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Influence of Menstrual Cycle on Aromatic Composition Behavior After Skin Application by Gas Chromatography/Mass Spectrometry

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Synopsis

Hormone secretion during the menstrual cycle changes per phase, influencing skin characteristics. These cutaneous modifications alter applied fragrances' behavior. This research aimed at analyzing the influence of menstrual cycle phases on a fragrance's volatile components' variation after skin application on women. We analyzed the variation of the emitted volatile components from an aromatic composition applied on the skin of 29 female participants during all menstrual cycle phases (follicular, ovulatory, luteal, and menstrual). The emitted volatiles were collected immediately after application and at intervals of 1 h 30 min, 3 h, 4 h 30 min, and 6 h, in a tube filled with Tenax® (Tenax Therapeutics, Morrisville, NC) coupled to a thermal desorption unit. The components were analyzed by gas chromatography/mass spectrometry. The raw ion-chromatogram data after "area normalization" were tested to principal components analysis and chemometrics tools. Statistical analysis showed no distinct variance pattern among the samples collected at different hormonal phases. However, the analysis of the individual scorer plots showed a distinct pattern for the release of aromatic compounds specific to each subject and associated with menstrual cycle phase. Although the results did not generate a model for skin's release of aromatic compounds in the population, the data showed that sex hormones have a unique effect on skin.

INTRODUCTION

Fine perfumery involves inspiration and sophistication and competes in the domestic and international markets. It is inserted in a competitive market with different fragrances

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launched every day and is increasingly searching for a more stable and reliable performance (1,2). The concern about long-lasting perfume requires more elaborate research regarding factors that affect performance when applied.

Many raw materials may be used in perfumery, which can be classified as natural (vegetal and animal) or synthetic (artificially produced). They can be divided into 13 olfactive groups: herbal, aldehydic, green, fruity, floral, spice, wood, leather, animal, musk, amber, and vanilla (3). A perfume can be defined as a homogeneous hydro-alcoholic dispersion containing 12%–30% of essential oils or aromatic compositions. The aromatic composition combines the raw materials to create a product that attends to consumers' expectations (4).

The structure of an aromatic composition is formed by three parts based on volatility: top, body, and bottom notes. Top notes are the first impression of the perfume and present the most volatile components. They remain on the skin for around 15 min. Body notes determine the identity of the composition and present intermediate volatility. They remain on the skin for 15 min to 4 h. Bottom notes represent the last step of the composition, with lowest volatility. They last between 4 and 8 h on the skin (5).

The hormonal fluctuation during the menstrual cycle is characterized by cyclical differences in luteinizing, follicle stimulating, and estrogen and progesterone hormone concentrations. Estradiol secretion increases in the plasma during the preovulatory phase causing vasodilation. Progesterone secretion increases in the postovulatory phase, inducing higher baseline body temperatures compared to the preovulatory phase due to its thermogenic effect. These changes affect skin physiology and can modify the duration of fragrances on skin (6,7).

Recent studies have shown that sex hormones (estrogen and progesterone) express several biological and immunological effects on skin (8,9). We observed fluctuations in perfume intensity perception along the cycle, but results were inconclusive (7,10–16).

Fragrance components can behave differently on skin. Their durability and characteristics change based on the mixture of numerous components containing functional groups such as ketones, aldehydes, esters, amides, and alkenes, which can be affected when changing the substrate on which they are applied (2,5,16,17).

Therefore, this study aimed at analyzing the variation of volatile components of the assessed aromatic composition after application on female volunteers' skin during the four phases of the menstrual cycle (follicular, ovulatory, luteal, and menstrual) by the dynamic headspace technique and gas chromatography/mass spectrometry (GC/MS).

MATERIAL AND METHODS

INVESTIGATIONAL PRODUCT

The floral, fresh, woody aromatic composition Ciclo® (Ciclo Cosméticos, São José, Brazil) 1910 used for this study was created by a perfumer from Givaudan® (Givaudan, Vernier, Switzerland) following International Fragrance Association (IFRA) norms. It is composed mainly of base olfactory notes (less diffusive) that deliver greater substantivity of perfume on the skin. The floral (body notes) and fresh (top notes) fractions are in lesser quantity and represent perfume identity.

The total fragrance consisted of 116 components in which the major constituents were: γ-methyl ionone (1.5%), 16-oxacyclohexadecan-1-one (Thibetolide® [Firmenich, Geneva, Switzerland], 2.6%), linalool (3.5%), linalyl acetate (4.4%), 1,4-dioxacycloheptadecane-5,17-dione (8.8%), 7-acetyl-1,2,3,4,5,6,7,8-octahydro-1,1,6,7-tetramethyl naphthalene (14.2%, Iso E Super® [International Flavors & Fragrances, New York, NY] major component) and methyl dihydrojasmonate (21.7%, Hedione® [Firmenich, Geneva, Switzerland] main component).

INVESTIGATION CENTER

The experiment was conducted at a research institute located in São Paulo, Brazil.

ETHICAL ASPECTS

The study protocol was previously approved by the ethics committee of the University of São Paulo, and was in accordance with Brazilian legislation, norms of International Conference Harmonization, Good Clinical Practice, and the Helsinki declaration. All participants signed informed consent terms before participating in the study.

PARTICIPANTS' VISITS

Twenty-nine female volunteers aged between 18 and 40 years old with regular menses (28–30 d) participated in the study. They did not use oral contraceptives for at least 6 mo before the study and fulfilled all inclusion and noninclusion criteria described in the ethics committee approved protocol.

Each female participant went to the institute for four visits according to the menstrual cycle phases. On the first visit (D1), ovulatory phase was detected using a noninvasive method through vaginal secretion smear to assess the format of oxalate crystals (5). Then, the investigational product was applied on the skin on a marked study site on the volar forearm during the morning period. After that, we collected headspace of the emitted volatiles on the study site at the following times: initial (immediately after product application), 1 h 30 min, 3 h, 4 h 30 min, and 6 h after product application (respectively t_0, $t_{1h30min}$, t_{3h}, $t_{4h30min}$, and t_{6h}). We repeated this procedure on each visit (D2, D3, and D4) to assess all four phases of the menstrual cycle.

CHEMICAL ANALYSIS

We submitted the volatiles collected from the volunteers to composition determination by the dynamic headspace technique according to the methodology described by Baydar, Mcgee, and Purzicky (1995). This technique quantifies the individual partition of the components of skin fragrance in the air and translates the information for an "olfactory perception profile" (18,19).

We applied 40 μL of the investigational product on the defined test site on the volunteers' forearm. Then, we added ethanol with a microsyringe to allow the fragrance to dry faster.

The treated area was sealed with a glass apparatus to collect volatiles on the experimental times (t_0, t_{1h30}, t_{3h}, t_{4h30} and t_{6h}). The volatiles were collected in a glass tube packed with a porous polymer resin based on 2.6-diphenylene oxide (Tenax®) using the dynamic headspace collection technique.

After collection, the Tenax® tubes were coupled to a thermal desorption apparatus (ATD 650® Turbo Matrix [PerkinElmer, Waltham, MA]), desorbed at 100°C for 10 min with a helium flow of 60 mL/min and injected in the GC/MS equipment (Agilent model 6890/ Mass Spectrometer 5973). The chromatographic conditions were:

- Temperature trap −30°C during the concentration, valve maintained at 150°C.
- During the desorption step of the trap, the trap temperature increased to 225°C with a helium flow at 1.5 mL/min.
- Injection split ratio 5:1 (total sample injected = 16.7%); interface temperature of 280°C.
- Volatiles were transferred to a capillary column HP Ultra 2, 50 m x 0.20 mm i.d. x 0.33-μm film thickness.
- Temperature gradient: 50°C/2 min for stabilization followed by a temperature increase of 3°C/min until 280°C.
- Carrier gas: helium at a flow rate of 1.5 mL/min.
- Mass spectra were registered after the column with an interface at 280°C and ion source temperature of 250°C.
- The ionization potential was adjusted to 70 eV and ions monitored with a range of 35–350 mass-charge (m/z).

We identified the compound by comparing retention indexes (Kóvats Index—determined relative to the retention times of a series of n-alkanes) and mass spectra with literature data (5,19,20).

MULTIVARIATE ANALYSIS OF CHROMATOGRAPHIC DATA

The methodology applied in this experiment was adapted from a study previously described by Pavon et al. (2006) for oil samples.

The basic principle of mass spectrometry consists of generating ions from compounds, organic or inorganic, through an appropriate ionization method, separating them through their m/z ratio in a mass analyzer, and qualitatively and/or quantitatively detecting the compounds from the ions' m/z ratio and their respective abundances by means of a detector. The detector "counts" the ions and transforms the signal into an electric current versus retention time. The magnitude of the electrical signal as a function of the m/z ratio is converted by a data processor, which generates the corresponding mass spectrum (21,22).

The data from the mass spectra of the raw material aromatic composition, specifically abundance of the different fragments (mass-load relation or m/z) versus the retention time of the constituents, were exported in an ASCII text file. The series of data were subjected to MATLAB 6.5 software to obtain the surface contour plots of m/z versus time for each sample or aromatic composition's raw material, as shown in Figure 1.

After visual analysis of the surface contour plots, the ranges of m/z fragments selected were submitted to principal components analysis using Unscrambler software version 9.5 (CAMO Process AS, Norway, 2002) (23). Data were previously submitted to area standardization

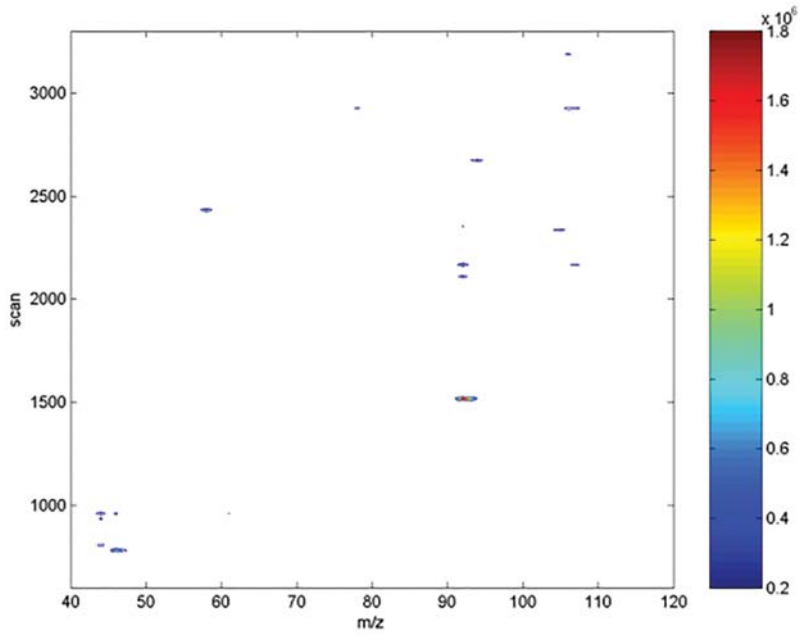

Figure 1. Surface contour plot from the volatile components of Ciclo® 1910 emitted by volunteer 19 during menstrual cycle follicular phase after GC/MS analysis. Figure 1 corresponds to the mass spectra data, specifically to the abundance data of the different m/z fragments in the abscissa axis (x) as a function of the retention time in the coordinate axis (scan-y).

(Equation 1). This transformation normalizes X_i spectrum by calculating the area under the curve Σx_{ij} and makes the area under the curve the same for all spectra (24).

$$\text{New } X_i = X_i / \sum_j x_{ij} \tag{1}$$

RESULTS AND DISCUSSION

The basic principle of mass spectrometry is to generate ions from organic and inorganic compounds through an appropriate ionization method, to separate them through their m/z ratio in a mass analyzer, and to identify and/or quantify the compounds from the m/z ratio of the ions using a detector that "counts" them and transforms the signal into an electric current. The magnitude of the electrical signal as a function of the m/z ratio is converted by a data processor, which generates the corresponding mass spectrum (21).

Headspace consists of the gaseous atmosphere surrounding a sample. The technique involves the collection of compounds from this atmosphere statically or dynamically to an adsorbent device. When using an adsorbent, the collected material must be released (extracted) with a solvent or temperature (25). It allows us to map the performance of an aromatic composition applied on the skin and identify degrading products during experimental times (18).

The headspace technique applied was semiquantitative because the peaks found in the chromatograms resulted from the suction generated by the vacuum pump, thus causing

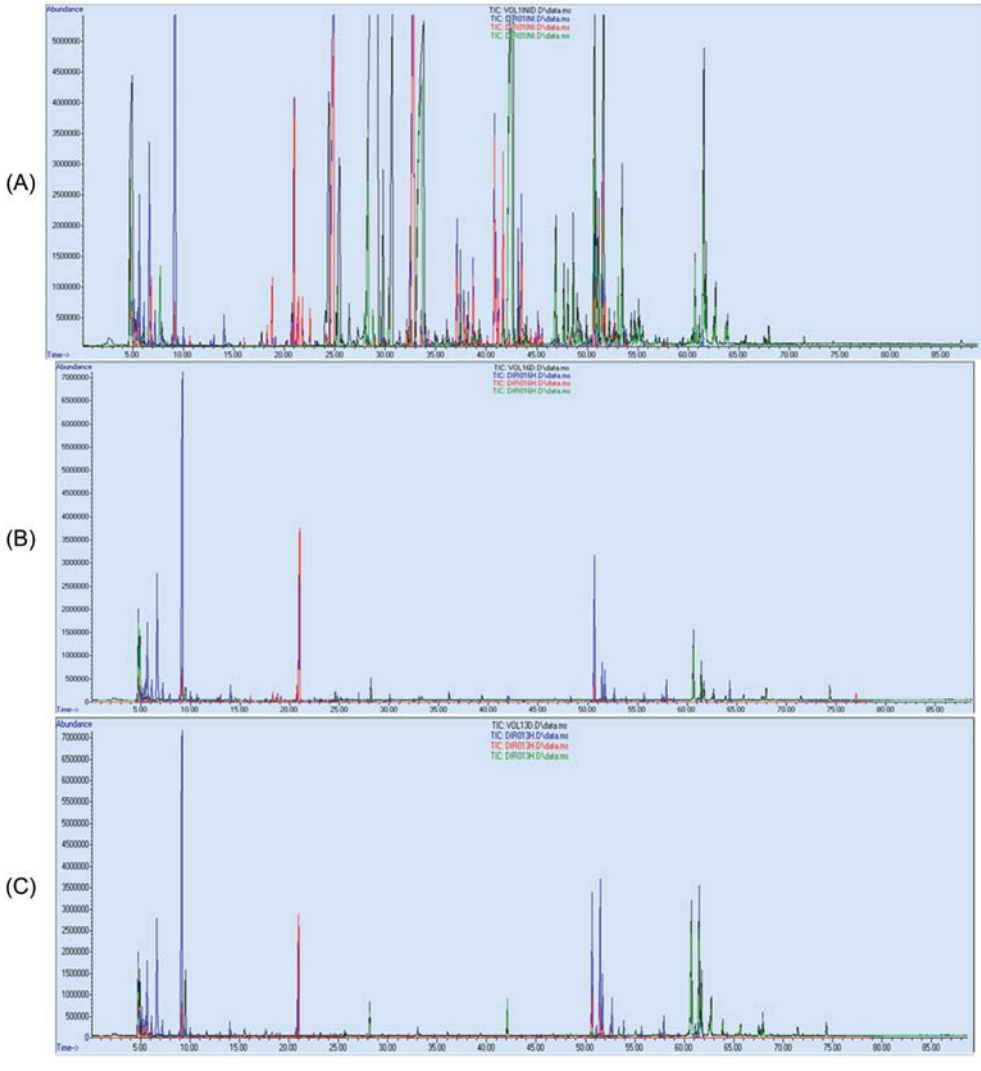

Figure 2. Chromatogram of volatile components of skin's composition for volunteer nb.1. (A) initial experimental time, (B) after 3 h, and (C) after 6 h. The black chromatogram corresponds to the follicular phase; the blue chromatogram corresponds to the luteal phase; the red chromatogram corresponds to the menstrual phase; and the green chromatogram corresponds to the ovulatory phase.

interferences (example: body odor and experimental variation during collection). The true quantitative composition is obtained by the direct injection of the aromatic preparation in the chromatograph. The interferences are most prevalent for the bottom notes of the fragrance, which can be easily hydrolyzed by sweat. Additionally, their release from the skin may not be efficient due to their higher molecular weight and lower volatility. Therefore, the analysis depends on the fragrance-substrate interaction (17,18). Studies show that other factors, such as pH, skin type (dry or oily), gender, and race affect the evaporation of perfumes applied on skin (26).

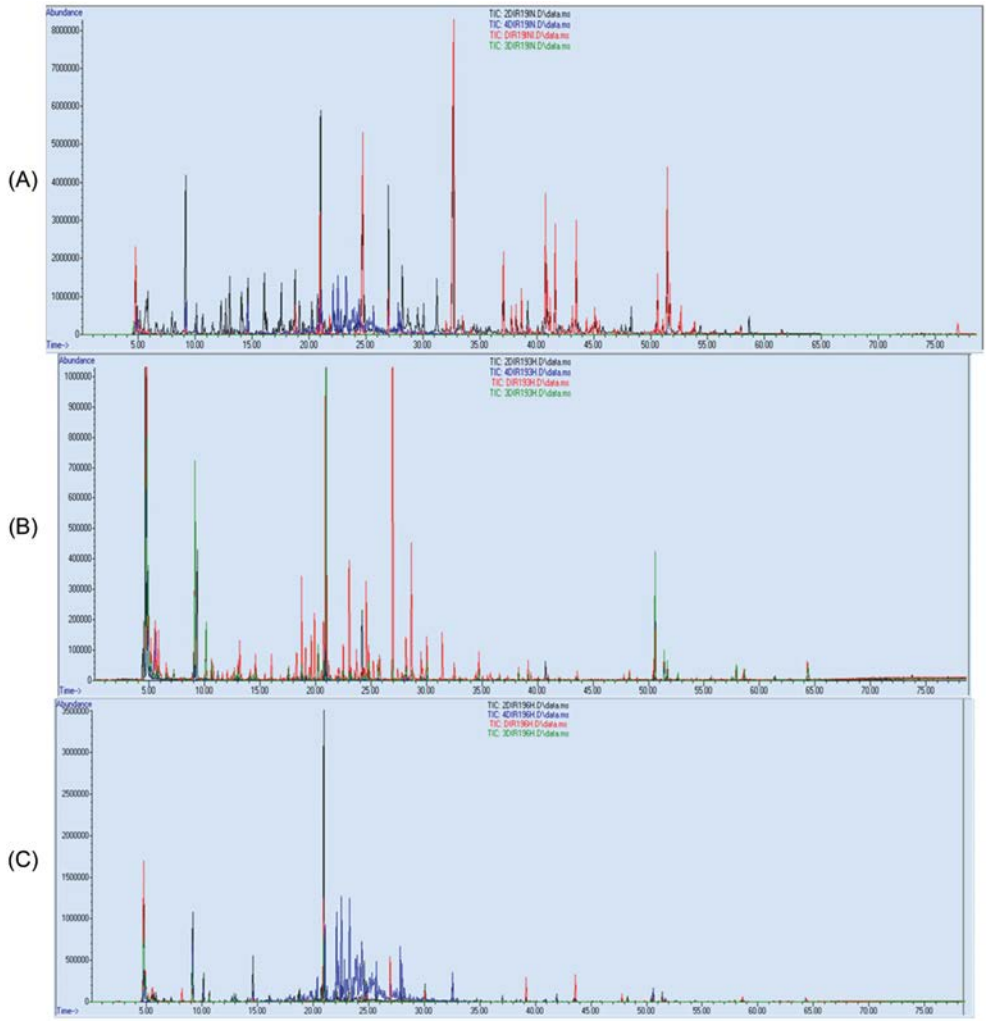

Figure 3. Chromatogram of volatile components of skin's composition for volunteer nb.19. (A) initial experimental time, (B) after 3 h, (C) after 6 h. The black chromatogram corresponds to the follicular phase; the blue chromatogram corresponds to the luteal phase; the red chromatogram corresponds to the menstrual phase; and the green chromatogram corresponds to the ovulatory phase.

Volunteers 1, 19, and 20 were chosen as examples of response profiles, as they were the most significant and didactic to present the different responses encountered for the whole panel. Figures 2, 3, and 4 present the chromatograms obtained for these volunteers in each experimental time (initial, t_{3h} and t_{6h}), which demonstrate the main differences in chromatographic profiles and fragment readings in the function of the menstrual cycle (5).

Figure 5 shows the total scores of all m/z fragments generated from the volunteers' skin's volatile components. There was a slight tendency to form a response pattern during the luteal and menstrual phases, but the scores explanation was low (69% and 27%, respectively). There were many luteal and menstrual phase points mixed to the observed remainders. However, for those with a slight pattern, we observed in the loadings that fragments 91 and

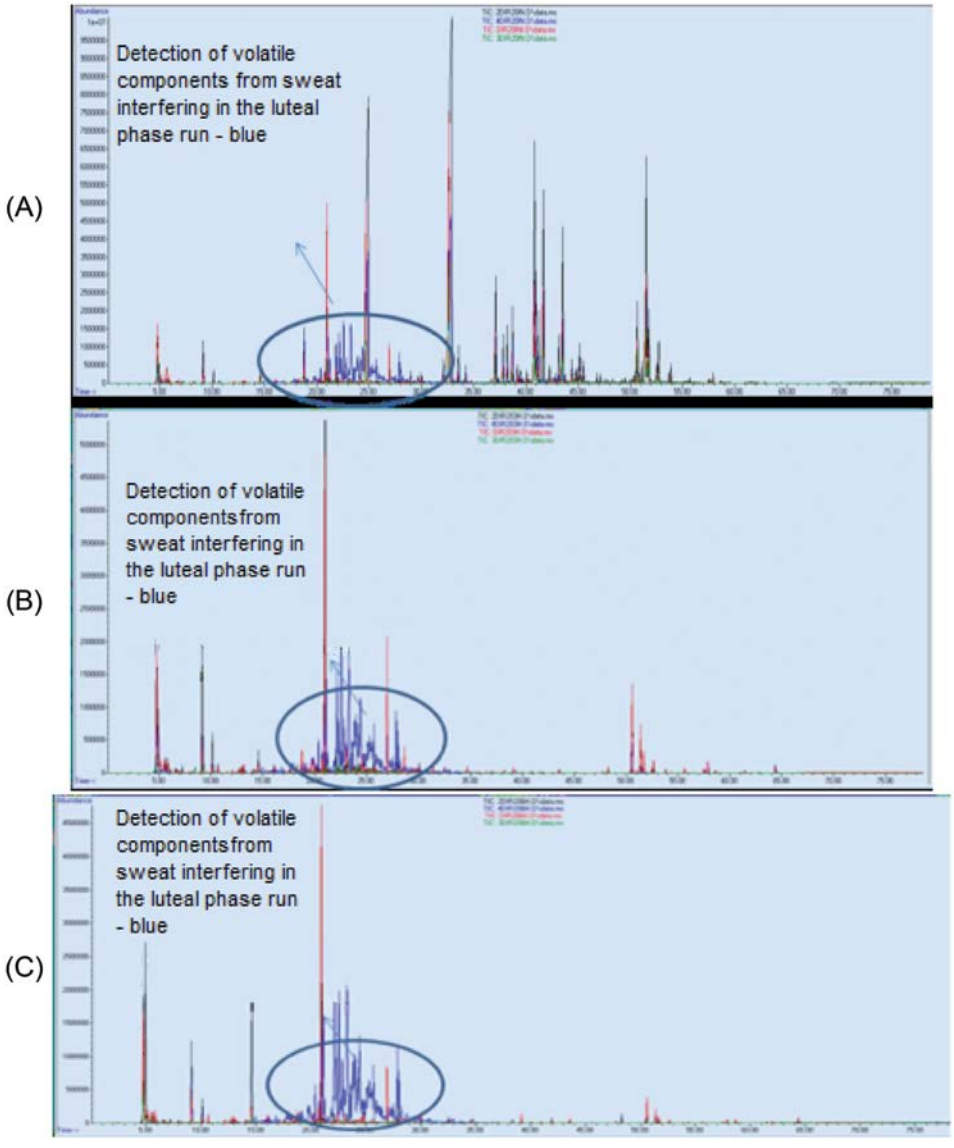

Figure 4. Chromatogram of volatile components of skin's composition for volunteer nb.20. (A) initial experimental time, (B) after 3 h, and (C) after 6 h. The black chromatogram corresponds to the follicular phase; the blue chromatogram corresponds to the luteal phase; the red chromatogram corresponds to the menstrual phase; and the green chromatogram corresponds to the ovulatory phase.

92 were the ones that demonstrated the response pattern for the profile found in the luteal and menstrual scores. Evaluating the aromatic composition, solvents, and environmental contaminants, we found these fragments in the following components: 91 = limonene and linalool; 92 = limonene, linalool, *cis*-ß-ocimene, *trans*-ß-ocimene; thujopsene, linalyl acetate; α-cedrene, and α-pinene. According to Baydar et al. (1995), these components were considered as fragrance top and body notes (18).

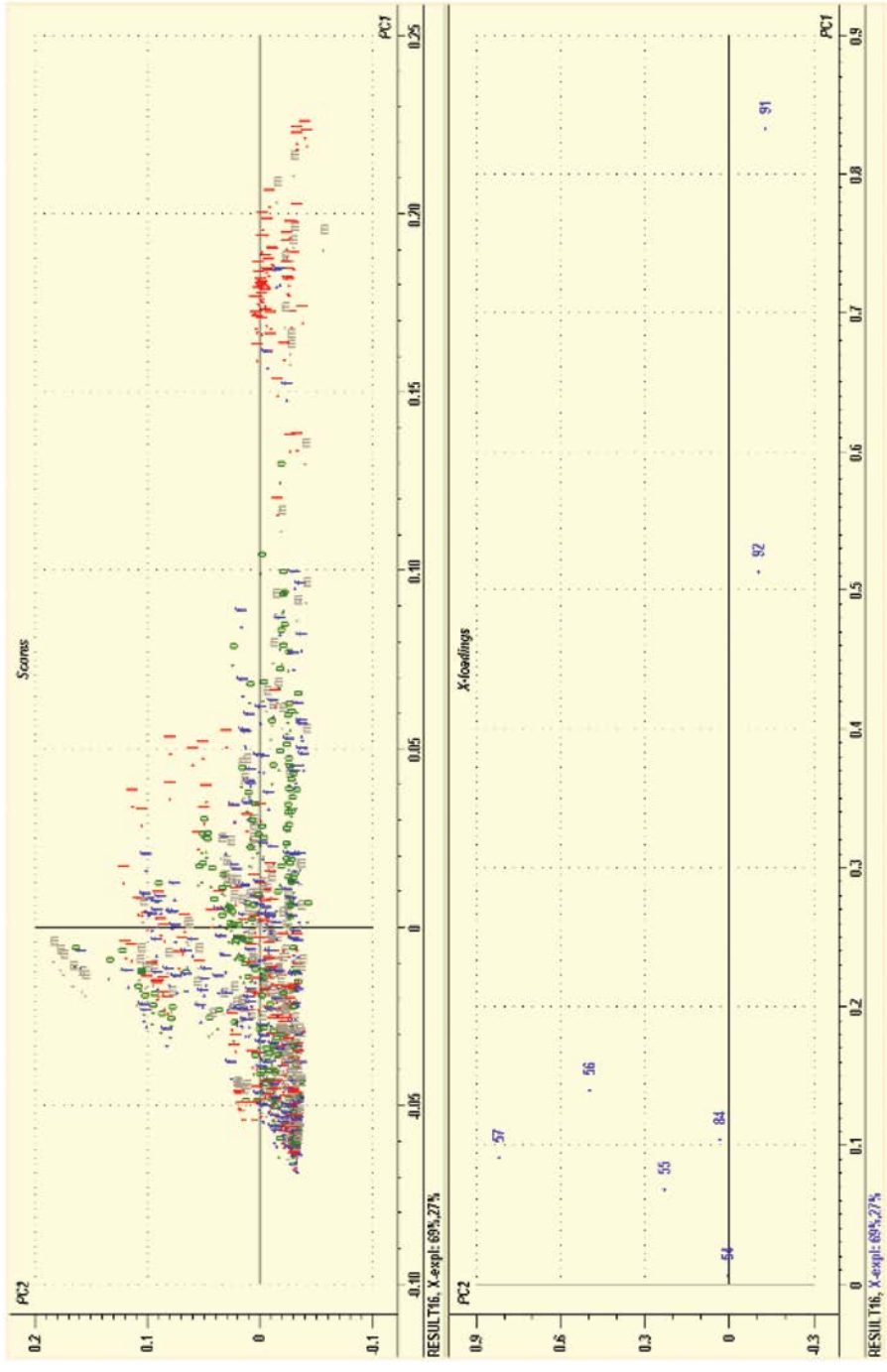

Figure 5. Total scores and loadings of selected m/z fragments from all volunteers according to the menstrual cycle. m (gray) corresponds to the menstrual phase; f (blue) corresponds to follicular phase; l (red) corresponds luteal phase; and o (green) corresponds to the ovulatory phase.

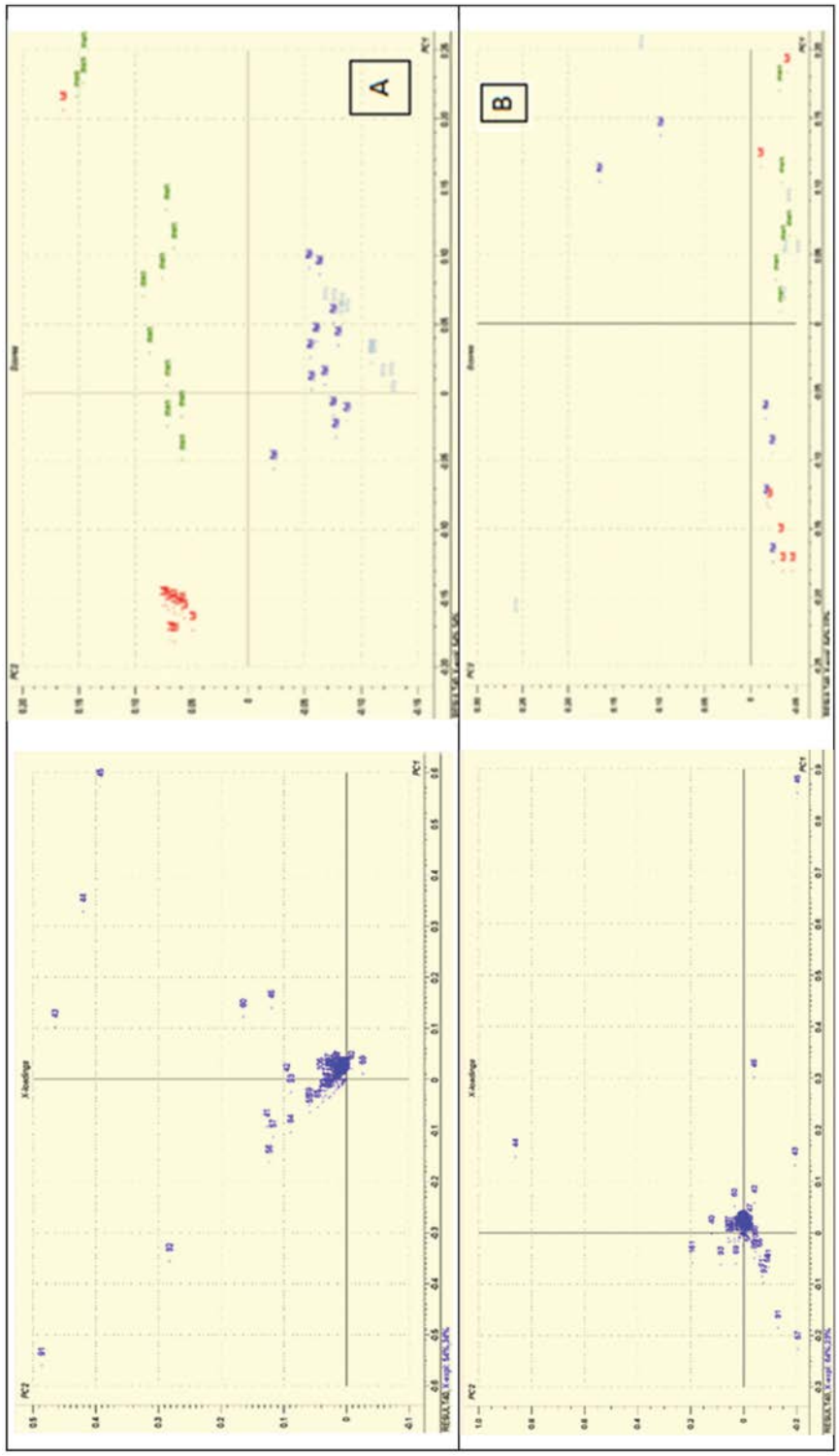

Figure 6. Total scores obtained in the principal components analysis for subject 1 (A) and 19 (B) for each menstrual cycle phase. Ovu (light blue) corresponds to the ovulatory phase; Fol (dark blue) corresponds to the follicular phase; Lut (red) corresponds to the luteal phase; Men (green) corresponds to the menstrual phase.

Several factors like participants' nutrition, metabolite release, and biotransformation products in the skin (sweat), might have interfered in the analysis causing the high variability detected among the individuals. Sweat was one of the most important interferents in the analysis, mainly for subject 20 during the luteal phase. A large part of the fragrance components for subject 20 were masked by fatty acid peaks (data not shown).

When we analyzed the chromatograms separately by individual, a specific pattern in the aromatic compounds release could be observed for each subject associated with the menstrual cycle. An example of these internal cycles is presented in Figure 6. Subject 1 showed a clear pattern for fragrance emission segmented according to the cycle phase, in which the follicular and ovulatory phases showed similar responses, while the luteal and menstrual phases showed a distinct emission pattern (Figure 6A). On the other hand, for subject 19 no defined pattern could be identified with the menstrual phase. In this case, there was a slight similarity between the follicular and luteal phases and between the menstrual and ovulatory phases.

The final results led us to understand that the menstrual cycle induces differences on skin conditions, influencing the behavior of applied fragrances. Still, we could not detect which exact ingredients were more susceptible to hormone oscillations. There was a distinct pattern in aromatic compounds release specific to each subject and associated with the menstrual cycle phase. Therefore, we can infer that sex hormones exert a unique effect on each participant's skin without a generic model concerning population (behavior was found to be individual).

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