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Abstract: Aroma release of two strawberry flavourings (one natural-NS and one nature-identical-NIS) at different concentration (0.15, 0.30 %) added to gummy candies made of pectin, gum arabic and gelatine as structuring agents was studied by gas chromatography/mass-spectrometry (GC-MS) and electronic nose (e-nose) equipped with two different sets of sensors to characterise and differentiate the products. By using GC-MS, different aroma patterns of the pure NS and NIS flavouring were obtained. The effect on the presence and amount of the volatile compounds of the strawberry flavours in the vapour phase was studied and discussed as a function of the candies formulation; the products were partly differentiated, in agreement also with the sensory analysis. The innovative e-nose instrument has differentiated candies based on flavour origin and concentration for both gelatine and gum arabic independently on the sensor array type; for the pectin-based products porphyrines and GNP showed lower and different performances in the discrimination ability.

Cover Letter

FACOLTÀ DI BIOSCIENZE E TECNOLOGIE AGRO-ALIMENTARI E AMBIENTALI

Object: Manuscript for publication on Journal of Food Engineering – Special Issue of the 3rd ISEKI_Food Conference – R2-revised version submission

Dear Editor-in-Chief,
Prof. Paul Singh

Dear Guest Editors
Prof. Cristina L. Silva
Dr. Rui Costa
Prof. Vassiliki Oreopoulou

On behalf of all the authors and in agreement with them, we would like to submit the revised version of the paper titled "Evaluation of aroma release of gummy candies added with strawberry flavours by gas-chromatography/mass-spectrometry and gas sensors arrays" (authors: Pizzoni D.[1], Compagnone D.[1], Corrado Di Natale[2], Nicola D'Alessandro[3], Pittia P.[1]) for publication in the Journal of Food Engineering.

This manuscript has been revised and improved accordingly to the reviewers comments and suggestions and now two new tables have been added. Specific replies to the referees are included.
We do hope that the revised version could have reached the expected quality and clarity and thus, be accepted.

Looking forward to hearing from you soon,

Our best regards

Paola Pittia and Dario Compagnone

Mosciano Sant'Angelo, 10th February 2015

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REPLIES TO THE REFEREE'S COMMENTS

Changes made in the manuscript and evidenced in the text by using the "revision" option.

Figure 1 needs also clarification in terms of 1 and 2 and A and B as in figure 2: "(A: natural; B:natural-identic; 1: 0.15%; 2 0,30%)"

Authors: Legend of Figure 1 has been corrected and the information requested included (line 626)

Table 4 is not cited in the text. Please cite table A and B where appropriate.

Table 4 is now cited in text (see Lines 315 and 334)

Highlights

- The aroma release of flavoring agents of different origin from food matrices needs to be studied
- Gummy candies made of different structuring were added with natural or nature-identical aroma
- Differences in aroma release of the differently formulated candies resulted by GC-MS analysis
- E-nose with two set of sensors discriminated samples by structuring agent and aroma origin.
- Sensory analysis showed results different from those obtained from instrumental analysis

Evaluation of aroma release of gummy candies added with strawberry flavours by gas-chromatography/mass-spectrometry and gas sensors arrays

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Abstract

Aroma release of two strawberry flavourings (one natural-NS and one nature-identical-NIS) at different concentration (0.15, 0.30 %) added to gummy candies made of pectin, gum arabic and gelatine as structuring agents was studied by gas chromatography/mass-spectrometry (GC-MS) and electronic nose (e-nose) equipped with two different sets of sensors to characterise and differentiate the products. By using GC-MS, different aroma patterns of the pure NS and NIS flavouring were obtained. The effect on the presence and amount of the volatile compounds of the strawberry flavours in the vapour phase was studied and discussed as a function of the candies formulation; the products were partly differentiated, in agreement also with the sensory analysis. The innovative e-nose instrument has differentiated candies based on flavour origin and concentration for both gelatine and gum arabic independently on the sensor array type; for the pectin-based products porphyrines and GNP showed lower and different performances in the discrimination ability.

Key words: strawberry flavor, gummy candies, electronic nose, porphyrins, aroma release

1. Introduction

Gummy candies are confectionery products and their texture is achieved by using various gelling agents such as gelatin, starch, gums and pectin. However, the most important ingredients from a quantitative point of view are the sweetener agents (i.e. sucrose, glucose and corn syrups) while colouring agents and aromas are specifically added to give the peculiar sensory attributes to this food commodity (Edwards, 2000; Lubbers and Guichard 2003).

The release of volatile compounds from food matrices is governed by kinetic and thermodynamic phenomena (Voilley and Souchon, 2006; Fabre et al., 2002). Thermodynamic factors determine the partitioning of volatiles between the food and air phases under equilibrium while the kinetic phenomena influence the rate at which equilibrium is achieved (de Roos, 1994) and may affect the rate of perception. Intrinsic properties of the food matrix and extrinsic factors (e.g. temperature, pressure) are involved in both the release rate and the aroma concentration under equilibrium.

Interactions of different nature could be established between matrix compounds and aroma molecules, reducing their concentration in the vapour phase. As a consequence, the same aroma can generate totally different odour sensations when used in different foods or even result in an imbalance in the aroma profile (de Roos, 2006; Guyot et al., 1996).

Several studies have been carried out to study and/or to characterize the aroma release of gelled candies taken in most of the cases as model systems in the attempt to understand the effect of the gelling agent on the retention of the volatile compounds and their release and perception upon mastication (Jangchud, 2013; Deleris et al., 2012; Boland et al., 2006; Lubbers and Guichard, 2003; Kailviainen et al., 1999; Baek et al., 1999).

Increasing amount of hydrocolloids in a food matrix has been shown to increase the thickness of the product associated to a reduction of the perception of flavor. This effect could be due to both specific interactions between the aroma compounds and the matrix, that decrease the partitioning in the vapour phase, and to an increased resistance to the mass transport and diffusion of the volatiles

from the food into the air phase that, in turn, depends on the structural properties and viscosity of the system (Renard et al., 2006).

Sweeteners and, in particular, small saccharides play also a main role in affecting not only the sensory properties of the product but also the textural properties of the hydrocolloids matrix, the viscosity of the aqueous phase and the water activity (a_w). These physical and physic-chemical properties may influence the release and partitioning of aroma compounds, even if they have been scarcely investigated.

In confectionery, flavor properties are due to the addition of different type of aromas that, according to the European Union (EU) legislation, are classified as natural, nature-identical (chemically identical to natural substances but obtained by chemical processes) and artificial (Demyttenaere, 2012). Besides compulsory regulations and labelling, in recent times there is an increasing interest in the use of natural flavourings. In fact, they meet the expectations of the consumers continuously looking for products made of raw and fresh-like materials, ingredients and additives. However this arises some issues for the producers as they are more costly and their stability over time may be lower.

The formulation of a complex formulated products to meet consumer acceptability is a main challenge for the confectionery industry and any change occurring in the recipe, including that of an aroma mixture has to be evaluated as it may affect the release of the volatile odorous compounds thus affecting their perception and the overall sensory quality. The optimization of the flavour quality of foods requires, in general, a deep knowledge and understanding of the of the nature and intensity of the interactions between aroma compounds and no volatile compounds that may occur at molecular level during formulation and processing as well as of the other intrinsic factors and phenomena affecting the aroma release. However, more recently in the candies sector, modern research approaches have pointed out the importance of the evaluation of the aroma profile by innovative ‘in nose’ instrumental techniques, because of the unique physiological factors of the

consumers (e.g. chewing rate, saliva flow, swallowing frequency) affect the release of flavours during eating and thus their perception (Yang et al., 2011).

Besides overall quality and acceptability, aroma compounds and pattern of foods are relevant aspects in relation to the origin of raw materials and ingredients and thus, their authenticity. This is a main issue for food producers even if consumers may not be able to discriminate among food products produced with ingredients and additives, flavouring agents included, of different type and origin.

A number of instrumental methods are used for the determination of single volatile aroma compounds and/or the overall aroma compounds pattern of food products. The known procedures used for the identification and determination of the authenticity of food products and more specifically the individual aroma volatile compounds employ high performance liquid chromatography, gas chromatography with mass selective detection, mass spectrometry, chromatography−mass spectrometry (Reyneccius, 2006), and other methods that require complex equipment and highly skilled personnel. The development of simple test methods based on the quali/quantitative estimation of aroma pattern using sensors is of particular importance for establishing the authenticity of food products and/or discriminate them on the basis of the flavouring origin and related composition. In these last years electronic noses have proved to be very useful tools for food and aromas analysis: applications were proposed both for quality control and study on aroma release in a wide range of food products such as cheese, wine, coffee, tomato and meat (Drake et al., 2003; Michishita et al., 2010; Miettinen et al., 2002; Pinheiro et al., 2002; Sinesio et al., 2000; Vestergaard et al., 2007).

Different kind of sensors can be used to built an electronic nose (e.g. in the cited works MOS, MOSFET, Quartz crystal and optical sensors were used). In this study a Quartz crystal microbalance (QCM) sensors array was used. In literature, QCMs sensors surface have been modified in different ways using porphyrins, peptides or molecular imprinted polymers (Dai et al., 2014; Di Natale et al., 1997; Pizzoni et al., 2014).

The objective of this study was to investigate the influence of the composition (i.e. gelling agents - gelatin, pectin- and sugars) on the release and sensory perception of aroma compounds from candy model systems added with two strawberry flavours, one nature-identical and one natural added in two different concentrations. Strawberry flavoured candies head-spaces were characterised and studied with both GC-MS and electronic nose; sensory analysis was used to test human abilities to discriminate products added with the two different aromas.

For this specific application, both a porphyrin and a peptide based QCMs sensors array were used. In previous studies these kind of modified sensors showed the ability to discriminate different aroma patterns (both in model and complex system) in a large field of application (food included) (Pennazza et al., 2013; Pizzoni et al., 2013; Piccone et al., 2011).

2. Material and Methods

2.1.Materials

All reagents and standards were purchased from Sigma-Aldrich (Milan, Italy). The natural and nature-identical strawberry aromas were from Symrise srl (Milan, Italy) that did not provide their composition. 20 MHz QCM sensors, were from Elbatech (Marciana, Italy).

2.2.Samples preparation

Model gummy candies (average weight of 6.3 g ± 0,1 g) of different formulation were kindly provided by Gelco srl (Castelnuovo Vomano, TE, Italy) that prepared them at laboratory scale by using ingredients and additives and by applying process conditions reproducing the industrial manufacturing procedures. Three different gelling agents were used: gelatin (Gel), gum arabic (GAr) and pectin (Pec). For each structuring gelling agent, two types of strawberry flavours (a natural – NS and a nature-identical- NIS one) at two different concentrations (0.15 $\%_{w/w}$ and 0.30 $\%_{w/w}$, coded as “1” and “2”, respectively) were used. In Table 1 the initial formulation of the model candies is reported. The aroma quantity to be added was computed on the total initial weight of the candy initial formulation. Thus, in total 12 type of samples were prepared, four for each gelling

agent. To avoid any interference on the evaluations, candies were formulated and manufactured without the addition of colouring agents.

The model candies were named as follows: NS-0.15 $\%_{w/w}$: A1; NS-0.30 $\%_{w/w}$ = A2; NIS-0.15 $\%_{w/w}$: B1; NIS-0.30 $\%_{w/w}$ = B2. Aroma was added to the candy mixture after the cooking step and following cooling at a temperature of 45°C. After flavour addition and mixing, the candies were poured in moulds and dried to set the gel at different conditions according to the structuring agent: GAr at 55°C for 41 h followed by a further equilibration step at 20 °C for 31h; Pec and Gel at 22-24°C for 48 h.

2.3 Mechanical properties analysis

Mechanical properties of the candies were determined by creep test by using a dynamometer (mod. 5542 Instron Universal Testing Machine, Wycombe, UK) equipped with a 500 N load head and fitted with a 66 mm diameter cylinder steel probe. Measures were carried out at a constant speed of 0.42 mm s^{-1} until a compression of the 30 % of the initial height of the sample was achieved at 20°C. From the stress/strain curves, the force at maximum deformation (N) and the ratio of elastic deformation to total one (expressed as %) taken as indices of the firmness and elasticity were determined. Data reported are the average of at least 10 repetitions obtained by different samples.

2.4 Chemical and physico-chemical analysis

Moisture was evaluated by gravimetric method at 100 °C in an air forced oven. Water activity (a_w) was measured by a dew-point hygrometer (Aqualab, Decagon, US).

Mono- and di-saccharide analysis was performed by HPLC using a System Gold Beckman 125 (Beckman, U.S.) apparatus equipped with RI detector and an NH_2 column (Lichro CART 250 x 4 mm Lichrospher 100, 5 μm). Analytical conditions were as follows: T_{column}: 25°C; elution solution: acetonitrile/water (75: 25 ratio) at a rate flux of 1.0 ml min^{-1}. Data reported of chemical and physic-chemical analysis are the average of three repetitions obtained on different samples.

2.5 GC - MS analysis

For GC-MS analysis, 500 µL of strawberry aroma or one candy was cut crosswise into four parts and inserted into a 20 mL vial hermetically closed with screw cap and silicone septum. Vials filled with samples was then kept at 40°C for 2 h in a temperature controlled apparatus to reach an equilibration state of the volatile compounds partition in the vapour phase of the head-space. This time was evaluated as sufficient by preliminary experiments (*data not shown*).

A volume of 500 µL of the vapour phase of the head-space were collected with a 1 mL gastight syringe (Hamilton Company, Bonaduz, Switzerland) and injected into a Thermo-Fischer Focus-ISQ (Waltham, MA, USA) single quadrupole GC-MS apparatus. A Thermo-scientific TRACE TR-5MS capillary column (0.25 mm inner diameter, 0.25µm film thickness, 30 m length) was used.

The analyses were carried out in temperature gradient as follow: 3 min at 38°C, temperature increase up to 150°C at 7°C min^{-1}, then final increase up to 250°C at 30°C min^{-1}. The carrier gas (He) flow was 1 mL min^{-1}. MS was working in full scan mode from 33.00 to 350.00 m/z. Ionization was achieved by electron impact working at 70 eV. XCalibur v2.0 software on a DELL elaborator was used for GC-MS data elaboration.

Compounds identification was carried out by using the library of the instrument and by comparison with standards. Area for each peak and total peak area were calculated using XCalibur software. Data reported for each differently prepared product are the average of three different measurements taken from different samples of the same candy type.

To evaluate the effect of the compositional and physical properties of the candy on the release/retention of key aroma compounds a relative aroma release/retention index (A.R. %, Aroma retention/release) was computed as follows:

A.R. (%): $A_{GCarea\ candy}/A_{GCarea\ pure\ aroma}$ (*100):

where $A_{GCarea\ candy}$ corresponds to the area of the specific aroma in the chromatogram of the candies head-space and $A_{GCarea\ pure\ aroma}$ to the area of the specific aroma in the chromatogram of the pure aroma head-space.

2.6 Electronic nose analysis

Electronic nose analyses were carried out on a TEN 2011 apparatus (Tor Vergata Sensors Group, Rome, Italy) connected to a N_2 source and to the sample through a lab made tubing system with two three-way stopcocks. The system was equipped with 8 20 Mhz quartz crystal microbalances (QCMs) as sensing elements. Two different sets of QCMs were used for the analysis: (i) porphyrins modified and (ii) gold nanoparticles(GNP) –peptides modified. In the former set the quartz crystals were coated with with butiloxy-tetra phenyl porphyrins (buti-TPP) coordinating eight different elements (Cu-buti-TPP, Co-buti-TPP, Zn-buti-TPP, Mn-buti-TPP, Fe-buti-TPP, Sn, -buti-TPP H_2-buti-TPP, Mg-buti-TPP) (Santonico et al., 2012). The GNP-peptides based sensors (ii) were functionalized by drop coating, according to the procedure described in Pizzoni et al. (2014). GNP-Peptides were deposited on 20 MHz QCM by drop casting 50 μL of the GNP suspension on each side of the crystal and drying in desiccator at room temperature. GNPs were functionalised as follows: GNP, GNP-Thioglicolic acid (GNP-TA), GNP-Glutathione (GNP-GSH), GNP-Cys, GNP-Cys-Gly, GNP-Cys–Ile– His–Asn–Pro, GNP-Cys–Ile–Gln–Pro–Val and GNP-Cys– Arg–Gln–Val–Phe.

Head-space analysis of samples was carried out as follows: 2 candies, cut crosswise into four parts (porphyrin based sensors) or 1/16 candy corresponding to about 0.4 g (GNP-peptides based sensors) were introduced in a 100 mL sealed glass laboratory bottle, connected through the three-way stop-cocks to the N_2 stream (66 mL min^{-1}). After 20 min stabilization at 37°C, the stop-cocks were opened in order to bring the head space to the sensors chamber. The interaction between volatile compounds and porphyrins/GNP-Peptide generate a decrease in the frequency values down to a steady-state. The stop-cocks were then closed in order to divert the N_2 flow directly in the measurement chamber for sensors cleaning.

The frequency shift (ΔF), taken as the analytical signal, was the difference between the average of the last 10 measurements (1 measurement/s) before injecting the gas sample (baseline) and the average of the last 10 values before closing the stop-cocks (after equilibration time).

Data were taken in triplicate repeating the entire procedure each time, using different candies.

2.7 Sensory evaluation

A triangle test was carried out to detect any overall flavour difference between samples made of Gel, Pec and GAr added with either NS or NIS strawberry flavour. Panel consisted of 20 faculty, staff, teachers and students of the Faculty of Bioscience and Technology for Food and Agriculture of the University of Teramo (aged between 20-50) experienced in sensory evaluations including aroma, but not trained for the specific product while used to consume candies. Panellists were asked to evaluate the samples only by sniffing the vapour phase of the candies. To this aim one candy, cut in 4 pieces was put in a 20 mL glass flask was hermetically sealed with its cup and allowed at 37°C for 30 min to equilibrate the volatiles in the head-space. All candies independently from the structuring agent were similar in shape and colour but the use of different thickeners led to structures with different transparency. Thus, to avoid effects on the evaluation, the glass container was completely covered with aluminium foil and panellist asked only to sniff the head-space after screwing the cup.

Samples were coded with three digit random letters, and each subject received one sets of three coded and randomised samples and asked to follow the sheet's instructions.

Two sessions for each candy type were performed, one in the morning and one in the afternoon.

2.8 Statistical analysis

Statistical analysis were carried out using MatLab R2009b (Mathworks, Natick, MA USA). ANOVA was used to analyse all the differences between group means and their associated variability. Principal component analysis (PCA) was used for multivariate data sets from electronic nose sensors array; PCA was also applied on GC-MS peak area data set.

3. Results and discussion

Moisture content and a_w as well as the main compositional parameters (protein and sugars) of the candies prepared with different structuring agents is reported in Table 2. Since no significant differences were observed due to both the origin of the flavouring agent (NS vs NIS) and its concentration ($p>0.05$), data are reported as the average of all the results obtained from the samples and referred to the gelling agent.

The GAr samples showed the lowest moisture content and water activity value while the highest ones were obtained for the Gel based product. These differences are due to the initial formulation of the candy and its manufacturing procedure that was slightly different according to the gelling agent and that allows to obtain final products with textural properties similar to the conventional reference commercial product.

Depending on the structuring agent, candies with different mechanical properties were obtained (Table 2). Gel samples resulted with a relative low hardness but the highest elasticity as also found by other authors in candies (Kalvianen et al., 1999). Products made of pectin and gum arabic showed similar mechanical properties despite their composition being their moisture and sugars content significantly different. Moreover, their hardness and elasticity were higher than those determined in the Gel candies. These results may depend on the specific formulation and concentration of the hydrocolloids used to produce the candies with textures similar to commercial products. Some studies have evidenced that the concentration of gelling/thickener agents as well as of sugars can affect the textural properties of gelled products, like candies, with effects on the retention and release of volatile compounds also upon mastication and/or chewing (Deleris et al., 2011; Boland et al., 2006; Kalvanien et al, 1999; Guinard and Marty,1995,).

GC – MS analysis

Gas chromatographic analysis of the vapour phase of both pure NS and NIS aroma mixtures was preliminarily carried out to evaluate their initial composition and in Table 3 the relative GC area

(%, on the total GC peak area) is reported. In total 29 volatile compounds were detected and 25 out of them identified. Relative standard deviation (RSD) was <12% for all the compounds.

Ethyl hexanoate, eucalyptol**,** ethyl octanoate and one not identified compound were detected only in the vapour phase of the NS flavouring, while ethyl propanoate**,** β-myrcene**,** 1,4-cineole and octanal only in the NIS one. Moreover, the relative concentration of the volatile compounds in the vapour phase of the two aromas was significantly different for the majority of them (e.g. ethanol, ethyl 2-methylbutanoate) confirming their dissimilarity due to both the different origin and manufacturing procedure affecting their final chemical composition.

Head-space analysis of candies under equilibrium conditions was then performed. Very different patterns of volatile compounds in terms of both presence and amount were found. This is evidenced in Figure 1 where the mean of the sum of the GC areas of the differently formulated products is shown. By comparing products added with the same aroma type, for both aromas (NIS and NS), the highest total GC area has been determined in the Gel samples while the lowest in the GAr candies. This result could be due to a different retention ability of the candies characterised by different thickening agents and composition as well as to the mechanical properties of the viscoelastic gelled candies. Increased hardness actually has been related to a more structured systems able to retain the volatile compounds also by entrapment in and/or obstruction of their mass transport the three-dimensional matrix (Kalvanien et al, 1999).

The addition of the aroma in a higher concentration (0.30% vs 0.15%) led to an overall higher release of the volatile compounds in the vapour phase even if the effect was not always proportional (i.e. no double sum of total GC peak area).

As regards the qualitative pattern of the volatile components present in the vapour phase of the candies, some differences were observed with respect to the composition of the initial NIS and NS aroma. In particular, two compounds (2,3-butanedione and 3-hydroxy-2-butanone) appeared in the vapour phase of the candies while they were not detected in that of the pure NS and NIS aroma. This result can be attributed to the contribution of volatile compounds present in other ingredients

used in the product formulation to the aroma pattern and/or the thermal conditions adopted during the production process that may have induced their formation from precursors.

Relative standard deviation (RSD) of the GC area of the volatile compounds in the vapour phase of the candies under study (*data not shown*) was <12% for all molecules except for 2,3-butanedione, cis-3-hexenol, limonene and cis-3-hexenyl-iso-butanoate in Pec structured candies being for all these volatiles significantly higher up to 50%. Despite the care taken in the manufacturing of the candies samples at lab scale and in the vapour sampling for the head-space analysis, the higher variability of the GC results may be index of a non uniformity of the Pec-made products that could also impair the flavour release in the vapour phase, by increasing the quantity of released aroma.

In order to better highlight the matrix effect on the vapour partition of the volatile aromas of the strawberry flavours added in candies at two different concentrations, ten compounds (ethanol, ethyl acetate, ethyl butanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, cis-3-hexenol, ethyl hexanoate, limonene, cis-3-hexenyl isobutanoate and decanal) were selected as representative of different chemical classes and polarity (based on the logP) and the Aroma release/retention index (A.R.%) was computed (Table 4).

For sake of clarity, A.R. (%) values above 100 indicate a higher release of the specific volatile compound from the matrix when present in the candy with respect to that occurred in the pure aroma; values below 100, a higher retention.

It can be noticed that some of the selected aroma compounds determined in the vapour phase of the pure aroma were not present (nd, not determined) in that of the of corresponding flavoured candies (e.g. decanal in NIS). In the GAr gelled candies, ethyl hexanoate and cis-3-hexenyl-iso-butanoate were also not detected, while they were in the Pec and Gel products.

In general, a A.R.% lower than 100 were observed for the majority of the aroma compounds with major differences due to the physico-chemical properties of the aroma compounds, moisture and composition of the matrix including potential interactions between the volatile and the no volatile compounds (in particular, the gelling agents) in the complex “candy” systems. In particular volatiles

like ethyl butanoate, limonene and ethyl hexanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate (when detected) showed a very low A.R.% (<15%); for other ones the A.R.% was higher but still below 100 (ethanol and decanal). On the other hand in NSI flavoured candies, A.R. % values of ethyl acetate, cis-3-hexenol (only in GAr B2 and Gel B2) and cis-3-hexenyl isobutanoate (in Pec B2)were higher than 100% to indicate a higher release than that occurred from the pure flavouring agent.

Differences of A.R.% of specific aroma compounds were observed also between the candies added with the strawberry natural (NS, Table 4A) or nature-identical type (NIS, Table 4B), likely affected by their different relative concentration in the pure flavouring. I.e., ethyl acetate showed an A.R.% ~~ratio~~ <4 % for the NS added candies while it was >90% for the NIS flavoured candies. Since candies were prepared according to the same protocol for each thickening agent, this could be the result of potential interactions with the no volatile compounds, (possibly concentration-dependent), and the effect of the other volatiles in the flavouring mixture that may could have differently affected their solubility and retention in the matrix during the candy manufacturing and/or analysis (Schober and Peterson, 2004).

Literature data report that the decrease of the aroma release in high viscous and/or gelled foods could be attributed to the thickener agent providing a barrier to diffusion affecting the kinetics of the volatile vapour partition or even obstruct their mass transport as well as to specific molecular interactions between the aroma compounds and the thickener (Terta et al. 2006, Renard et al., 2006; Roberts et al., 1996). Differences in the retention behaviour highly depend on the polarity and/hydrophobicity of the volatile compounds. Polar compounds (e.g. ethanol, ethyl butanoate) are more soluble in water and can diffuse more easily through the matrix with a general lower retention. Presence of solutes (e.g. low molecular saccharides) that can affect water state may also either favour or hinder the retention (Dalla Rosa et al., 1994) of the more polar compounds that are more sensitive to the availability of free water with solvent ability. As regards the more hydrophobic ones (e.g. limonene) it is likely that they form hydrophobic inclusion complexes through hydrogen

bonding of water molecules with the equatorial hydroxyl groups of gelling macromolecules as evidenced by Roberts et al (1996) in the case of polysaccharides.

In our study thickeners of different origin and gelling performances were used; the mechanism that induces a reduced flavour release from pectin, gelatine and gum arabic could be thus different and still under investigation. Gelatin is a proteic gelling agent obtained from collagen that together with water forms a semi-solid colloidal gel that exhibit a coil-to-helix or disordered-to- ordered transition upon cooling (Renard et al., 2006). Several studies have evidenced that the reduced release of aroma compounds do not depend on specific interaction at molecular level molecular but rather by entrapment mechanisms that obstruct the mass transfer (Zafeiropoulou et al., 2012; Baek et al., 1999; Harrison and Hills, 1996). Also for pectin several studies have evidenced that no or limited interactions may occur between the volatile compounds and this hydrocolloid in gelled system and that the main mechanism reducing aroma release is the hindered molecule migration through the three-dimensional structure (Rega et al., 2002). However, Tromelin et al., (2010) observed a decrease of the partition coefficient of a series of volatile compounds in pectin gels and by a quantitative structure–activity/structure– property relationships reached the conclusion that interactions with pectin may occur and involve positively charged surface areas in the retention phenomena.

On the other side gum arabic is a complex mixture of polysaccharide and glicoproteins (Sutherland, 2006). Besides its gelling abilities, it shows unique properties (emulsification, acid stability, low viscosity at high concentrations, etc.) thereby used for flavor encapsulation exerting interesting retention abilities concentration-dependent (Rosenberg et al., 1999).

In our study we observed quite different and significant effect due to the structuring agent used to manufacture the candies as different retention effects were observed in candies made with the same thickening agent as affected by the strawberry flavouring agent origin (NS vs. NIS). For the NS added candies, the A.R.% of most of the selected volatiles showed similar values between Pec and

Gel products, while in the NIS samples the value of A.R. % was not significantly different when considered in GAr and Pec samples.

On the basis of these results it could be concluded that the release/retention of the volatile compound of the two strawberry flavouring agents in the differently made candies could have been the result of the complex combination of several factors and phenomena including the intrinsic properties (chemical and physico-chemical and structural-mechanical properties) and the potential interaction between the aroma compounds and the other components in the matrices.

The effect of the process conditions of the candies manufacturing has to be also considered as the GAr samples, due to the structuring agent, were subjected to a drying step at 55°C not applied for the Pec and Gel ones. GC analyses were carried out to evaluate the effect of the drying step on the loss of the specific volatiles and in Table 5 the change of concentration of selected volatile compounds of differently formulated candies added with NIS (0.15%) after the drying step (in respect to the corresponding product just after aroma addition) are reported. The relative loss varied depending on the aroma compound, candy formulation and related gelling agent, thus drying conditions.

In conclusion, the obtained GC-MS data demonstrated that different molecules can give very dissimilar behaviour and their release in the head-space of candies is dependent on the structuring agent, the origin of the aroma used (and thus by all the volatiles present in the latter) and its concentration that can affect the head-space in dissimilar and not easily predictable ways.

Principal components analysis (PCA) was used to study the main sources of variability between the different candies based on the aroma composition of the vapour phase in equilibrium evaluated by GC analysis. PCA has been calculated by autoscaling the data obtained to have comparable data and score plot are reported in Figure 2.

The first two principal component explained most of the variance among the samples (72%) while by including the third one it was possible to represent over 87% of the total variance.

403 Results evidence that on the basis of the GC-MS data the NSI added-samples are clearly
404 distinguished from the NS-added candies along the PC1 (A *vs*. B samples).
405 Moreover, the increase of the concentration of added aroma in the formulation (1 *vs*. 2 samples)
406 increases the distance in the plot and the corresponding classification ability, for both the natural
407 and nature-identical strawberry flavoured candies. This behaviour of the GC dataset was expected
408 by taking into account the main differences in the presence and relative concentration of the volatile
409 compounds in the head-space for both aroma types. Moreover the different concentration of the
410 aroma added in the candy formulation was discriminated along the PC3 even if the trend was not
411 linear. The different structuring agents were not clearly discriminated being located in different
412 places of the score plot.
413 The higher variability of the release of the volatile compounds in the head-space of the samples
414 made of pectin candies led to a higher dispersion of the data in the PCA score plot while those
415 obtained from the gum arabic or gelatine candies appear more close and grouped.
416
417 **Electronic Nose analysis**
418 The use of gas sensors arrays to evaluate the vapour phase generated by the differently flavoured
419 candies made of three different gelling agents was carried out in order to evaluate the feasibility of
420 no conventional and alternative instrumental techniques and methods for process and product
421 quality control. To this regard, e-noses represent ideal candidates because of the very limited cost of
422 instrumentation and ease of the procedure of the analysis.
423 Analysis of the samples was carried out using two different set of sensors, equipped with either
424 different porphyrins as binding agents or peptide modified gold nanoparticles (GNP-peptide).
425 While the use the former kind of sensors is well established in the literature, the peptide-based
426 sensor array has been recently developed by some researchers of this study (Compagnone et al.,
427 2013). It was thus of interest to evaluate the performances of both the sensor arrays on the
428 differently formulated candies added with strawberry aroma of different origin. In preliminary tests

429 different sensitivity of the two set of sensors was observed, confirming the results obtained in
430 previous studies (Compagnone et al., 2013). For these reason the sample used for the analysis was
431 significantly different, being corresponding to 1/16 of a candy and 2 candies for the GNP-peptide-
432 and porphyrin-based sensors, respectively.
433 The PCA score plots from the e-noses results are reported in Figure 3. The first three principal
434 components explained most of the variance along the samples with the higher one explained by the
435 GNP-peptide e-nose (95,05%) than that observed for the results obtained from the porphyrin-based
436 instrument (69,18%).
437 Under the conditions used, the GNP-peptide sensor array was able to better discriminate the
438 samples on the basis of the gelling agent used clearly evidenced by the distinct position of the data
439 in different parts of the three-dimensional score plot. This could be attributed either to the improved
440 sensitivity obtained by using the nanostructured material or the high variability of the peptides
441 interacting with the set of the volatiles of the headspace, in agreement with the results obtained in
442 other studies (Compagnone et al., 2013).
443 However, in a previous study carried out on candies of different thickening agents including also
444 pectin and gelatine, but not gum arabic by using the same porphyrin based e-nose, Piccone et al
445 (2011) found significant differences among all the gummy samples with an explained variance of
446 95%; in particular the ΔF signals of the pectin and gelatin products resulted located in opposite
447 regions and, thus, inversely correlated. The ability to discriminate samples based on the different
448 structuring agent was not the main aim of this study and could have a limited interest also for a
449 potential application in the quality control.
450 The e-nose data obtained from both the porphyrins and the GNP-peptide sensors sets were then
451 statistically processed according to the structuring agent of the candies in order to evaluate their
452 discriminant ability based on the strawberry flavouring origin and concentration by taking into
453 account the pattern of the volatile compounds release and the correspondent sensor array output.

The resulting three PCA scores plot for both series of sensors array set are reported in figure 4. For the GNP-peptide e-nose data (b, d, f) the first 3 principal components explained 91.14%, 93.54% and 97.59% of the total variance for GAr, Gel and Pec data, respectively while for those obtained from the porphyrin based one (a, c, e) the total variance for the same candy matrices obtained by the first two (Gel) or three PCs was slightly lower (88.08%, 74.39%, 80.45%, respectively).

From the plots obtained it is evident that both sensor arrays clearly able to discriminate among data obtained from samples added with NSI and NS flavour mixture at the two different concentration for the products made with gum arabic and gelatine while no clear discrimination is obtained for pectin based formulations. This latter result may be also be related to those obtained from the GC-MS analysis where a higher variability of the chromatographic areas for the aroma compounds in the vapour phase of the Pec-made candies (both with NS and NIS added samples) was observed even if in that case a statistical differentiation was achieved.

A cluster analysis obtained matching the e-nose results of the four different combination of aromas (NS and NSI) and concentrations (0.15% and 0.30%) confirmed a complete differentiation of the Gel-candies by both sensors arrays, and a good differentiation among those made of gum arabic. In the latter case, the results obtained from candies at the two different concentrations of natural identic aroma which were not completely separated by the GNP-peptide based array.

Sensory analysis

Triangle sensory test was carried out to evaluate the ability of human olfactory system to discriminate candies made with the same structuring agent and added with the same aroma concentration, but of different origin. Results evidenced a 75% of the correctness of the panellist were able to discriminate samples based on the aroma type (NS and NSI) with a risk error lower than 0.1%, independently from the structuring agent when candies added with the highest concentration (0.30%) were tested.

In the case of the product added with the lowest concentration, a lower degree of correctness was observed (62, 40 and 68 %, for GAr, Gel and Pec, respectively) with a α index, correlated to the probability to evidence a difference between the samples made with the flavouring of different origin of 5 %, 32 % and 1% for GAr, Gel and Pec, respectively. Gelatin made candies added with NSI or NS seem thus, are not easily discriminated by human senses.

This result seems to be opposite to those obtained by the instrumental analysis based on both the GC-MS and e-noses, since the aroma pattern of the Gel-candies made with NS and NIS was discriminated. However, sensory analysis takes into account the overall odour quality that is due the combination of that of each volatile compound present in the headspace and its relative concentration that cannot be predicted by both GC-MS and e-nose measurements. For this reason, it appears of paramount importance the instrumental control of the gas phase both for quality control by the food industry and for traceability and prevention of potential frauds on the consumers side.

Conclusions

The results of this study, where conventional and innovative instrumental techniques were used to characterise the aroma pattern of gummy candies made of different thickening agents and added with strawberry flavourings of different origin, highlight the complexity of the aroma release in gelled systems like candies.

The role of the gelling agent and the composition of the products on the release of the aroma compounds present in the flavouring mixture, each of them characterised by different chemical and physico-chemical properties, was evidenced by GC-MS and e-nose analysis, allowing a significant discrimination, in particular for the products made of gelatine and gum arabic.

GNP-peptide based electronic nose sensors sets provided a better discrimination between samples structured with different texturing agents than the porphyrin based one, while the latter showed slightly better performances for the discrimination of different kind of aroma at different concentration in candies structured with the same agent. This result could foresee the feasibility of

the application of both types of e-nose for the discrimination of the candies added with flavour mixtures of different origin.

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Legends of Figures

FIGURE 1: Total gas-chromatographic area of the vapour phase of the differently formulated candies (A: natural; B: natural-identic; 1: 0.15%; 2: 0.30 %).

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FIGURE 2: PCA plots of GC-MS data of candies prepared with different thickening agents and added with either NS or NIS strawberry flavour at two different concentrations (A: natural; B: natural-identic; 1: 0.15%; 2: 0.30 %).

FIGURE 3.A-B: PCA scores plot of porphyrin based (A) and GNP-Peptide based (B) electronic nose data of the vapour phase of all differently formulated candies (A: natural; B: natural-identic; 1: 0.15%; 2: 0.30 %).

FIGURE 4. PCA scores plot of porphyrin (a, c, e) and peptide based (b, d, f) electronic nose data for gum arabic (a, b), gelatin (c, d) and pectin (e, f) candies (A: natural; B: natural-identic; 1: 0.15%; 2: 0.30 %).

Table

Ingredient/additive	Concentration % (w/w)		
	Gel	Pec	GAr
Water	18,0	17,0	-
Sucrose	22,0	39,0	20,0
Glucose syrup	51,5	40,0	19,0
Citric acid solution	2,0	2,0	-
Sorbitol	-	-	3,5
Glycerol	-	-	1,5
Gelatin	6,5	-	-
Pectin	-	2,0	-
Arabic gum solution (50 %GAr)	-	-	56,0

TABLE 1: Initial formulation of gelatin (Gel), pectin (Pec) and gum arabic (GAr) candies.

Parameter	Gel	Pec	GAr
Moisture (%)	18.90±0.23[a]	13.97±0.58[b]	11.34±0.49[c]
a_w	0.709±0.004[a]	0.689±0.005[b]	0.616±0.012[c]
Proteins (% d.m.)	9.40±0.45	nd	nd
Mono- and di-saccharides (% d.m.)	68.20±2.11 [b]	85.30±1.35 [a]	49.45±0.45 [c]
Maximum force (N)	9.01±0.59 [c]	20.13±2.35 [a]	16.20±1.64 [b]
Elasticity (%)	77.19±1.77 [a]	40.44±2.10 [b]	42.99±2.68 [b]

[a-c]: Different letters for the same compound indicate samples with significant statistical differences of the means ($p<0.05$)
nd: not determined; a_w: water activity: d.m.: dry matter

TABLE 2: Compositional and mechanical properties of candies made of gelatin (Gel), pectin (Pec) and gum arabic (GAr)

	GC Peak area (%)	
Volatile compounds	**NS**	**NIS**
Ethanol	0.28	5.61
2-Methylbuthanal	0.69	0.29
Ethyl acetate	0.40	0.04
Not identified	0.77	0.17
Not identified	0.20	0.003
Ethyl propanoate	nd	0.04
Not identified	0.09	0.03
1,2-Propandiol	0.11	0.03
Ethyl isobutanoate	0.08*	0.10*
Not identified	0.04	nd
Ethyl butanoate	81.90*	90.83*
Ethyl 2-methylbutanoate	5.67	0.04
Ethyl 3-methylbutanoate	3.81	0.02
cis-3-Hexenol	0.14	0.43
α-Pinene	0.20	0.07
β-Pinene	0.22	0.11
β-Myrcene	nd	0.05
Ethyl hexanoate	0.46	nd
Octanal	nd	0.01
1,4-Cineole	nd	0.01
o-Cymene	0.09	0.003
Limonene	3.58	1.87
Eucalyptol	0.01	nd
γ-Terpinene	0.14	0.05
α-Terpinolene	0.05	0.02
Nonanal	0.15	0.04
cis-3-Hexenil isobutanoate	0.28	0.06
Ethyl octanoate	0.51	nd
Decanal	0.14	0.08
Total	100	100

Nd: not determined

*: samples with no significant statistical differences ($p>0.05$)

TABLE 3: Composition of the natural (NS) and nature-identical (NIS) strawberry flavor expressed as GC-MS peak area (% on total GC area)

	Natural Strawberry (NS) flavor candies					
	GAr A1	**GAr A2**	**Pec A1**	**Pec A2**	**Gel A1**	**Gel A2**
Ethanol	31 ± 5 [b]	91 ±9 [a]	16 ± 1 [c]	29 ±5 [b]	15.3 ±0.9 [c]	31 ± 2 [b]
Ethyl acetate	1.33 ± 0.03 [h]	3.2 ±0.8 [f,g]	2.8 ± 0.3 [f]	2.9 ±0.3 [f]	1.6 ±0.2 [h]	2.01 ± 0.02 [g]
Ethyl butanoate	0.09 ± 0.02 [i]	0.24 ±0.04 [g]	2.1 ± 0.1 [c]	3.2 ±0.8 [a,b,c]	1.6 ±0.1 [d]	3.5 ± 0.2 [a,b]
Ethyl 2-methylbutanoate	0.9 ± 0.1 [c]	3.2 ±0.2 [b]	3.3 ± 0.2 [b]	5 ±1 [a,b]	2.66 ±0.07 [c]	5.3 ± 0.1 [a]
Ethyl 3-methylbutanoate	1.0 ± 0.2 [c]	3.4 ±0.3 [a]	3.4 ± 0.2 [a]	6 ±2 [a,b]	3.1 ±0.2 [a]	5.6 ± 0.3 [b]
cis-3-Hexenol	95 ± 16 [e]	489 ±47 [a]	217 ± 31 [c]	404 ±110 [a,b]	146 ±9 [d]	492 ± 28 [a]
Ethyl hexanoate	nd	nd	6.5 ± 0.6 [b]	11 ±3 [a,b]	6.22 ±0.02 [b]	11.9 ± 0.3 [a]
Limonene	0.52 ± 0.09 [k]	1.5 ±0.2 [d,i]	2.0 ± 0.2 [f,h,i,j]	3.1 ±0.4 [e,c,g]	2.1 ±0.1 [b,i]	3.5 ± 0.4 [a,e]
cis-3-Hexenyl isobutanoate	nd	nd	13 ± 2 [d]	22 ± 8 [c,d]	16 ±3 [c,d]	21 ± 2 [c]
Decanal	nd	nd	17 ± 1 [c]	28 ± 8 [b,c]	41 ±10 [a,b]	53.4 ± 0.8 [a]

A)

	Nature-identical Strawberry (NIS) flavor candies					
	GAr B1	**GAr B2**	**Pec B1**	**Pec B2**	**Gel B1**	**Gel B2**
Ethanol	5.0±0.9 [e]	14±1 [c]	3.2±0.4 [f]	14±1 [c]	4.7±0.5 [e]	11±1 [d]
Ethyl acetate	165±16 [a,c,d]	120±28 [c,d,e]	170±17 [b,c]	188±32 [a,b]	120±9 [e]	106±6 [e]
Ethyl butanoate	0.15±0.01 [h]	0.37±0.01 [f]	1.2±0.1 [e]	3.2±0.4 [b]	1.9±0.1 [c]	4.1±0.3 [a]
Ethyl 2-methylbutanoate	nd	nd	nd	nd	nd	nd
Ethyl 3-methylbutanoate	nd	nd	nd	nd	nd	nd
cis-3-Hexenol	66±10 [e,f]	311±24 [b]	66 ± 6 [f]	256±54 [b,c]	92±10 [e]	250±42 [b,c]
Ethyl hexanoate	np	np	np	np	np	np
Limonene	1.0±0.3 [d,k]	1.7±0.1 [i,j,l]	1.4 ± 0.2 [d,l]	7±4 [a,b,c,d,e,f,i]	2.4±0.3 [b,g,h]	4.8±0.8 [a]
cis-3-Hexenyl isobutanoate	nd	nd	40 ± 1 [b]	227±113 [a]	93±21 [a]	213±73 [a]
Decanal	nd	nd	nd	nd	nd	nd

[a-i]: Different letters for the same volatile compound (including both NS and NIS added candies) indicate samples with significant statistical differences ($p<0.05$)
np: not present
nd: not determined

TABLE 4.A-B: Aroma release/retention index (A.R.) values (%) of selected aroma compounds of candies added with NS (A) and NIS (B) (1: 0.15%; 2: 0.30 % added aroma concentration).

	Concentration (%)		
Volatile compound	**Gel**	**Pec**	**GAr**
Ethyl acetate	98.2±0.9[b]	99.5±0.1[a]	97.5±1.0[b]
Ethyl butanoate	23.6±1.7[c]	64.3±2.0[a]	45.1±2.9[b]
cis-3-Hexenol	82.5±3.4[b]	92.3±2.1[a]	86.0±1.7[b]
Limonene	47.6±4.3[b]	99.2±0.3[a]	38.8±3.8 [c]

[a-c]: Different letters for the same compound indicate samples with significant statistical differences of the means ($p<0.05$)

TABLE 5: Effect of the drying step on the concentration of selected volatile compounds in candies made of gelatin (Gel), pectin (Pec) and gum arabic (GAr) added with NIS (aroma: 0.15%)

Figure 1

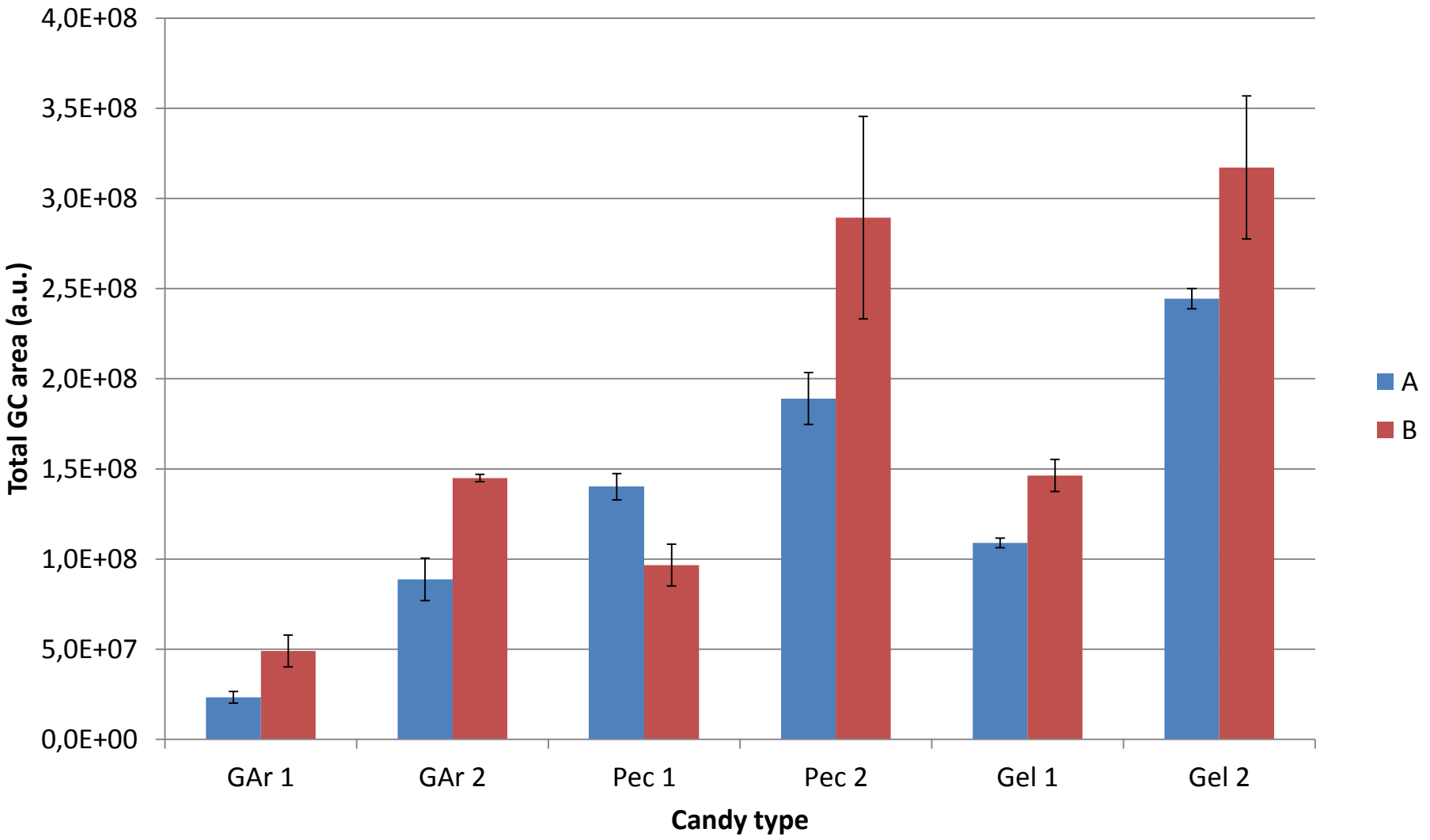

4,0E+08
3,5E+08
3,0E+08
2,5E+08
2,0E+08
1,5E+08
1,0E+08
5,0E+07
0,0E+00
Total GC area (a.u.)
GAr 1
GAr 2
Pec 1
Pec 2
Gel 1
Gel 2
Candy type
A
B

Figure 2

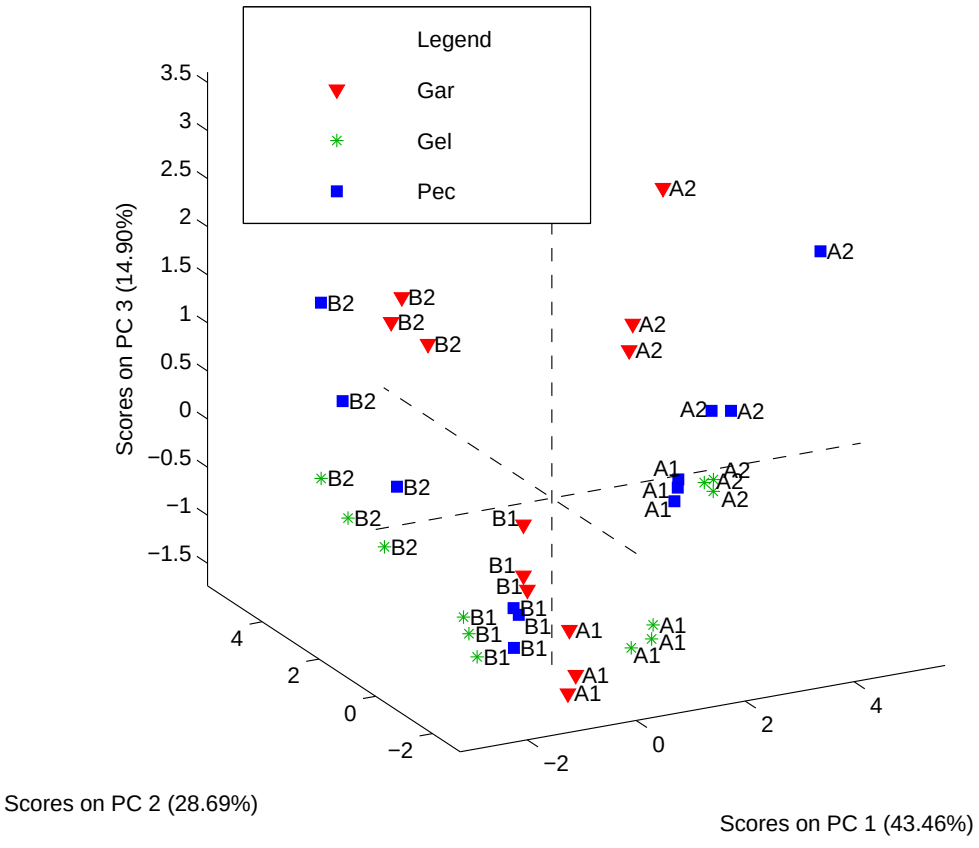
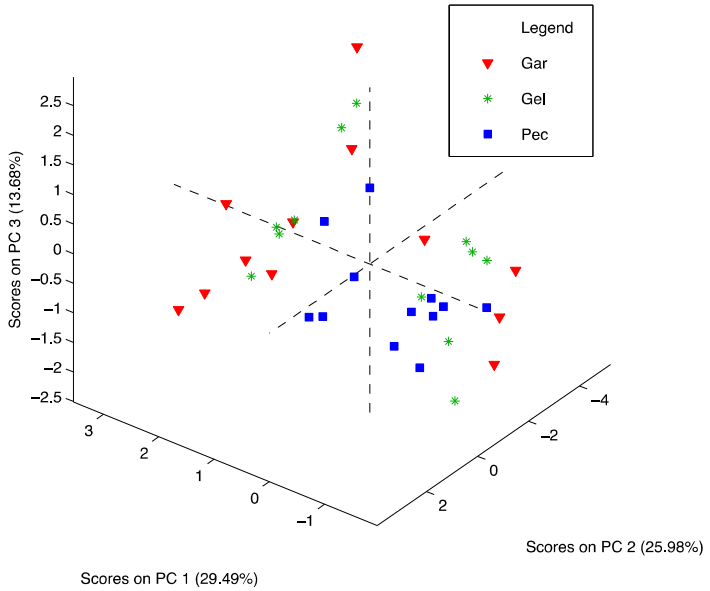
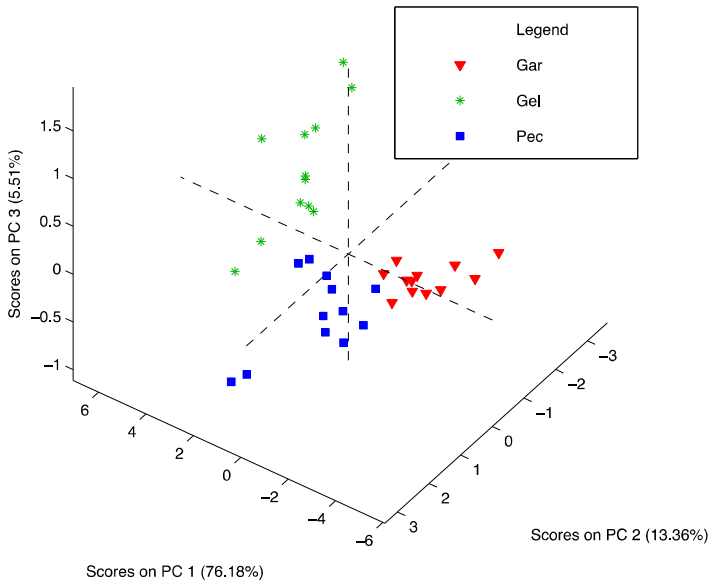

Legend
Gar
Gel
Pec
Scores on PC 3 (14.90%)
Scores on PC 2 (28.69%)
Scores on PC 1 (43.46%)

Figure 3 total



A)



B)

FIGURE 3.A-B: PCA scores plot of porphyrin based (A) and GNP-Peptide based (B) electronic nose data of the vapour phase of all differently formulated candies (A: natural; B: natural-identic; 1: 0.15%; 2: 0.30 %).

Figure 3a

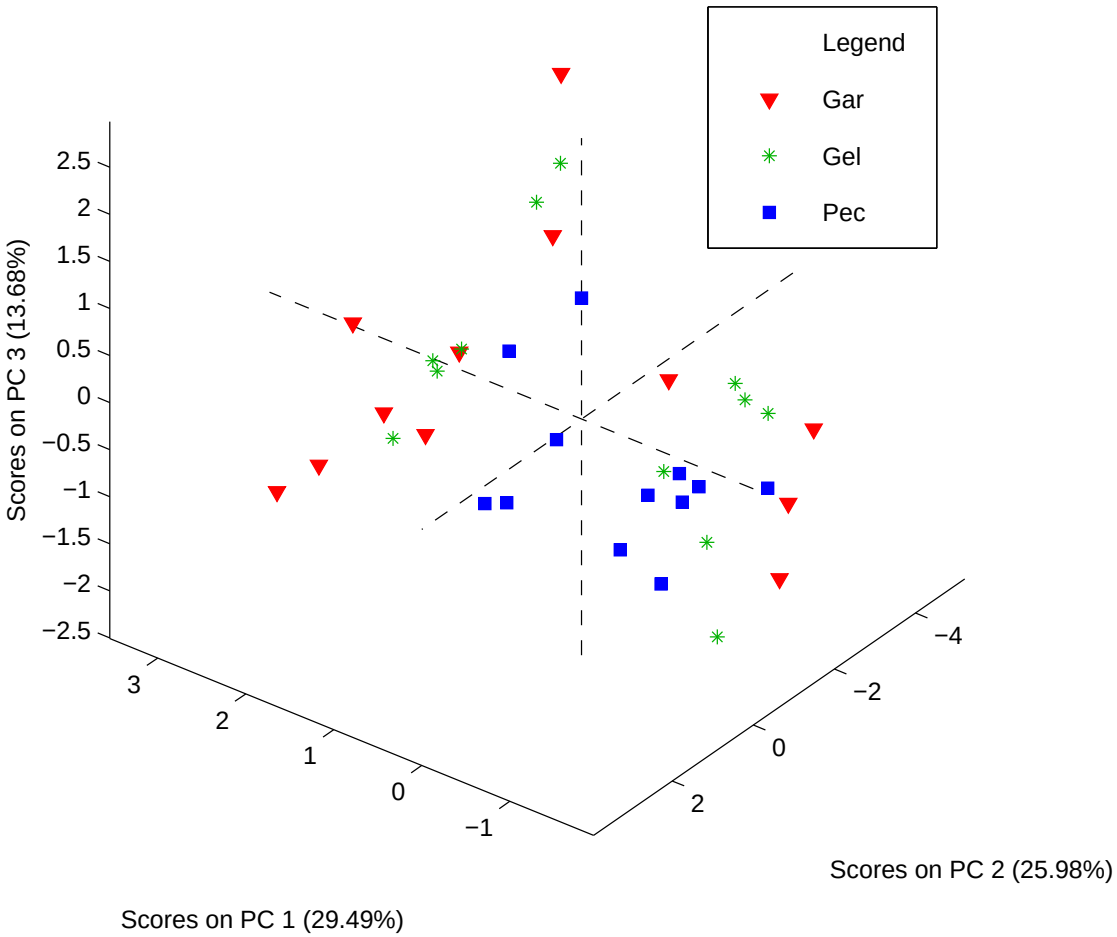

Legend
Gar
Gel
Pec
Scores on PC 3 (13.68%)
2.5
2
1.5
1
0.5
0
−0.5
−1
−1.5
−2
−2.5
3
2
1
0
−1
2
0
−2
−4
Scores on PC 1 (29.49%)
Scores on PC 2 (25.98%)

Figure 3b

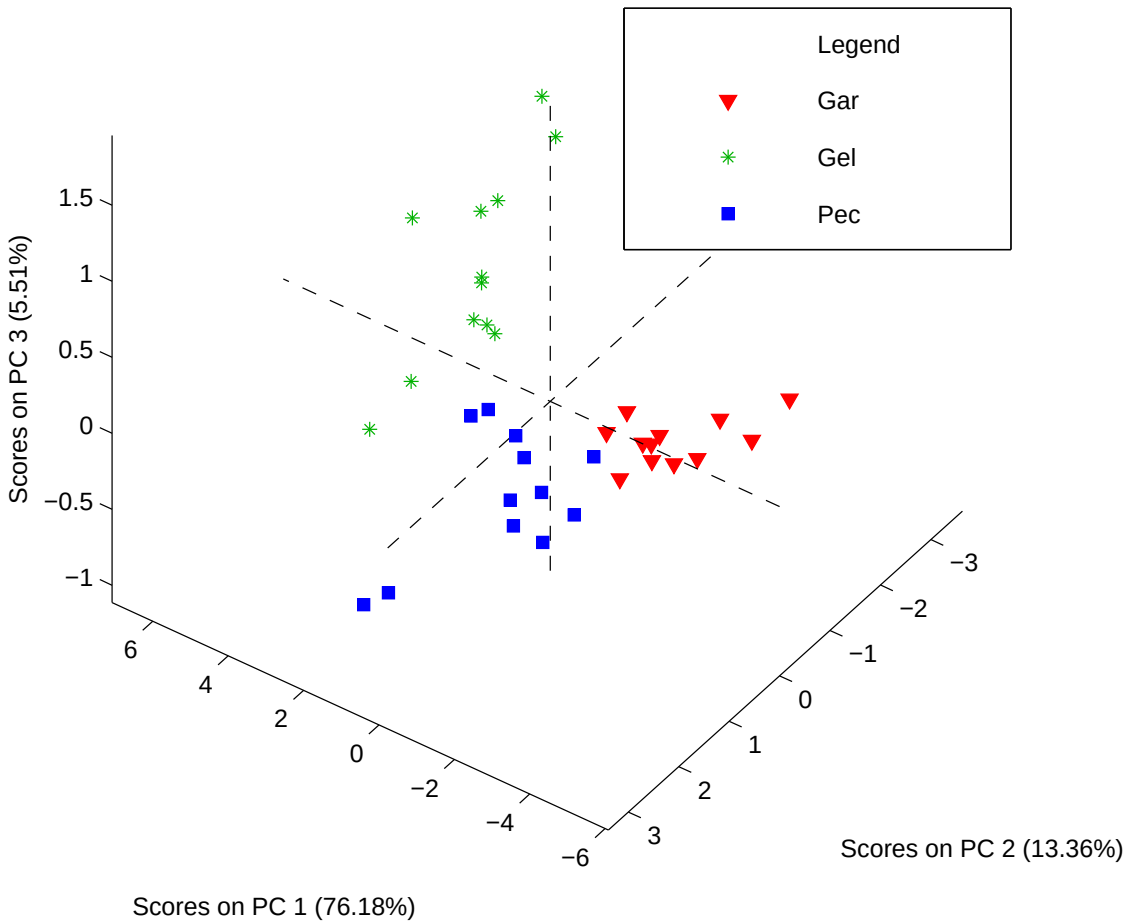

Legend
Gar
Gel
Pec
Scores on PC 3 (5.51%)
1.5
1
0.5
0
−0.5
−1
6
4
2
0
−2
−4
−6
Scores on PC 1 (76.18%)
3
2
1
0
−1
−2
−3
Scores on PC 2 (13.36%)

Figure 4 total

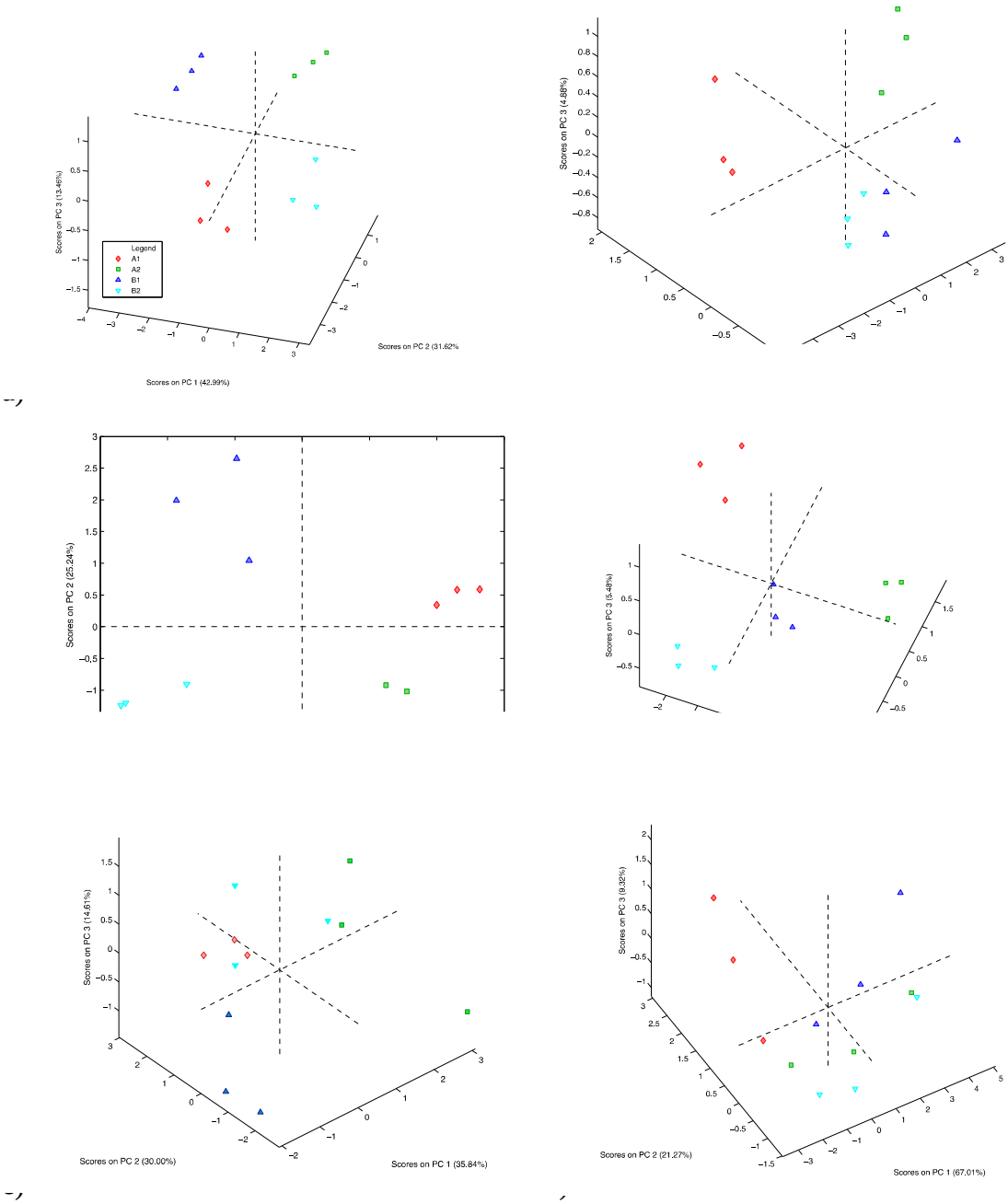


FIGURE 4: PCA scores plot of porphyrin (a, c, e) and peptide based (b, d, f) electronic nose data for gum arabic (a, b), gelatin (c, d) and pectin (e, f) candies (A: natural; B: natural-identic; 1: 0.15%; 2: 0.30 %).

Figure 4a

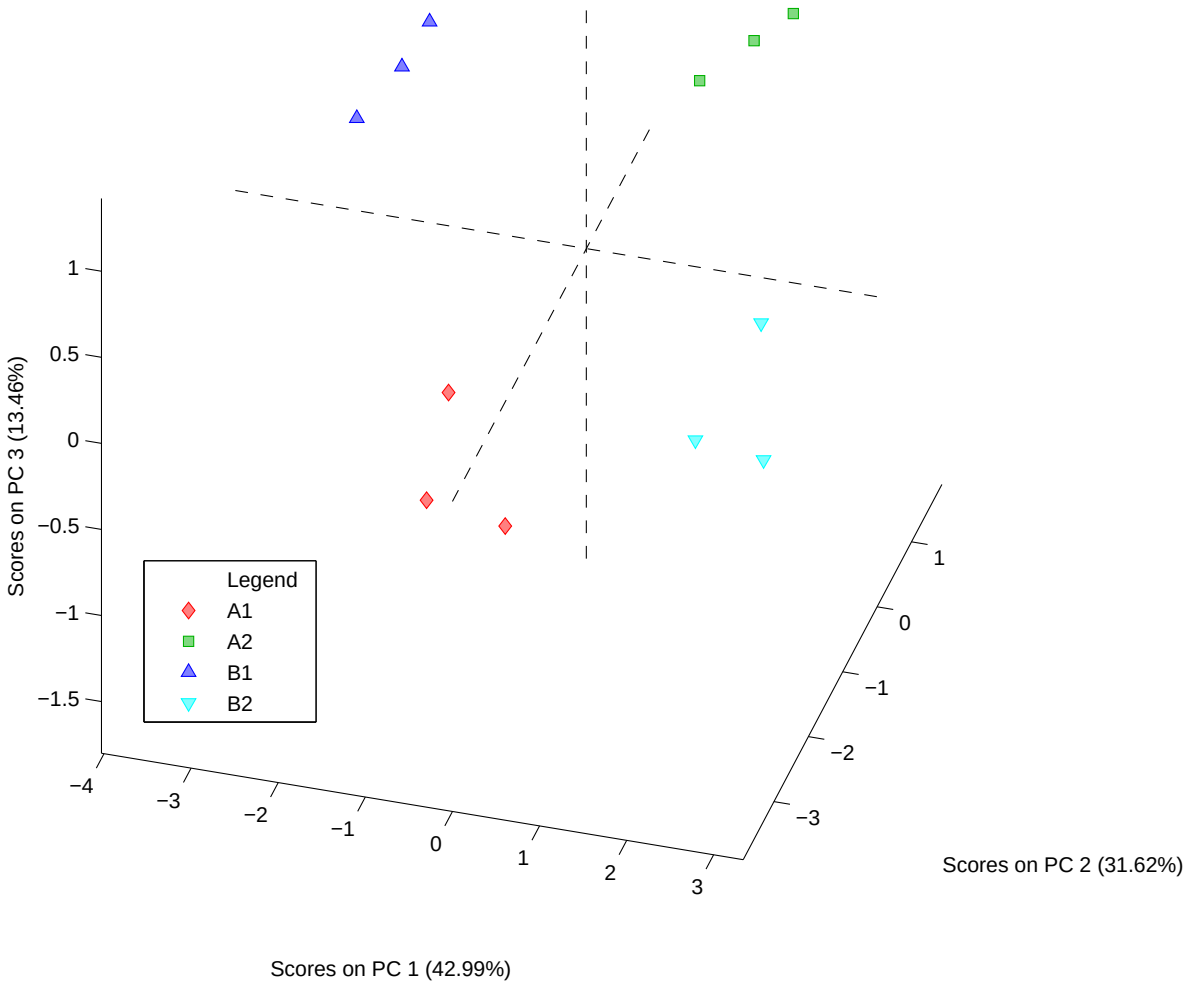

Legend
A1
A2
B1
B2
Scores on PC 3 (13.46%)
1
0.5
0
−0.5
−1
−1.5
−4
−3
−2
−1
0
1
2
3
Scores on PC 1 (42.99%)
1
0
−1
−2
−3
Scores on PC 2 (31.62%)

Figure 4b

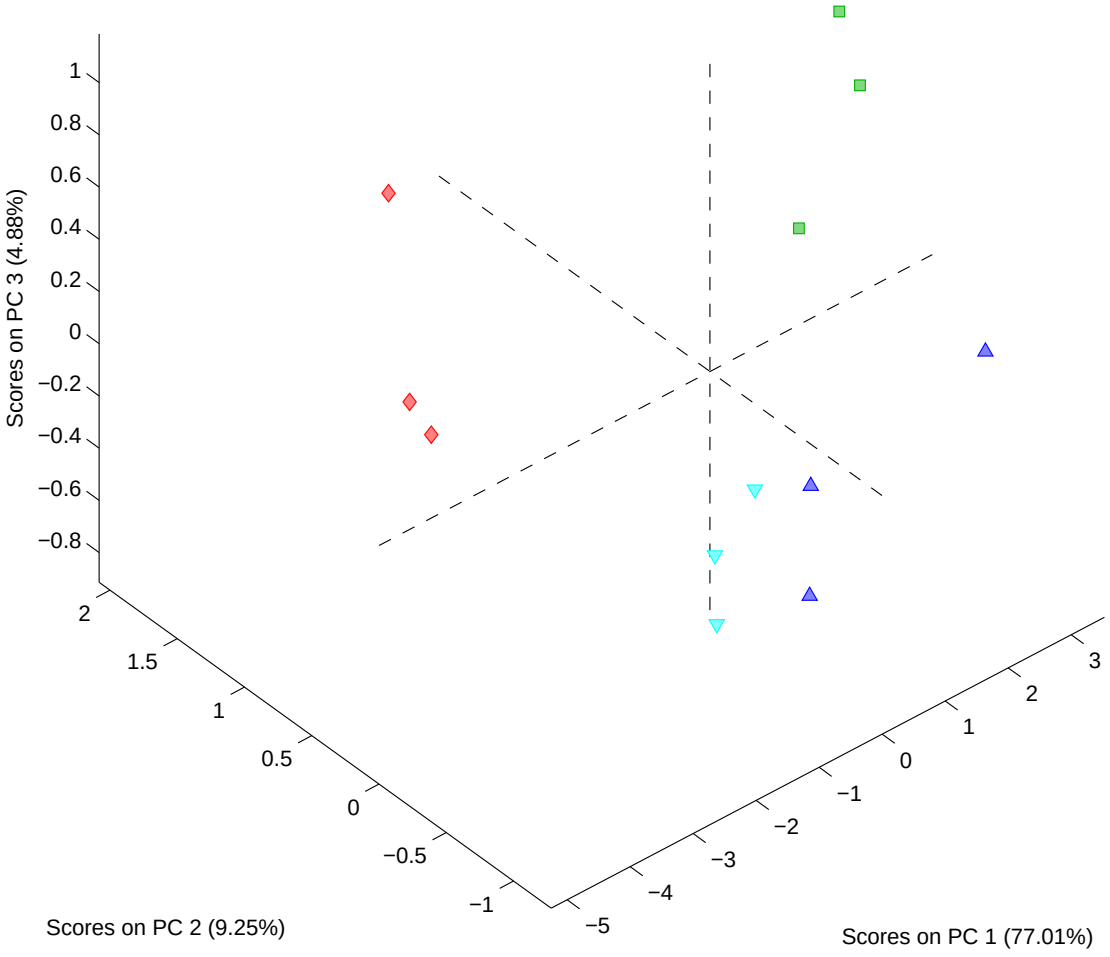

Scores on PC 3 (4.88%)
1
0.8
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
−0.8
Scores on PC 2 (9.25%)
2
1.5
1
0.5
0
−0.5
−1
Scores on PC 1 (77.01%)
3
2
1
0
−1
−2
−3
−4
−5

Figure 4c

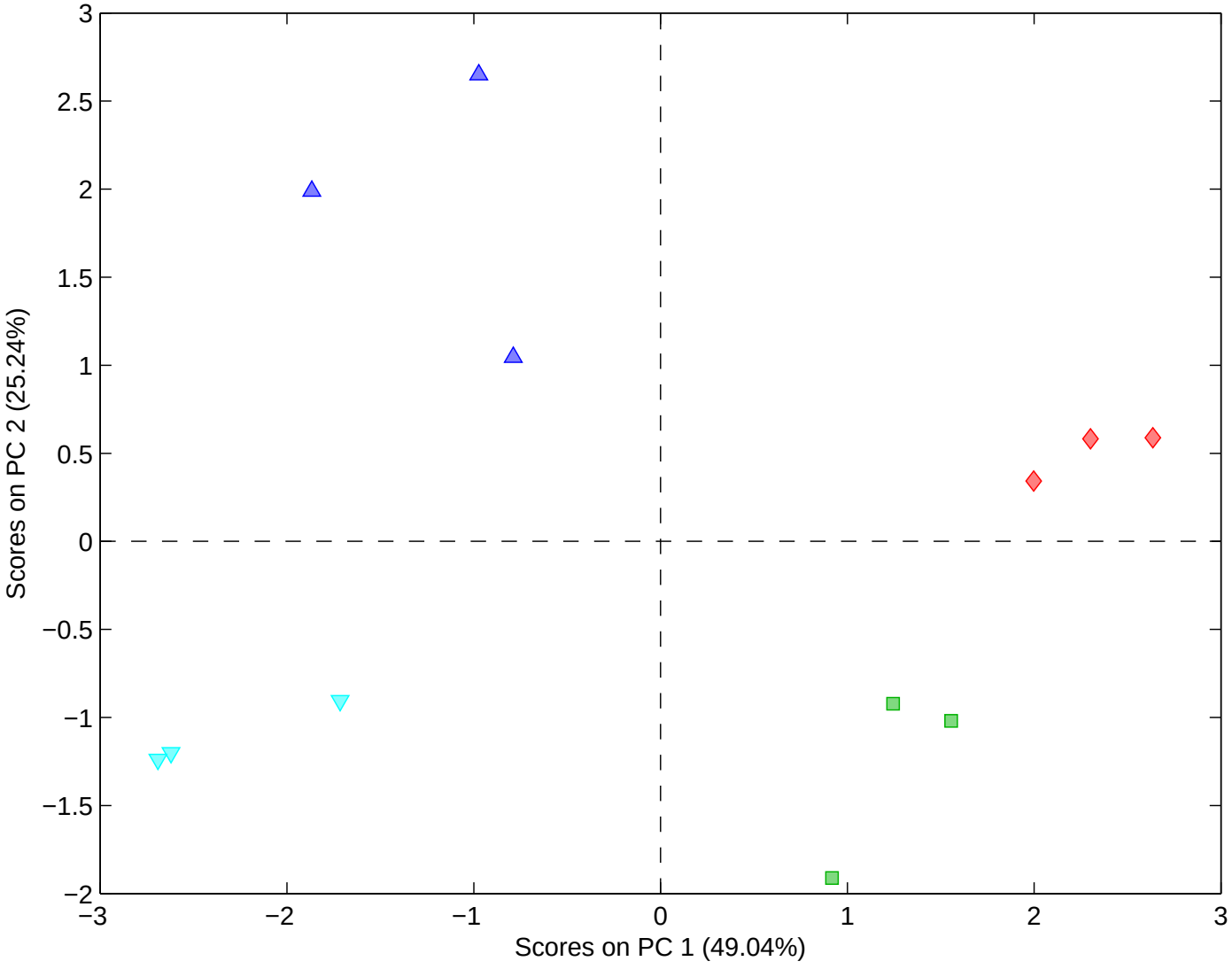

Scores on PC 2 (25.24%)
Scores on PC 1 (49.04%)
3
2.5
2
1.5
1
0.5
0
−0.5
−1
−1.5
−2
−3
−2
−1
0
1
2
3

Figure 4d

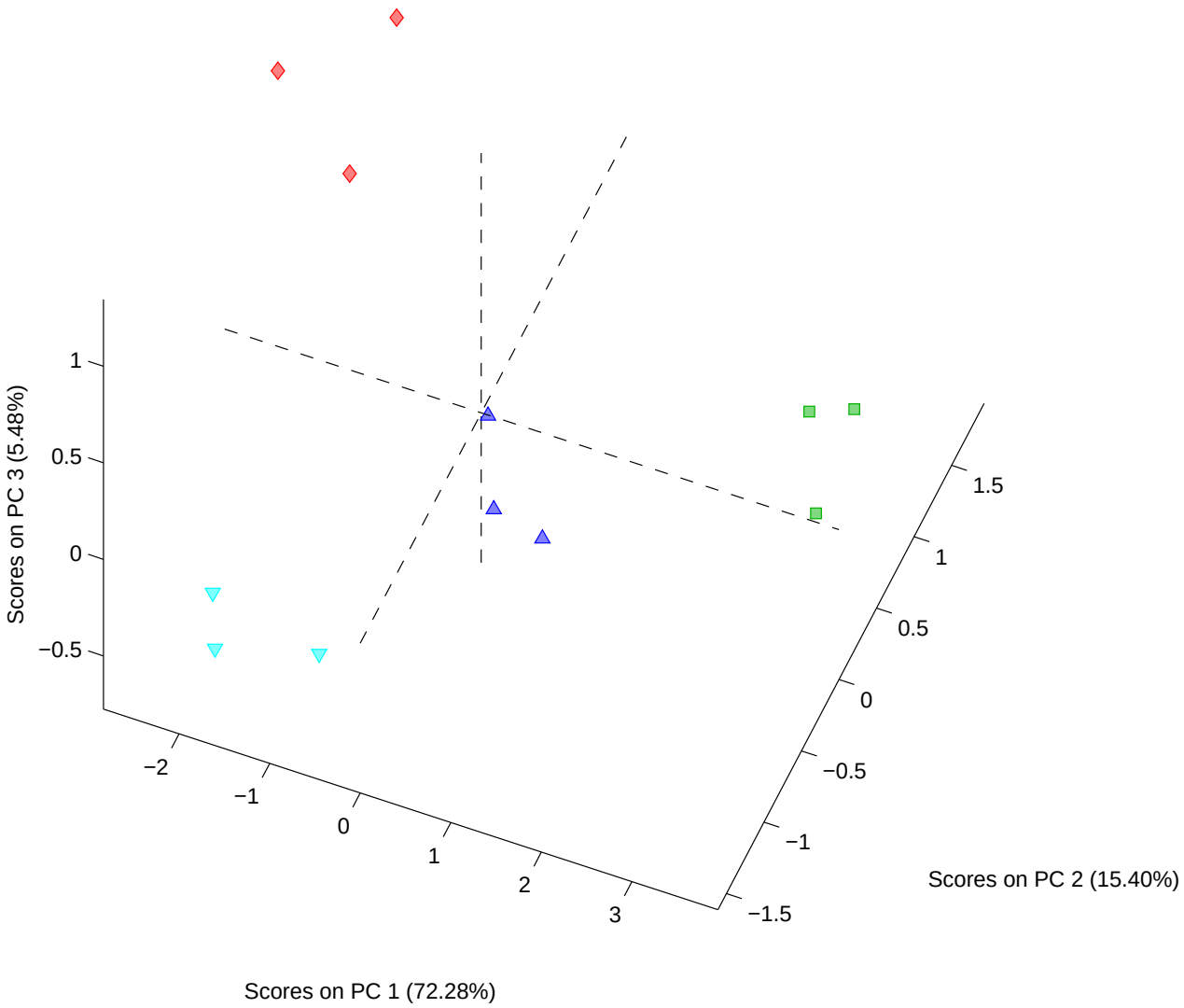

Scores on PC 3 (5.48%)
1
0.5
0
−0.5
−2
−1
0
1
2
3
Scores on PC 1 (72.28%)
1.5
1
0.5
0
−0.5
−1
−1.5
Scores on PC 2 (15.40%)

Figure 4e

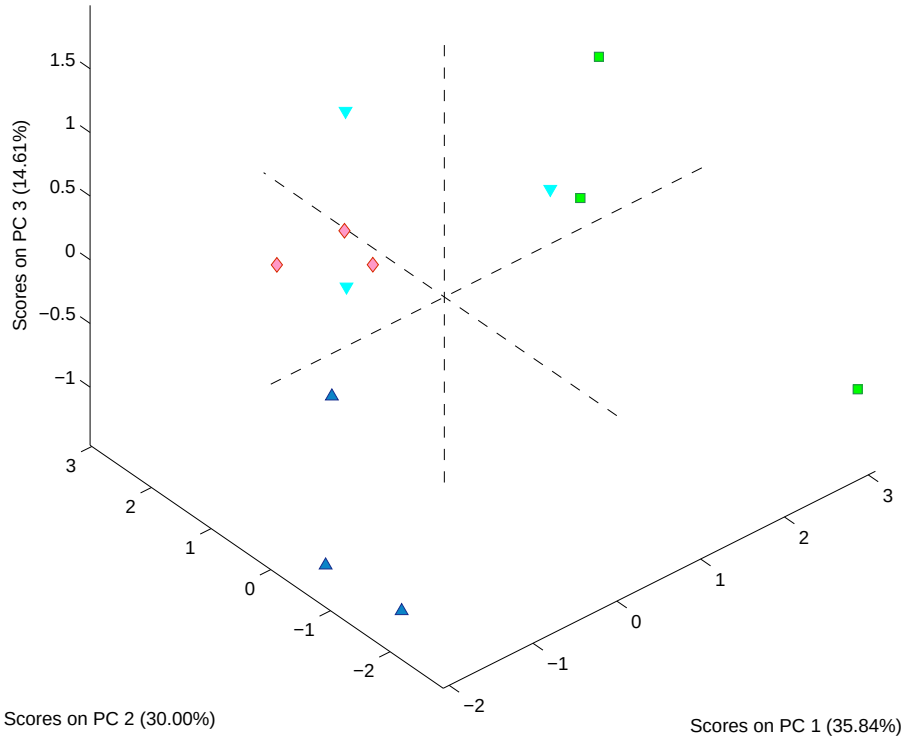
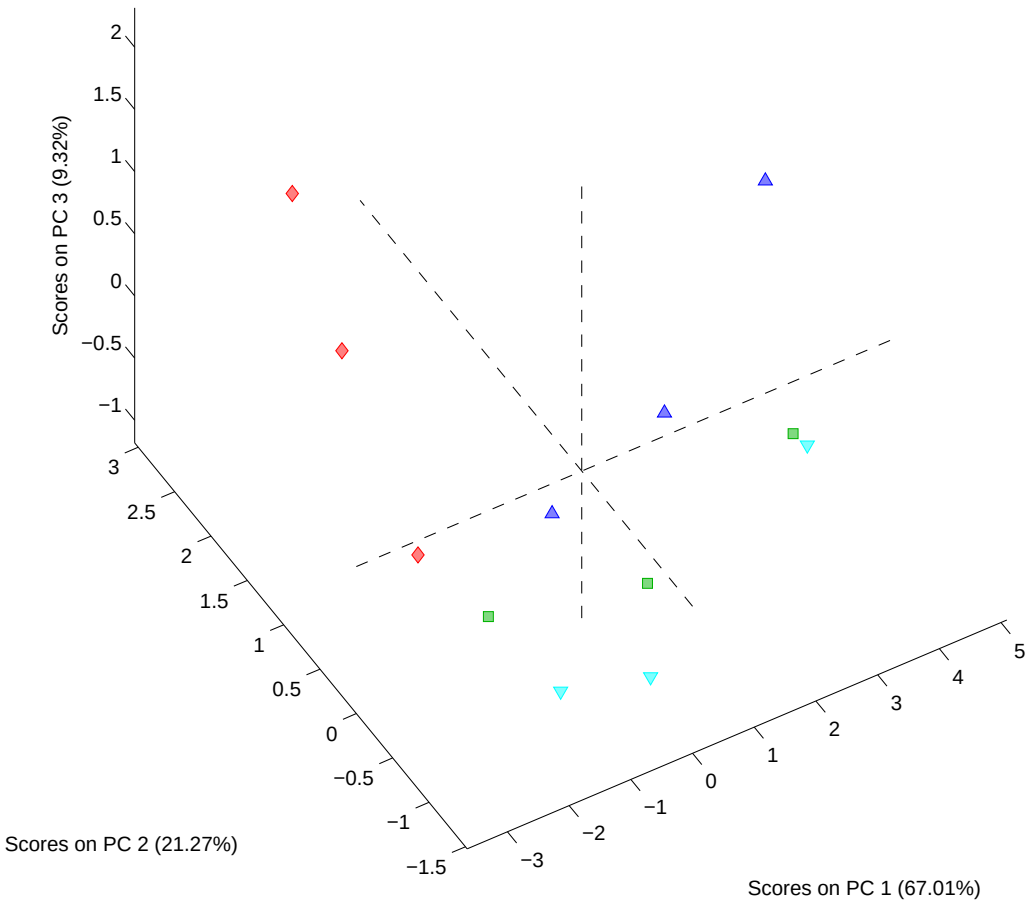

Scores on PC 3 (14.61%)
1.5
1
0.5
0
−0.5
−1
3
2
1
0
−1
−2
Scores on PC 2 (30.00%)
−2
−1
0
1
2
3
Scores on PC 1 (35.84%)

Figure 4f


Scores on PC 3 (9.32%)
2
1.5
1
0.5
0
−0.5
−1
Scores on PC 2 (21.27%)
3
2.5
2
1.5
1
0.5
0
−0.5
−1
−1.5
Scores on PC 1 (67.01%)
−3
−2
−1
0
1
2
3
4
5