

Descriptors	Sources of variation (F-values)						Perilla samples (mean ratings)					
	S	J	R	J*S	S*R	J*R	smooth green 1	smooth green 2	crisp red 1	crisp red 2	crisp green 1	crisp green 2
Aroma												
Grassy	5.76 ***	15.52 ***	0.07 n.s.	18.80 ***	0.47 n.s.	0.93 n.s.	5.36[a]	5.34[a]	5.53[ab]	5.21[a]	5.88[bc]	5.99[c]
Cumin	1.57 n.s.	15.23 ***	0.60 n.s.	12.95 ***	1.85 n.s.	0.96 n.s.	4.36	4.32	4.15	4.42	4.54	4.73
Floreal	13.97 ***	82.75 ***	1.89 n.s.	9.39 ***	0.75 n.s.	0.78 n.s.	3.94[a]	3.98[a]	3.74[a]	4.17[a]	5.15[b]	5.01[b]
Taste												
Bitter	15.03 ***	342.38 ***	0.90 n.s.	8.64 ***	1.04 n.s.	1.75 n.s.	3.40[a]	3.45[a]	3.94[b]	3.91[b]	4.04[b]	3.88[b]
Flavour												
Grassy	17.15 ***	207.27 ***	1.79 n.s.	50.93 ***	1.07 n.s.	1.20 n.s.	4.91[c]	4.81[bc]	4.68[ab]	4.52[a]	5.34[d]	5.22[d]
Cumin	1.21 n.s.	39.30 ***	1.70 n.s.	15.69 ***	2.02 *	0.89 n.s.	4.18	4.20	4.09	4.34	4.14	4.47
Trigeminal sens.												
Astringent	8.21 ***	65.27 ***	0.33 n.s.	8.42 ***	1.62 n.s.	1.01 n.s.	6.70[b]	6.67[b]	6.20[a]	6.18[a]	6.81[b]	6.74[b]
Pungent	13.86 ***	377.89 ***	0.01 n.s.	8.89 ***	0.29 n.s.	1.29 n.s.	4.97[b]	5.02[b]	4.33[a]	4.44[a]	5.10[b]	4.99[b]
Cooling	9.29 ***	555.42 ***	0.29 n.s.	6.04 ***	1.23 n.s.	1.17 n.s.	6.11[c]	6.13[c]	5.57[a]	5.74[ab]	5.84[b]	5.84[b]

Figure 1 black&white

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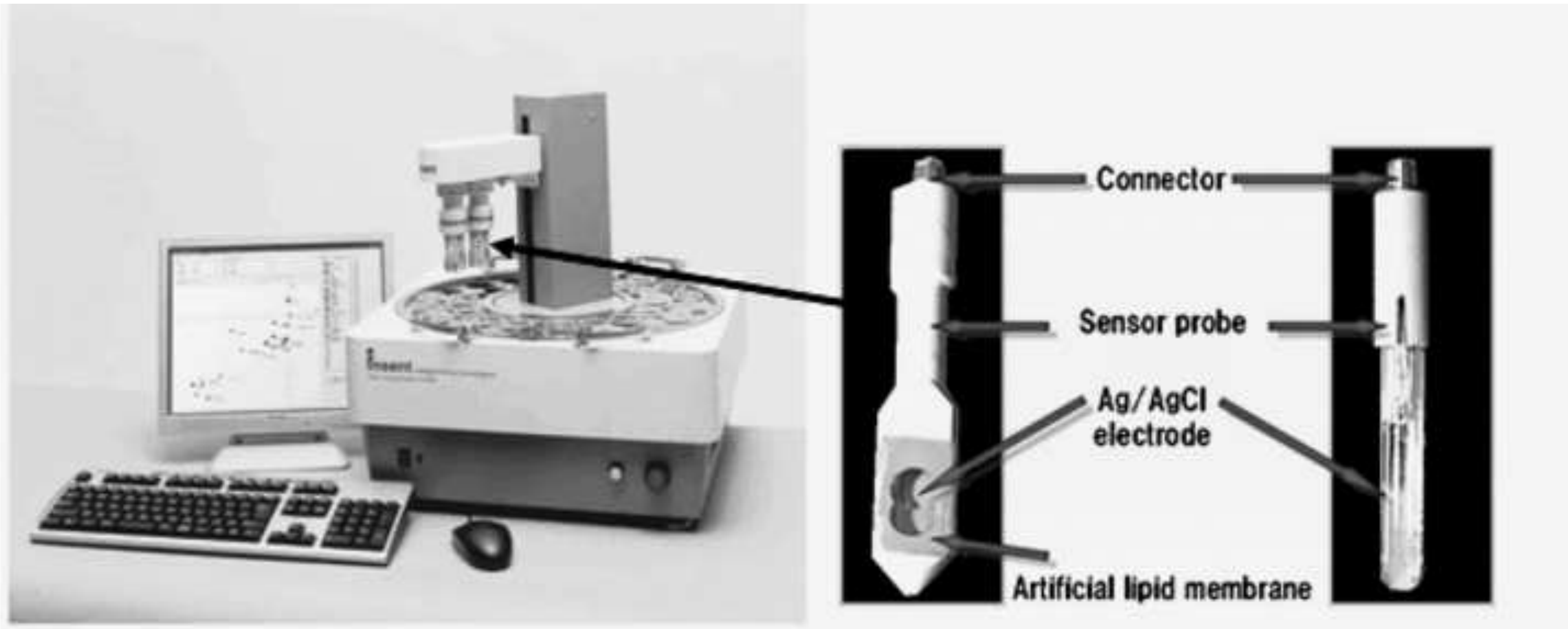

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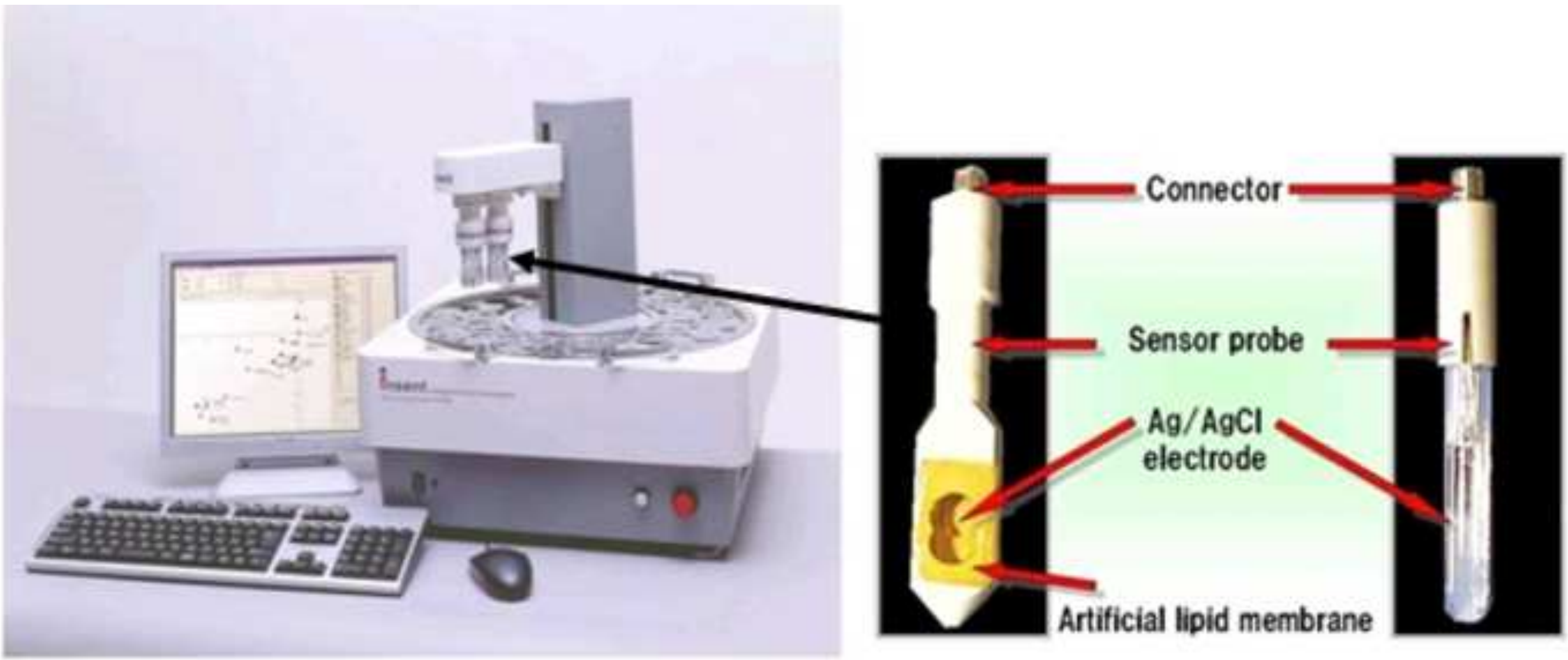

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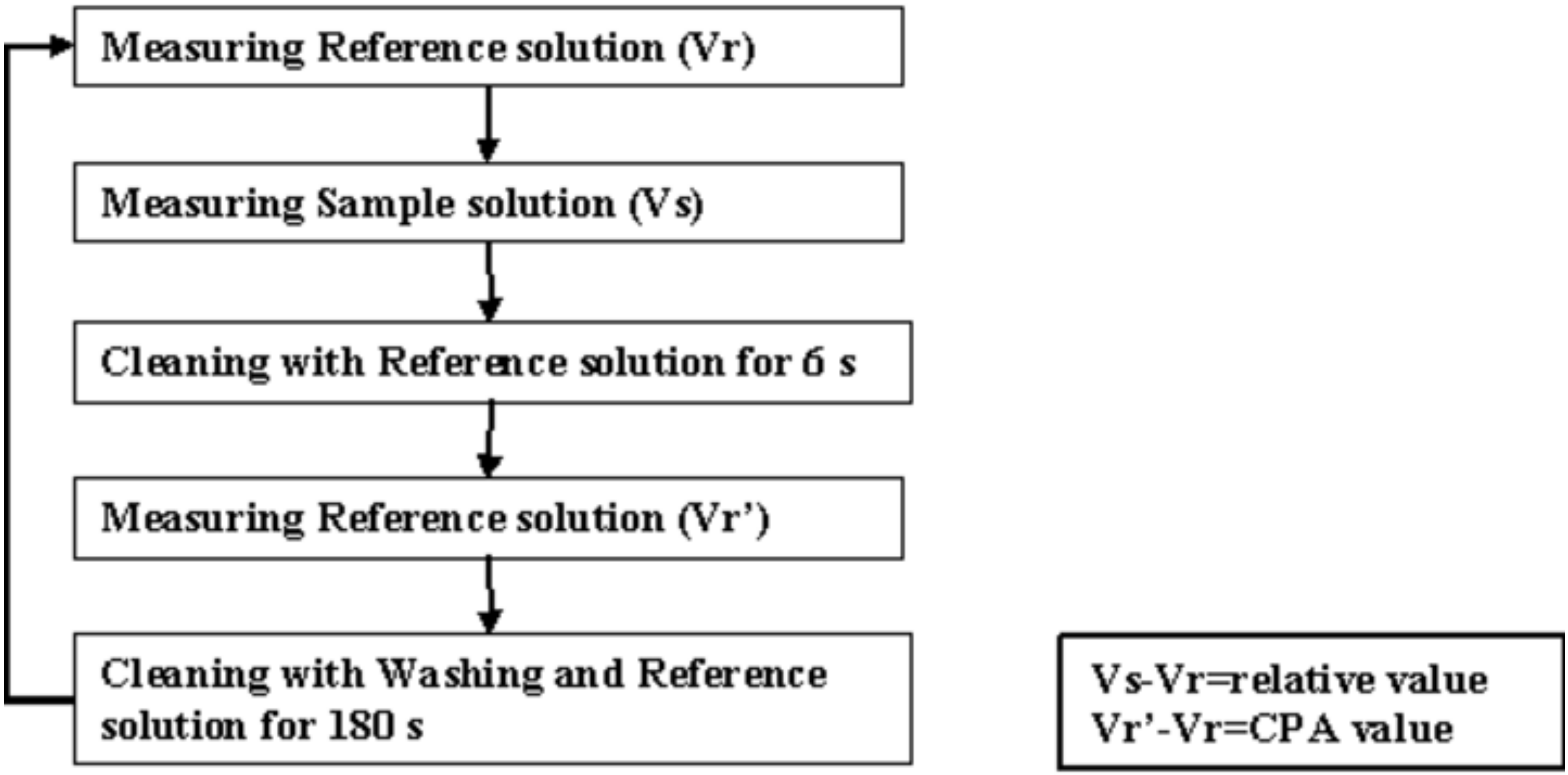

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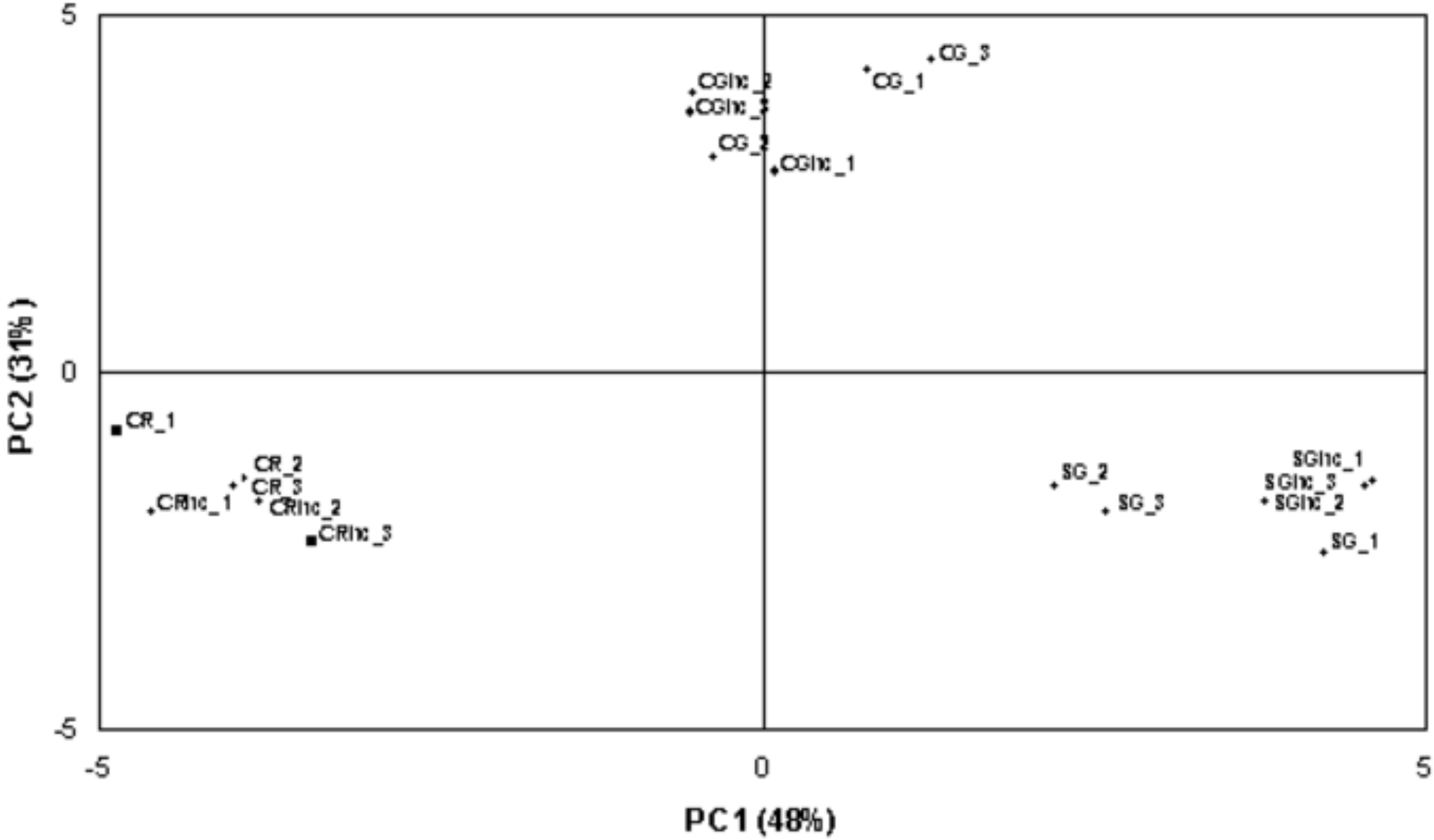

Figure 4
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