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"UPLC-HRMS for discriminant metabolomic studies of Tequila"

A thesis presented by

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Dedication

To my mother, who always supports me in every step of the way, no matter what or how. My grandparents, who with their love and support, I'm the woman I am today. My father and sister, who always have encouraging words for me and celebrate my achievements like their own. My advisor, Dr. Teresita Martín del Campo, who inspired me and taught me, with patience, even more than I expected. My committee, Dr. Mirna Estarrón and Dr. Anaberta Cardador, who believed on the project and shared their knowledge with me. My friends, who believed in me and always be there for me.

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1. Introduction

1.1 Motivation

CULTURAL IMPORTANCE

Tequila is the most representative beverage in México. It takes its name from the place where it was born: Tequila, Jalisco. It is a beverage with an ancestral origin, that has been produced since the XVI century (Orozco, 2020). Starting with the Nahuatl tribe, the *tecuilas*, they inhabited the now-known Tequila region. The initial and only process that they applied was the fermentation of the agave nectar (Sánchez, 2017). Later, in the first decades of the Conquest, with the Spanish influence, a new process was added to the production, the distillation, and the production that is used today began (Gallardo, 2015). First known as "Tequila mezcal wine" derived from the plant´s heart called "mezcal", which means "the house of the moon". It was until 1821, when the independence of México was consumed, that alcoholic beverages from Europe presented difficulties to be imported, giving rise to the Tequila to increase its sales and to be known as the Mexican drink par excellence (CRT, 2019).

There are about 295 *agave* species, of which approximately 75% are in México (Garay et al., 2015). According to the Official Mexican Norm, NOM-006-SCFI-2012, Alcoholic Beverages-Tequila-Specifications, Tequila must only be elaborated with one *agave* species: *Agave tequilana* Weber Blue variety (SEGOB, 2012). Despite the specifications and requirements that Tequila must meet to be a drink now recognized internationally, between 1980 and 1990 Tequila was seen as a medium-quality drink; nevertheless, the industry, including the entire production chain, got organized to improve the quality and marketing, and the sector was boosted (Romo, 2019).

DESIGNATION OF ORIGIN

According to article 156 of the Mexican Industry Property Law, the Appellation of Origin is the name of a determined geographic zone of the country used to designate a product originally from thereof, and whose quality or characteristics are due exclusively to the geographic medium (CRT, 2020b). The Mexican Institute of Industrial Property (IMPI) is the maximum authority that emits the declarations of the designation of origin and it authorizes its use (SEGOB, 2015).

Nowadays, México has 18 products elaborated and protected under the Appellation of Origin due to its natural and cultural richness (Castro, 2020). The products with Appellation of Origin have a 100% bigger market value than generics, according to the director of the Appellations of Origin National Institute (INAO) (Romo, 2019). Tequila was the first alcoholic beverage that obtained the designation of origin. In May 1973, the Tequila's denomination of origin included only four states: Jalisco, Guanajuato, Michoacán, and Nayarit. For the four states, the number of municipalities is specific, all municipalities in Jalisco, six in Guanajuato, twenty-nine in Michoacán, and eight in Nayarit (Avedoy, 2018). The municipalities are shown in Table 1.

Table 1. Municipalities included in Tequila's designation of origin (Avedoy, 2018).

States	Municipalities
Jalisco	All of them
Guanajuato	Abasolo, Cuerámaro, Manuel Doblado, Huanímaro, Purísima del Rincón, and Pénjamo
Michoacán	Briseñas from Matamoros, Chilchota, Chavinda, Jacona, Cotija, Ecuandureo, Churintzio, New Parangaricutiro, Maravatío, Jiquilpan, Numarán, Peribán, La Piedad, Pajacuarán, Régules, Tancítaro, Los Reyes, Sahuayo, Tanhuato, Tangamandapio, Tangancícuaro, Venustiano Carranza, Tocumbo, Tingüindín, Zamora, Yurécuaro, Vistahermosa, and Villamar
Nayarit	Ahuacatlán, Amatlán de Cañas, Jala, Jalisco, Ixtlán, San Pedro de Lagunillas, Santa María del Oro, and Tepic

In 1974, the state of Tamaulipas entered to the Designation of Origin; but it was until 1976, that the municipalities of Altamira, Antiguo de Morelos, Aldama, Gómez Farías, Llera, González, Mante, Nuevo Morelos, Ocampo, Xicoténtatl, and Tula were recognized as the only ones where Tequila can be made (Avedoy, 2018).

ECONOMIC IMPORTANCE

The first step in the Tequila production chain is the agave harvest; therefore, the 17 thousand farmers and the 70 thousand families that depend on them are the first benefited from the sales expansion of this beverage, with an income of $2,600 million per year (ElEconomista, 2017). It also contributes to the national economy with $4,200 million (Mexican pesos) through the Service and Production Special Tax (IEPS by its initials in Spanish) (Romo, 2018).

Statistics of the Tequila Regulatory Council demonstrate that this industry grew in the fields of agave consumption, production, and exportation exponentially in 20 years, as shown in Table 2. Besides, Tequila houses increased from 36 in 1995 to 155 in 2018 (Romo, 2019).

According to the Consumer Federal Attorney (PFC, 2018), Tequila is the second most consumed alcoholic beverage in México with 26% of preference; after the beer with 52%, as shown in Figure 2. The rising in the sales and production of Tequila continues; 2021 was stated as "the most successful year for the agave-Tequila productive chain", according to the history data of The Tequila Regulatory Council (CRT, 2022). Given that, in the twelve months, the liters produced by the national beverage reached 351 million, the equivalent of 1,004 liters produced per minute, with a growth of 40.9% compared to the previous year (Amador, 2022).

Table 2. Tequila indicators from 2000 to 2020 (CRT, 2022).

	Tons/Liters Increased	Growth
Agave consumption	792,000 tons	229%
Production	192.4 million (L)	206%
Exportation	187.9 million (L)	290%

L= Liters.

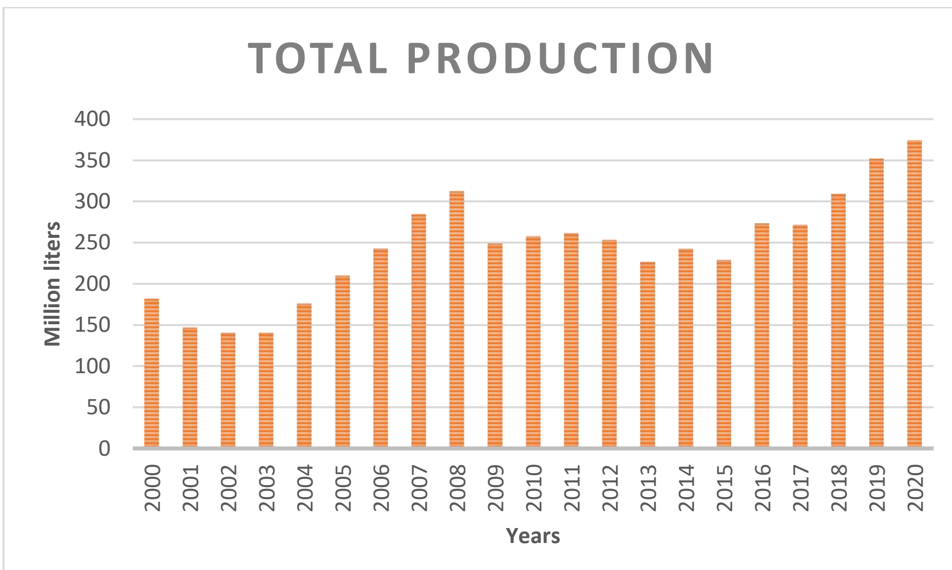

Figure 1. Total Tequila production in Vol. million liters (CRT, 2022).

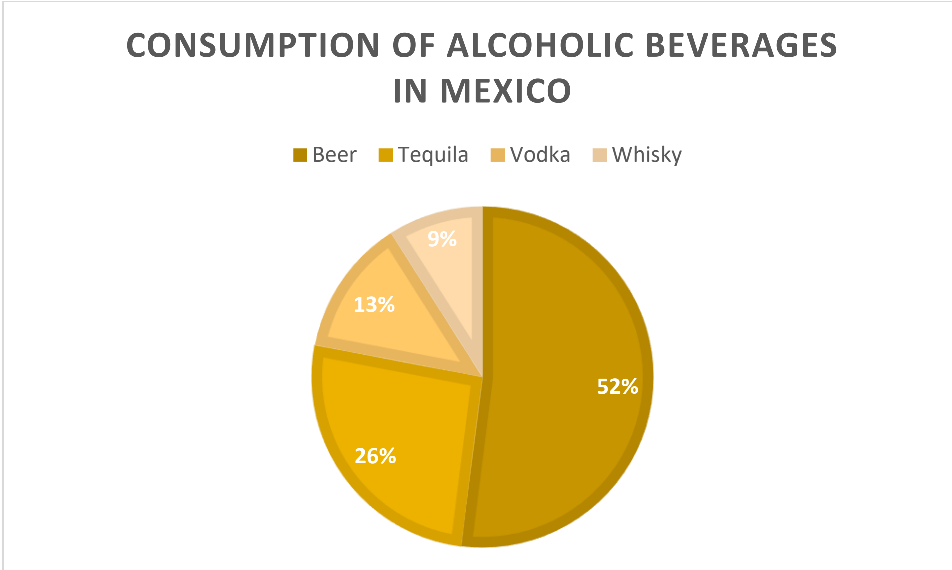

Figure 2. Percentage of alcoholic beverages consumption in México.

The exportations represent an important income to the Mexican economy, higher than $28,000 million (Mexican pesos) (Romo, 2018). The 72.6% of the Tequila production is intended for the exportation to more than 120 countries, being the United States the principal buyer, as shown in Table 3 and Table 4 (CRT, 2022).

Table 3. Tequila quantity exported per country in 2020 (CRT, 2021).

Country	Exportation in thousand liters
Unitated States	254,212.9
Germany	4,147.3
Spain	2,819.1
Canada	2,233.9
France	2,133.8
Australia	1,837.9
Colombia	1,304.7
China	844.6
Chile	611.7
Brazil	408.5
Ecuador	407.2

Table 4. Tequila quantity exported per country in 2021 (CRT, 2022).

Country	Exportation in thousand liters
Unitated States	288,291.8
Germany	6,446.7
Spain	4,708.5
Canada	3,132.9
France	3,110.2
Australia	2,868.2
Chile	1,168.7
China	1,349
Brazil	683.8
Ecuador	647.6
Belgium	581.5

1.2 Problem Statement and Context

Since the increase in the production and sales of Tequila, the knockoffs and falsifications started, which caused health hazards to the person who ingested it. In June 2020, it was reported that the death of 18 people causing by adulterated agave distilled, an alcoholic beverage similar to Tequila, but it does not meet the standards of the Designation of Origin (Aguilar, 2020).

Damage to health is caused by methanol poisoning, levels above 300 mg/100 mL of anhydrous alcohol according to the NOM-006-SCFI-2012. When consumed, it is converted into formaldehyde and then into formic acid, which produces the blood to become acid, known as metabolic acidosis. The intoxication symptoms are abdominal pain, diarrhea, nausea, breathing difficulty, blindness, seizures, or even death (MI, 2016).

The economic sector is also affected by piracy; 36% of the total sales are adulterated beverages, a quantity equal to 61.2 million liters of illicit Tequila, a loss of $19,430 million (Mexican pesos) for the industry, and $6,000 million to the Service and Production Special Tax (IEPS by its initials in Spanish) (Aguilar, 2020).

For these reasons, the Tequila Regulatory Council was invited to the international Producers Union Council (Unifab), which teaches the consumers about the consequences of adulterated products, both economic and health issues. The principal objective of Unifab is to protect the products that are under the protection of the Designation of Origin, looking after the cultural heritage of every member country (Romo, 2020).

In Table 5, it is shown that the most common analytical technique for Tequila analysis is gas chromatography coupled with mass spectrometry, which determines the amount of the physicochemical compounds limited by NOM-006-SCFI-2012, declared by the Ministry of the Interior of the Mexican Federal Government, indicated in Table 6. This technique was also used to determine plasticizer substances such as phthalates contaminating Tequilas during manufacturing or storage (Balderas-Hernández et al., 2020). Another application for this technique is to evaluate the changes that the maturation process confers to the sensorial characteristics of Tequila (Martín-del-Campo et al., 2019).

Furthermore, the SPME-GC methodology (Vallejo-Cordoba et al., 2004) could be a good alternative for the classifying Tequila categories according to the carbohydrates used; the disadvantage is that multiple samples are required for the analysis. Another alternative methodology is the aromagram (González-Robles and Cook, 2016) which achieves the differentiation of mature Tequila from white Tequila owing to the presence of cask-extractive compounds and the increased flavor dilution factors of certain terpenes, acetals, and higher alcohols. UV-vis absorption spectroscopy allows discrimination among different Tequila brands, as well as 100% agave and Tequilas (Barbosa-García et al., 2007).
These are the only methodologies used to provide limited information about Tequila composition and quality, but they need to be performed separated to have a complete Tequila analysis, and neither of them could discriminate between Tequila categories or identify the whole beverage, just stages in the process.

Table 5. Comparison between Tequila analyses from 2004 to 2020.

Objective	Analytical Method	Results	Reference
Monitor phthalate content in Tequila and determine if it is related to the age of maturation and year of production	Gas Chromatography-Mass Spectrometry	22% of 295 samples were phthalate free, but after 201, none of them exceeded the maximum permitted limits	(Balderas-Hernández et al., 2020)
Evaluate the changes in the profile of minor volatile compounds throughout the maturation process of Silver Tequila in new oak barrels	Gas Chromatography-Mass Spectrometry	173 compounds were identified, of which 50 showed significant variations respect to the maturation time	(Martín-del-Campo et al., 2019)
Use for the first time Ion Chromatography and Fourier Transform Infrared Spectroscopy to assess the authenticity of Tequila	Ion Chromatography and Fourier Transform Infrared Spectroscopy	A differentiation between Tequila “100% agave” and Tequila was possible. The concentrations of methanol and isobuthanol were significantly higher in “100% agave” than in Tequilas	(Lachenmeier et al., 2005)
Establish a Solid Phase Microextraction (SPME) and Gas Chromatography method that allows Tequila volatile characterization and ethyl esters quantitation	SPME, Gas Chromatography-Mass Spectrometry	Alcohols, esters, and ketones were the mainly identified volatiles. The coefficients of variation were <10% so the technique is reproducible. Quantitative differences in ethyl esters were found for Tequila categories	(Vallejo-Cordoba et al., 2004)

Table 5. Comparison between Tequila analyses from 2004 to 2020 (continue).

Objective	Analytical Method	Results	Reference
Use the Gas Chromatography-Mass Spectrometry and Gas Chromatography/ Aroma Extract Dilution Analysis to identify and quantify the key aroma compounds in the extract of white and mature Tequila	Gas Chromatography-Mass Spectrometry and Gas Chromatography Olfactometry/Aroma Extract Dilution Analysis	The main different odour-active regions between white and mature Tequila were: phenethyl alcohol, guaiacol, 4-ethyl guaiacol, vainillin, and cis/trans whisky lactones	(González-Robles & Cook, 2016)
Present a fast, simple and inexpensive spectroscopic method that allows the discrimination of Tequilas	UV-vis spectroscopy and multivariate analysis	Different brands of white Tequila were identified, and 100% agave and Tequilas were discriminated	(Barbosa-García et al., 2007)

Table 6. Tequila physicochemical specifications (NOM-006-SCFI-2012).

Parameters	Minimum	Maximum
Alcoholic content at 293K (%Alc. Vol.)	35	55
Dry extract (g/L)	0	5
Superior alcohols	20	500
Methanol	30	300
Aldehydes	0	40
Esters	2	200
Furfurals	0	40

Values expressed in mg/100 ml of anhydrous alcohol.

2. Hypothesis, Objectives and Research Plan

2.1 Hypothesis

If there are methods for obtaining **certain** metabolites at **certain** stages of the Tequila process, then there may be a method for determining the metabolic footprint of the final drink.

2.2 General objective

Verify the adequacy of the method to identify Tequila by class, category, and geographical origin, through a mathematical statistical model of metabolomics given by an UPLC-MS study.

2.2.1 Specific Objectives

1. Implement the UPLC-MS method to determine the metabolomic footprint in different tequilas, from previous works of distillates with Appellation of Origin.
2. Obtain the metabolomic information of 27 certified samples from stores and distilleries.
3. Discriminate by class and category of Tequila samples through the metabolomic fingerprint.

2.3 Research Plan

1. Investigate and collect information regarding the methodology previously used in distillates with characteristics similar to Tequila for the UPLC-MS.
2. Through the UPLC-MS, obtain data from Tequila samples, based on their class and category, and create chromatograms with retention time for each peak.
3. Perform the corresponding statistical analysis in the STATISTICA software with the UPLC-MS data, performing Analysis of Principal Components, ANOVA, and General Discriminant Analysis.

3. Theoretical Framework

3.1 Tequila

The production process of Tequila begins with the inulin hydrolysis, the principal carbohydrate in the head of the agave, by an enzymatic and thermal procedure. The extraction of the agave's carbohydrates is the next step; this is carried out by a roller mill train. The extraction result is the fresh wort that is fermented by yeast into ethanol and carbon dioxide. This step depends on the carbohydrates required for a determined Tequila category, according to the NOM-006-SCFI-2012. Then, the first distillation is performed with the aim of non-desirable components elimination as yeasts, solids, salts, water, and methanol; these are called vinasse. Another distillation is necessary to obtain the final product, Tequila (CRT, 2020c).

The maturation process depends on the category of Tequila. Tequila has two categories: category and class. The categories of Tequila are two: the "100% agave" that is subjected to a fermentation only with sugars from the Agave *tequilana* Weber Blue variety; and the so-called "Tequila" which undergoes fermentation enriched with other sugars in a proportion not greater than 49% of reducing sugars. The classes depend on the time of contact of the drink with the barrel, which are: Silver, known as the "purest", which has minimal or no contact with the wood; Gold, which is a mixture of white Tequila and rested or Aged; Aged, with a maturation process of minimum two months in barrel; Extra-aged, with a maturation process of minimum one year in barrel; and Ultra-aged, with a maturation process of minimum three years in barrel. The barrel must be oak or oak wood, new or previously used for wine or whisky, with a maximum capacity of 600 liters (CRT, 2020a).

Figure 3. The production process of Tequila.

Tequila's chemical composition is determined by water, agave, category of sugar used, yeasts, operation conditions, category of barrel, and time maturing in the barrel (López-Ramírez, 2015). As the three other spirits mentioned before, Tequila major volatiles are alcohols, acetals, aldehydes, esters, phenols, acids, furans, terpenes, and lactones, all of them involved in the sensory characteristics of the final product (Warren-Vega et al., 2021). The most abundant alcohols in Tequila are isoamyl alcohol, isobutanol, and methanol; the most abundant furans are furfural and acetaldehyde; and the most abundant esters are ethyl lactate and acetate (Espinosa, 2019).

The minority compounds with the highest concentration of alcohols are 2-hexanol, oct-2-en-1-ol, 3,4-dimethylpentan-1-ol, 3-phenylpropan-1-ol, and cyclohexen-2-en-1-ol. The most abundant acetals are 1,1-diethoxyisobutane, 1,1-diethoxymethane, 2-(diethoxymethyl)furan. The compounds with the highest concentration of aldehydes are 2,6,6-trimethylcyclohexen-1-carbaldehyde, 4-propan-2-ylcyclohexen-1-calbaldehyde, 2-

phenylacetaldehyde, and 4-hydroxy-3,5-dimethoxybenzaldehyde. The compounds with the highest concentration of esters are 1-phenylpropyl acetate, methyl hexadecanoate, methyl nonanoate, ethyl formate, and ethyl 2-methyl butanoate. The compounds with the highest concentration of phenols are 2-methoxy-4-propylphenol, 2-methoxyphenol, and 2,6-dimethoxyphenol. The compounds with the highest concentration of furans are 5-hydroxymethyl, 2-acetylfuran, and tetrahydrofuran-3-one (Martín-del-Campo et al., 2019).

The maximum alcoholic volume in Tequila es 55 degrees v/v, and the minimum is 35 degrees v/v (Contreras et al., 2010). The most used technique to adulterate this distilled spirit is the incorporation of methanol and water; in the case of methanol must not exceed the 3g/L in México, 0.28g/L in the US, and 0.2g/L in Brazil (Necochea-Chamorro et al., 2019).

3.2 Metabolomic fingerprint

The metabolomic fingerprint is one of the main approaches of metabolomics; which objective is not to identify or quantify each metabolite in the sample but to establish a total profile, a fingerprint, as a unique pattern that characterizes a print of the metabolism in a particular biological sample (Shulaev, 2006). The importance of metabolomic fingerprint lies in the identity and quantification of alterations as descriptors of differences in specific biological samples. Also, this approach compares patterns of the fingerprints of metabolites that change in response to toxic or environmental alterations. Spectroscopic techniques are used to perform fingerprinting, such as Fourier transform infrared spectroscopy (FT-IR), nuclear magnetic resonance (NMR), Fourier transform ion cyclotron resonance mass spectroscopy (FTICR-MS), or mass spectrometry (MS) by directly obtaining physical spectra without previous separation techniques like electrophoresis or chromatography (Dettmer et al., 2007).

3.3 Metabolomic fingerprint in spirits

Whisky.

The most practiced analytical method for metabolomic analysis in whiskies is Fourier transform ion cyclotron resonance coupled with mass spectrometry (Stupak et al., 2018) (Roullier-Gall et al., 2018) (Roullier-Gall et al., 2020) (Kew et al., 2017), as shown in Table 7, this technique is established on the determination of the mass-to-charge ratio (m/z) of ions based on the cyclotron frequency of the ions in a stable magnetic field (Marshall et al., 1998). Along with, principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) as the major statistical analysis. PCA is a multivariate technique that examines a data set whose interpretations are related by several correlated quantitative dependent variables (Abdi & Williams, 2010); and PLS-DA is the regression of a given Y of many variables on a collection of X predictor variables, representing the categories of a categorical variable (Pérez-Enciso & Tenenhaus, 2003).

Rum.

Table 7 show that techniques in rum variate between proton nuclear magnetic resonance spectroscopy and liquid/ gas chromatography (Franitza et al., 2016) (Belmonte-Sánchez et al., 2019) (Belmonte-Sánchez et al., 2020). Proton nuclear magnetic resonance spectroscopy is a non-invasive approach that applies nuclear magnetic resonance in terms of hydrogen nuclei within a substance's molecules, so that the structure of its molecules may be determined (Naruse et al., 1982). The other methods are liquid or gas chromatography; these are separation techniques, that are a result of the sample interaction with the mobile and stationary phases, given by the affinity of the sample (Hyötyläinen & Riekkola, 2003). Furthermore, principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA), as in whisky, are the principal statistical analysis.

Brandy.

Gas chromatography coupled with mass spectrometry is the analytical method used in Brandy metabolomic analyses, as shown in Table 7. The ones that variate between methods are ion mobility spectrometry and flame ionization detection (Li et al., 2020) (Ivanović et al., 2021). In Ion mobility spectrometry, ionized molecules in the gas phase are separated and identified depending on their mobility in a carrier buffer gas (Borsdorf & Eiceman, 2006). In flame ionization detection, the eluting chemicals are burned by a flame that is surrounded by air an oxygen-rich environment, so the carrier gas is exiting the column combined with hydrogen (Dodds et al., 2005). A gas-phase ion is produced in approximately one out of 10,000 organic molecules; a collector electrode, which is located above the flame, detects these ions (Becker et al., 2019). Furthermore, principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA), as in whisky and rum, are the principal statistical analysis.

Table 7. Detection methods and analysis of significant markers in spirits

Distillate	Analytical Method	Statistical analysis	Significant markers	Discrimination achieved	Reference
Whisky	Gas chromatography-Tandem high-resolution mass spectrometry	Principal component analysis and Partial least squares discriminant analysis	N-(3-methylbutyl) acetamide and 5-oxooxolane-2-carboxylic acid	Separation of malt whiskies by the type of cask used during its maturation	(Stupak et al., 2018)
Whisky	Fourier transform ion cyclotron resonance and liquid chromatography-Tandem mass spectrometry	Partial least squares discriminant analysis and Hierarchical cluster analysis	Bourbon casks: flavonols, oligolignols, and fatty acids Sherry casks: quercetin-glucuronide and myricetin-glucoside Whiskies: high alcohols and fatty acids	Separation of malt whiskies by the type of cask used during its maturation and discrimination of rums and whiskies	(Roullier-Gall et al., 2018)
Whisky	Fourier transform ion cyclotron resonance- Mass spectrometry and UPLC	Partial least squares discriminant analysis and Hierarchical cluster analysis	Geographical origin: (2S,3S)-2,3,4-triacetyloxybutanoate, polyphenols, higher alcohols, and carbohydrates Distillery brand: polyphenols, carbohydrates, sulfur and nitrogen compounds, and higher alcohols Maturation time: syringic acid, gallic acid and scopoletin	Geographical origin, distillery brand and maturation time impact of whisky	(Roullier-Gall et al., 2020)
Whisky	Fourier transform ion cyclotron resonance- Mass spectrometry	Principal component analysis and Orthogonal Projections to Latent Structures Discriminant Analysis	Malt: syringic acid, ellagic acid, and gallic acid Sherry cask: ellagic acid, glucono delta-lactone, gallic acid and syringic acid Bourbon: decanoic acid, dodecanoic acid, disaccharide, hexadecanoic acid, hexadic-9-enoic acid, and tetradecanoic acid	Discriminate malts and blends, and separation of malt whiskies by the type of cask used during its maturation	(Kew et al., 2017)

Table 7. Detection methods and analysis of significant markers in spirits (continue).

Distillate	Analytical Method	Statistical analysis	Significant markers	Discrimination achieved	Reference
Whisky	High resolution nuclear magnetic resonance	Independent, Principal component analysis, and Orthogonal partial least squares discriminant analysis	Blends and malts: 3-methylbutanol Maturation wood types: higher alcohols, carbohydrates including glucose, syringaldehyde, and furfural Real samples: higher alcohols and carbohydrates Fakes: glycerol and sugars	Discriminate malts and blends, separation of malt whiskies by the type of cask used during its maturation, and generic whisky authentication	(Kew et al., 2019)
Whisky	Ultrahigh pressure liquid chromatography-Quadrupole time-of-flight mass spectrometry	Linear discriminant analysis	Wood derived phenolic compounds, lignan derived compounds, and several C8 and larger lipids	Differentiate Tennessee whiskeys from bourbon and rye, and discriminate between younger and older whiskeys	(Collins et al., 2014)
Rum	Two-dimensional gas chromatography-time-of-flight mass spectrometry	Partial least squares discriminant analysis	1-decanol, γ-dodecalactone, ethyl 3-methylbutanoate, ethyl nonanoate, 3-furancarboxaldehyde, 1-hexanol, β-ionone, 2- and 3-methylbutanol, methyl decanoate, 3-octanol, and 2-undecanone	Discrimination of sugar cane juice rums and rums from sugar cane molasses	(Franitza et al., 2018)
Rum	Proton nuclear magnetic resonance spectroscopy-Mass spectrometry	Principal component analysis and Partial least square discriminant analysis	A-D-fructofuranose/β-D-fructofuranose, α-D-glucopyranose, and sucrose	Classification by fermentation barrel, raw material, and distillation method and aging	(Belmonte-Sánchez et al., 2020)

Table 7. Detection methods and analysis of significant markers in spirits (continue).

Distillate	Analytical Method	Statistical analysis	Significant markers	Discrimination achieved	Reference
Rum	Liquid chromatography-High resolution mass spectrometry	Principal component analysis and Partial least square discriminant analysis	Furfural derivates (hydroxymethylfurfural) and sugars (glucose, mannitol)	Classification based on fermentation barrel, raw material, and aging	(Belmonte-Sánchez et al., 2019)
Brandy	Gas chromatography-Mass spectrometry and Gas chromatography-Ion mobility spectrometry	Partial least squares regression	Acetals, furans, phenols, lactones, terpenes, C13-norisoprenoids, nonanal, esters, and alcohols	Relation between volatiles and brandy age	(Li et al., 2020)
Brandy	Gas chromatography with Flame Ionization Detection-Mass spectrometry	Principal component analysis and Orthogonal partial least squares discriminant analysis	Unsaturated fusel alcohols, unsaturated aldehydes, monoterpene derivatives, lactones, fatty acid ethyl esters, ethyl butyrate, ethyl stearate, ethyl laurate, and ethyl benzoate	Discrimination based on plum varieties, pH of the mash, and yeast or enzymes added during fermentation	(Ivanović et al., 2021)

3.4 UPLC-MS

Ultra Performance Liquid Chromatography is a **separation science** that breaks a compound or mixture into its constituent parts on a stationary phase column. This technique uses narrow LC columns packed with sub-2μm small hybrid particles and high back-pressures for mobile phase delivery methods (Gruz et al., 2008). The UPLC system consists of a mobile phase, pump (back-pressure >15,000 psi), autosampler, column, column heater, UV

detector, and the chromatography data system. The last one can create peaks just like in gas chromatography; with widths, at half-height, of less than a second (Yu et al., 2006).
The advantages that UPLC has that HPLC do not are that while UPLC maintains the practicality and chromatographic principles, the peak resolution and sensitivity are superior, the operating costs are lower, the analysis times are shorter, and the separation efficiency is increased (Swartz, 2005).

The performance qualities of the UPLC technique are improved significantly by the mass spectrometry detection due to the sensitivity of the analytes present at the detection stage, where they are more concentrated (Yu et al., 2006). This detection technique provides a reproducible and accurate quantification of the metabolites in a biofluid or organism, with a capacity to cover extent ranges. For that, MS-based metabolite profiling (metabolite fingerprint) can be done as the metabolite concentrations are higher than nine orders of magnitude in an organism, and the different molecular species such as organic and amino acids, vitamins, lipids, peptides, and carbohydrates are present (Want et al., 2010).

4. Materials and Methods.

4.1 Materials

27 samples of Tequila were obtained from convenience stores and recognized distilleries, including all classes and categories. The Tequilas were 3 Silvers: “Don Jacinto” from Arandas, Jalisco; “Cien años” from Tequila, Jalisco; and “San Matías” from Tepatitlán, Jalisco. One Aged Tequila: “Sauza” from Tequila; and one Ultra-aged Tequila: “San Matías” from Acatic, Jalisco. The 100% agave Tequilas were 7 Silvers: “Campo Azul” from Jesús María, Jalisco; “Regional” from Tequila; “Don Eduardo” from Tequila; “Don Julio” from Atotonilco El Alto, Jalisco; “Antiguo” from Tequila; “Arette” from Tequila; and “El Jimador” from Tequila; 9 Aged Tequilas: “30-30” from Capilla de Guadalupe, Jalisco; “Don Julio” from Atotonilco El Alto; “Sauza” from Tequila; “Don Jacinto” from Arandas; “El Jimador” from Tequila; “Campo Azul” from Jesús María; “Don Eduardo” from Tequila; “Antiguo” from Tequila; and “San Matías” from Tepatitlán; and 6 Extra-aged Tequilas: “30-30” from Capilla de Guadalupe; “Don Julio” from Atotonilco el Alto; “El Jimador” from Tequila; “Antiguo” from Tequila; “Regional” from Tequila; and “Campo Azul” from Jesús María.

4.2 Chemicals and reagents

UPLC water by a Milli-Q® Integral system was used. Acetonitrile for use in Liquid Chromatography and Mass Spectrometry BAKER ANALYZED® LC-MS Reagent (J.T, Baker®, Phillipsburg, New Jersey) and Millipore Sigma formic acid, 98%-100% for LC-MS, (LiChropur®, Burlington, Massachusetts).

4.3 Sample preparation

All the Tequila samples were filtrated with a PTFE membrane (Ks-Tek®) with 0.22 μm pore size and 13 mm membrane diameter, and stored at -20 °C. All samples were measured by five biological replicates and pooled in one additional sample.

4.4 UPLC process

The samples were directly injected into the UPLC-MS without any dilution. The separation of metabolites was performed by a Waters Acquity UPLC® system (Waters corporation, Milford, Massachusetts) coupled to a MALDI SYPNAPT G2-Si High-Definition Mass Spectrometer (Waters corporation, Milford, Massachusetts). The method for separation was achieve using an Acquity UPLC® HSS T3 1.8 μm, 2.1 x 150 mm column (Waters corporation, Milford, Massachusetts). The temperature for the column was set to 25 °C. The temperature of the sample compartment was set to 4 °C. For seal, it was used 90/10 water/acetonitrile and for wash 50/50 water/acetonitrile. A reversed-phase gradient was employed, with 0.1% acid formic in water as solvent A, and 0.1% acid formic in acetonitrile as solvent B. Elution starting concentration was 95% A / 5% B. After 25 minutes, the gradient concentrations changed linearly to 50% A / 50% B, for 40 to 43 minutes the concentration changed linearly to 0% A / 100% B, and for 43 to 47 minutes the concentration was the same as the start of 95% A / 5% B. Forty-seven minutes was the total time for each analysis. The flow rate was set at 0.4 mL/min. The blank was 95/5 water/acetonitrile. All the samples were ionized in Electro Spray (ESI) in positive and negative mode to ensure the ionization of all compounds.

The tramp gas was argon and for the API was nitrogen. The source capillary voltage was 2.5 kV, for the sampling cone 40 V and for the source off set 80 V. The temperature of the source was 150 °C, and the solvation 500 °C. The gas flow for the cone was 50 (L/h), for the desolvation gas was 1000 (L/h), and the nebulizer 6.5 Bar.

4.5 Statistical analysis.

The data obtained from the UPLC-MS process were separated in 2 data set: the negative ionized and the positive ionized; and both were analyzed separately by working with STATISTICA v13 (TIBCO Software INC, USA). Analysis of variance (ANOVA) multivariate tests of significance, univariate results, and Fisher's least significant difference were carried out to compare the means and discriminate between category, class, origin, and (in the case of Fisher's LSD) code, code is described in Table 8. Subsequently, General Discriminant Analysis (GDA) was performed to obtain the squared Mahalanobis distances to separate from groups centroids the different Tequila categories, classes, and origins; and forward stepwise (p inclusion 0.05, p exclusion 0.05) was used to include the peaks that had a significant F value ($p<0.05$), in order to reduce the model. Lastly, Principal Component Analysis was used to observe the projection of the variables and cases of each Tequila category, class, origin, and code.

Table 8. Tequila's code by class, category, and origin.

Code	Class	Category	Origin	Tequila House
MB09	Silver	Tequila	JA	Don Jacinto
CB05	Silver	100% agave	JA	Campo Azul
CB12	Silver	100% agave	JT	Regional
CB07	Silver	100% agave	JT	Don Eduardo
CB04	Silver	100% agave	JA	Don Julio
MB01	Silver	Tequila	JA	100 años
MB02	Silver	Tequila	JA	San Matías
CB06	Silver	100% agave	JT	Antiguo
CB01	Silver	100% agave	JT	Arette
CB03	Silver	100% agave	JT	El Jimador
CR08	Aged	100% agave	JA	30-30
CR04	Aged	100% agave	JA	Don Julio
CR10	Aged	100% agave	JT	Sauza
CR09	Aged	100% agave	JA	Don Jacinto
CR03	Aged	100% agave	JT	El Jimador
CR05	Aged	100% agave	JA	Campo Azul

JA: Tequila from the region Los Altos, Jalisco, México.

JT: Tequila from the region Tequila, Jalisco, México.

Table 8. Tequila's code by class, category, and origin (continue).

Code	Class	Category	Origin	Tequila House
CR07	Aged	100% agave	JT	Don Eduardo
MR10	Aged	Tequila	JT	Sauza
CR06	Aged	100% agave	JT	Antiguo
CR02	Aged	100% agave	JA	San Matías
CA08	Extra-aged	100% agave	JA	30-30
CA04	Extra-aged	100% agave	JA	Don Julio
CA03	Extra-aged	100% agave	JT	El Jimador
CA06	Extra-aged	100% agave	JT	Antiguo
CA12	Extra-aged	100% agave	JT	Regional
CA05	Extra-aged	100% agave	JA	Campo Azul
MEA02	Ultra-aged	Tequila	JA	San Matías

JA: Tequila from the region Los Altos, Jalisco, México.

JT: Tequila from the region Tequila, Jalisco, México.

5. Results and Discussion.

5.1 UPLC-MS.

The samples were ionizing in positive and negative to have a complete evaluation of all the compounds; given that some important compounds for the Tequila's sensory characteristics, such as cinnamaldehyde that can only be ionized in positive, and vanillin that can only be ionized in negative. Each sample was measured by five biological replicates. The total time of each analysis was forty-seven minutes. Each retention time was assigned with a peak number, and this was made for the negative and positive mode.

104 compounds (peaks) were obtained in the negative mode, Table 9 shows each compound with its retention time. The compounds present in most samples were P1, P2, P3, P4, P6, P19, P24, P25, P27, P38, P42, P46, P48, P49, P52, P56, P58, P60, P62, P65, P68-P87, P89, P91, P93, P94, P98, P101-P104. The Extra-aged and Ultra-aged Tequilas are the ones that contained almost all the compounds found.

Table 9. Compounds ionized in negative mode with its retention time.

Compound (peak)	Retention time (min)	Compound (peak)	Retention time (min)	Compound (peak)	Retention time (min)
P1	0.71	P36	13.15	P71	27.80
P2	0.89	P37	13.35	P72	27.94
P3	0.93	P38	13.48	P73	28.26
P4	1.04	P39	13.89	P74	28.63
P5	1.14	P40	14.30	P75	29.04
P6	1.25	P41	14.63	P76	29.57
P7	1.74	P42	15.11	P77	29.83
P8	2.39	P43	15.36	P78	30.23
P9	2.84	P44	15.74	P79	30.86
P10	3.00	P45	16.13	P80	30.99
P11	3.49	P46	16.96	P81	31.43
P12	3.80	P47	17.30	P82	32.16
P13	4.01	P48	17.74	P83	32.25
P14	4.99	P49	18.24	P84	32.70
P15	5.37	P50	18.52	P85	32.94
P16	5.80	P51	19.47	P86	33.83
P17	6.10	P52	19.75	P87	34.77
P18	6.55	P53	20.07	P88	35.07
P19	6.80	P54	20.43	P89	36.07
P20	8.23	P55	20.75	P90	36.51
P21	8.42	P56	21.27	P91	37.87
P22	8.83	P57	21.5	P92	38.22
P23	9.28	P58	21.82	P93	38.41
P24	9.65	P59	22.37	P94	38.60
P25	10.38	P60	22.60	P95	38.92
P26	10.76	P61	22.95	P96	39.17
P27	11.00	P62	23.33	P97	39.29
P28	11.50	P63	23.61	P98	40.33
P29	11.77	P64	23.83	P99	40.53
P30	12.03	P65	24.24	P100	40.72
P31	12.12	P66	24.46	P101	41.23

Table 9. Compounds ionized in negative mode with its retention time (continue).

Compound (peak)	Retention time (min)	Compound (peak)	Retention time (min)	Compound (peak)	Retention time (min)
P32	12.38	P67	25.43	P102	41.37
P33	12.46	P68	25.86	P103	41.52
P34	12.62	P69	26.38	P104	41.97
P35	12.85	P70	26.74		

In positive mode 123 compounds (peaks) were obtained, Table 10 shows each compound with its retention time. The compounds present in most samples were P1-P7, P11, P13, P14, P16, P18, P20, P27, P28, P30, P33, P34, P38, P40, P44, P46, P48, P49, P52, P54-P122. The Extra-aged and Ultra-aged Tequilas are the ones that contained almost all the compounds found.

Table 10. Compounds ionized in negative mode with its retention time.

Compound (peak)	Retention time (min)	Compound (peak)	Retention time (min)	Compound (peak)	Retention time (min)
P1	0.67	P42	12.94	P83	29.78
P2	0.71	P43	13.05	P84	29.82
P3	0.87	P44	13.39	P85	30.12
P4	0.97	P45	13.54	P86	30.23
P5	1.04	P46	13.96	P87	30.48
P6	1.10	P47	14.15	P88	30.76
P7	1.45	P48	14.50	P89	30.94
P8	1.60	P49	14.86	P90	31.36
P9	2.00	P50	15.09	P91	31.81
P10	2.90	P51	15.39	P92	31.90
P11	3.87	P52	15.81	P93	32.16
P12	4.12	P53	16.07	P94	33.01
P13	4.48	P54	16.39	P95	33.51
P14	5.80	P55	16.71	P96	33.84
P15	6.10	P56	17.18	P97	34.05
P16	6.43	P57	17.56	P98	34.14

Table 10. Compounds ionized in negative mode with its retention time (continue).

Compound (peak)	Retention time (min)	Compound (peak)	Retention time (min)	Compound (peak)	Retention time (min)
P17	6.74	P58	18.34	P99	34.38
P18	6.97	P59	18.71	P100	35.23
P19	7.01	P60	20.4	P101	35.21
P20	7.38	P61	20.76	P102	35.31
P21	7.53	P62	21.42	P103	35.46
P22	8.06	P63	21.56	P104	36.06
P23	8.15	P64	22.00	P105	36.17
P24	8.33	P65	22.42	P106	36.49
P25	8.47	P66	22.77	P107	36.79
P26	8.62	P67	23.07	P108	36.90
P27	8.80	P68	23.23	P109	38.25
P28	9.08	P69	23.82	P110	38.45
P29	9.52	P70	24.04	P111	38.77
P30	9.73	P71	24.59	P112	39.09
P31	9.92	P72	25.14	P113	39.85
P32	10.42	P73	25.31	P114	40.02
P33	10.75	P74	25.72	P115	40.42
P34	10.95	P75	25.99	P116	40.63
P35	11.39	P76	26.3	P117	40.72
P36	11.62	P77	26.52	P118	40.80
P37	11.80	P78	27.00	P119	41.28
P38	12.14	P79	27.45	P120	41.49
P39	12.37	P80	27.96	P121	41.64
P40	12.59	P81	28.24	P122	41.7
P41	12.79	P82	29.21	P123	43.14

5.2 ANOVA.

In the Table 11 and Table 12 they are shown, with a 95% level of confidence, that all the variables: category, class, and origin are statistically significant ($p < 0.05$); all three double interactions are also statistically significant, for both ionization modes, positive and negative.

This demonstrate that all the effects and its interactions affect the tequila characterization, so it is possible to identify each tequila by category, class, or origin. This differentiation has already been reported before in the study of whisky from different origin, brand, and maturation time (Roullier-Gall et al., 2020), where Fourier Transform- Ion Cyclotron Resonance was used to identify 1,182 molecular formulas with an increase in their peak intensities as a function of the maturation time, also 467 formulas were recognized that differentiates the origin of the barrel used for the maturation process.

Table 11. Multivariate Tests of Significance (negative mode).

Effect	F	Error	p
Category	1364.003	18	0.00
Class	622.498	54.96	0.00
Origin	519.502	18	0.00
Category*Class	1162.462	18	0.00
Category*Origin	260.653	18	0.00
Class*Origin	183.504	36	0.00

Table 12. Multivariate Tests of Significance (positive mode).

Effect	F	Error	p
Category	301.2	3.0	0.00
Class	159.5	10.0	0.00
Origin	270.0	3.0	0.00
Category*Class	650.8	3.0	0.00
Category*Origin	238.5	3.0	0.00
Class*Origin	303.8	6.0	0.00

In this ANOVA and Fisher's LSD analysis, the variables and the 104 peaks of the chromatogram obtained by the UPLC-MS process are displayed in Table 13, where the samples are ionized in negative mode. This analysis was made to obtain the peaks that show a significant difference between category, class, origin, and its interceptions. It can be observed that according to the variable category, the peaks that show a significant difference ($p<0.05$) are 32, according to the variable class are 79, and according to the variable origin are 36. According to the interaction category*class are 36, according to the interaction category*origin are 19, according to the interaction class*origin are 68. The classes of the Tequila include most of the peaks, even when it is interacting with the origins; on the other hand, Tequila categories are the ones with the fewer number of peaks.

The ANOVA and Fisher's LSD analyses are in Table 14 with the variables and the 123 peaks of the chromatogram obtained by the UPLC-MS process, in positive mode. It can be observed that according to the variable category the peaks that show a significant difference ($p<0.05$) are 42, according to the variable class are 94, according to the variable origin are 48, according to the interaction category*class are 39, according to the interaction category*origin are 43, and according to the interaction class*origin are 75. The classes of the Tequila include the majority of the peaks, even when it is interacting with the origins; on the other hand, Tequila categories are the ones with the fewer number of peaks, even interacting with origin.

In both modes, positive and negative, Tequila classes are those containing the highest number of peaks that are statistically significant, this can be because classes are separated according to the time that are contained in the barrel, where acquires different chemical composition, due to the evolution of their compounds over time. That difference in composition is represented in the number of peaks that identify each class. An increase in volatile compounds in tequilas with a longer maturation time in barrels has already been study, like ethyl esters (Vallejo-Cordoba et al., 2004), isoamyl alcohol, furfural, 5-methyl furfural, vanillin, and decanoic acid (González-Robles and Cook, 2016), cyclopentanone, 2-phenylethyl acetate, and methyl-2-furancarbocaldehyde (Martín-del-Campo et al., 2019).

The samples ionized in negative mode, shown in Table 13, that has some peaks with different groups according to the Tequila category are in total 51 peaks, which represents more than 49.0% of the total peaks. According to origin are 45 peaks, which represent the 43.2% of the total peaks. The Tequila class that has the most different groups are the Silver and Ultra-aged. Those variations are obtained by the Fisher's LSD analysis, and that mean that those peaks that are grouping differently one another, so that allow to classify each Tequila category by category and origin. In the case of the classes that the Silver and Ultra-aged Tequila are the ones with the most different means, so it is possible to separate each Tequila by its class because the data allow to differentiate the Tequila classes that has the most different maturation time.

Table 13. Univariate results for each dependent variable (negative mode).

	Category*			Class*					Origin*					
	p	T	HT	p	W	A	EA	UA	p	JA	JT	Category*Class	Category*Origin	Class*Origin
P1	0.9	29398^{a}	24076^{a}	0.0	37509^{b}	17104^{a}	19741^{a}	8100^{a}	0.5	24381^{a}	2577^{a}	0.0	0.2	0.9
P2	0.0	104058^{b}	22613^{a}	0.0	64028.7^{b}	20146^{a}	17274^{a}	68688^{b}	0.0	27324^{a}	48503^{a}	0.0	0.0	0.7
P3	0.7	62327^{a}	94512^{a}	0.7	124791^{a}	54703^{a}	63506^{a}	188360^{a}	0.6	52810^{a}	122338^{a}	0.1	0.2	0.0
P4	0.0	158762^{b}	61026^{a}	0.0	60950^{a}	70713^{a}	89230^{a}	221745^{b}	0.0	73609^{a}	85474^{a}	0.8	0.0	0.0
P5	0.0	11049^{a}	12495^{a}	0.0	4138^{b}	14003^{a}	15091^{a}	55246^{c}	0.2	12538^{a}	11914^{a}	0.1	0.2	0.6
P6	0.0	11322^{a}	20507^{b}	0.2	17112^{a}	21655^{a}	14942^{a}	27337^{a}	0.7	15054^{a}	22289^{b}	0.9	0.0	0.2
P7	0.0	1368^{b}	384^{a}	0.0	428^{a}	238^{a}	1077^{b}	2560^{c}	0.1	803^{b}	351^{a}	1.0	0.0	0.0
P8	0.3	5503^{a}	4762^{a}	0.0	2319^{a}	1193^{a}	16530^{b}	4327^{b}	0.2	2796^{a}	6917^{a}	0.8	0.2	0.0
P9	0.0	1218^{b}	325^{a}	0.0	485^{c}	0.0^{b}	1278^{a}	1241^{a}	0.3	488^{a}	503^{a}	1.0	0.0	0.0
P10	0.4	495^{a}	569^{a}	0.0	247^{a}	84^{a}	2083^{b}	0^{a}	0.2	258^{a}	839^{a}	1.0	0.4	0.0
P11	0.7	1187^{a}	2249^{a}	0.6	593^{a}	4513^{a}	640^{a}	0^{a}	0.2	3919^{a}	258^{a}	1.0	0.7	0.1
P12	1.0	1578^{a}	1836^{a}	0.3	429^{b}	0^{a}	762^{a}	0^{ab}	0.8	743^{a}	2785^{a}	0.1	0.0	0.1
P13	1.0	569^{a}	1188^{a}	0.0	0^{b}	1543^{a}	1864^{a}	2847^{a}	0.9	1473^{a}	684^{a}	1.0	1.0	0.0
P14	1.0	139^{a}	446^{b}	0.0	0^{a}	0^{a}	1751^{c}	696^{b}	0.0	135^{a}	628^{b}	1.0	1.0	0.0
P15	0.0	8015^{b}	1852^{a}	0.0	1585^{b}	3743^{a}	3978^{a}	5318^{a}	0.1	964^{a}	5000^{b}	0.0	0.1	0.0
P16	1.0	94^{a}	1145^{b}	0.0	0^{a}	938^{b}	2793^{c}	472^{b}	0.0	845^{a}	1040^{a}	1.0	1.0	0.0
P17	1.0	67^{a}	356^{a}	0.0	0^{a}	0^{a}	1399^{b}	335^{a}	0.0	61^{a}	530^{b}	1.0	1.0	0.0
P18	0.5	1622^{b}	736^{a}	0.0	236^{a}	796a^{b}	1267^{b}	6710^{c}	0.8	1306^{b}	523^{a}	0.8	0.3	0.0
P19	0.6	3547^{a}	5726^{b}	0.0	2079^{a}	2595^{a}	14637^{b}	13870^{b}	0.0	4321^{a}	6256^{b}	0.6	0.1	0.0
P20	0.9	592^{a}	676^{a}	0.0	54^{a}	152^{a}	2276^{b}	2963^{b}	0.0	453^{a}	857^{b}	0.9	0.9	0.0
P21	0.6	7263^{a}	17248^{a}	0.0	5196^{a}	11640^{ab}	37176^{c}	35188b^{c}	0.1	12143^{a}	1839^{a}	0.8	0.6	0.0
P22	0.0	6287^{b}	4252^{a}	0.0	399^{a}	2015^{b}	14290^{c}	20685^{d}	0.0	4632^{a}	4649^{a}	0.0	0.5	0.0
P23	1.0	25904^{b}	5626^{a}	0.0	134^{a}	694^{a}	20579^{b}	129522^{c}	0.0	11022^{a}	8038^{a}	1.0	1.0	0.0
P24	0.1	47843^{a}	57355^{a}	0.0	17953^{a}	44209^{b}	112506^{c}	234828^{d}	0.0	49880^{a}	60945^{a}	0.8	0.3	0.0
P25	0.9	8320^{b}	4479^{a}	0.0	715^{a}	2211^{a}	12760^{b}	38825^{c}	1.0	7130^{b}	3379^{a}	1.0	0.8	0.2
P26	0.8	769^{a}	3480^{a}	0.0	0^{a}	418^{a}	12903^{b}	3848^{a}	0.2	2278^{a}	3616^{a}	0.8	1.0	0.1
P27	0.9	3538^{a}	5559^{a}	0.0	0^{a}	2155^{a}	18617^{b}	13895^{b}	0.0	4057^{a}	6240^{b}	0.9	1.0	0.0
P28	0.0	2062^{a}	1406^{a}	0.0	1504^{b}	33^{a}	4495^{c}	479^{ab}	0.0	1194^{a}	1852^{a}	0.1	0.6	0.0
P29	0.7	4585^{a}	6231^{a}	0.0	3849^{a}	0^{a}	17337^{b}	22924^{b}	0.9	5974^{a}	5862^{a}	0.4	0.4	0.2
P30	0.5	1226^{a}	3567^{a}	0.0	0^{a}	1635^{ab}	11036^{c}	6131^{bc}	0.6	3835^{a}	2437^{a}	0.5	1.0	0.1
P31	0.8	2459^{a}	4730^{b}	0.0	0^{a}	1587^{a}	15795^{b}	11722^{b}	0.0	1829^{a}	6653^{b}	0.8	1.0	0.0
P32	0.2	92^{a}	5369^{b}	0.0	0^{a}	2147^{a}	17231^{b}	189^{a}	0.0	543^{a}	8010^{b}	0.2	1.0	0.0

Table 13. Univariate results for each dependent variable (negative mode) (continue).

	Category*			Class*					Origin*					
	p	T	HT	p	W	A	EA	UA	p	JA	JT	Category*Class	Category*Origin	Class*Origin
P33	1.0	1139[a]	14147[a]	0.0	363[a]	0[a]	55095[b]	4470[a]	0.0	1586[a]	21291[b]	1.0	1.0	0.0
P34	0.6	1744[a]	1478[a]	0.0	291[a]	59[a]	5578[b]	6434[b]	0.0	931[a]	2099[b]	1.0	0.5	0.0
P35	0.7	561[a]	1626[a]	0.0	1444[b]	123[a]	3821[c]	1005[ab]	0.0	78[a]	2708[b]	0.8	0.7	0.0
P36	0.5	1070[a]	2618[a]	0.0	2100[a]	0[c]	6391[b]	5351[ab]	0.0	894[a]	3688[b]	0.2	0.2	0.0
P37	0.8	2692[a]	1819[a]	0.0	457[a]	0[a]	6578[b]	11925[c]	0.1	2028[a]	1945[a]	0.7	0.4	0.0
P38	0.0	9044[b]	5146[a]	0.0	5977[c]	83[b]	13924[a]	18538[a]	0.2	5108[a]	6637[a]	0.9	0.0	0.0
P39	0.1	9134[b]	4745[a]	0.0	5714a	6521[a]	4617[a]	276[a]	0.7	5846[a]	5331[a]	0.0	0.7	0.0
P40	0.5	219[a]	1595[a]	0.6	2363[b]	0[a]	2008[ab]	441[b]	0.1	34[a]	2573[b]	0.2	0.2	0.1
P41	0.4	3340[a]	3162[a]	0.0	1460[a]	336[a]	9101[b]	16703[c]	0.0	2564[a]	3800[a]	0.5	0.3	0.0
P42	0.4	992[a]	4224[b]	0.0	3245[a]	1379[a]	8071[b]	4960[ab]	0.1	2220[a]	4932[b]	0.2	0.1	0.0
P43	0.2	15517[b]	6642[a]	0.0	1965[a]	3127[a]	20452[b]	57655[c]	0.6	9630[a]	7099[a]	0.3	0.9	0.8
P44	0.3	705[a]	8646[a]	0.5	13092[b]	5156[ab]	901[a]	506[ab]	0.5	1548[a]	12463[b]	0.3	0.1	0.1
P45	0.7	3933[a]	13905[a]	1.0	21153[a]	0[a]	15418[a]	19665[a]	1.0	6571[a]	17189[a]	0.4	0.4	0.3
P46	0.4	4185[a]	24849[b]	0.0	8331[a]	3525[a]	75729[b]	20925[a]	0.0	4634[a]	36448[b]	0.9	1.0	0.0
P47	0.6	4579[a]	9310[a]	0.0	6485[ab]	2466[a]	20064[c]	22895[bc]	0.3	6235[a]	10482[a]	0.3	0.3	0.0
P48	0.5	6465[a]	60405[a]	0.8	109464[a]	10293[a]	19019[a]	14687[a]	0.6	7076[a]	91219[a]	0.3	0.2	0.2
P49	0.2	1783[a]	15429[b]	0.3	14040[ab]	7888[a]	20581[b]	7176[ab]	0.1	6117[a]	19233[b]	0.2	0.1	0.0
P50	0.6	2678[a]	7009[a]	0.0	3297[a]	6278[a]	12441[b]	293[a]	1.0	6048[a]	6310[a]	0.2	0.5	0.0
P51	0.8	13812[a]	18467[a]	0.6	38765[b]	5323[a]	757[a]	16664[ab]	0.7	1510[a]	32928[b]	0.0	0.1	0.0
P52	0.2	16057[a]	28501[a]	0.1	2930[b]	43120[a]	28316[ab]	79712[a]	0.5	35348[a]	17317[a]	0.3	0.9	0.1
P53	0.3	183362[b]	98534[a]	0.0	218601[c]	82332[b]	2227[a]	821[ab]	0.0	103754[a]	125199[a]	0.0	0.0	0.9
P54	0.1	299[a]	27258[b]	0.3	18536a[b]	36368[b]	6684[a]	1495[ab]	0.7	15512[a]	28418[a]	0.8	0.4	0.5
P55	0.0	22861[b]	9675[a]	0.0	8044[a]	17562[b]	9059[a]	17952[ab]	0.9	7190[a]	16969[b]	0.0	0.1	0.0
P56	0.4	22860[a]	22296[a]	0.0	39549[b]	12408a	12901a	232a	0.0	9423[a]	34804[b]	0.0	0.0	0.0
P57	0.4	2293[a]	12694[a]	0.6	21765[b]	3893[a]	4431[a]	841[ab]	0.3	192[a]	20755[b]	0.0	0.0	0.0
P58	1.0	69531[a]	61013[a]	0.0	3160[a]	55545[b]	133206[c]	345891[d]	0.0	120461[b]	7406[a]	0.9	0.9	0.0
P59	1.0	55125[a]	138049[b]	0.5	73390[a]	196961[b]	88560[a]	59915[ab]	0.4	148616[a]	97013[a]	0.1	0.3	0.0
P60	0.0	514321[b]	101813[a]	0.0	331415[b]	112084[a]	52477[a]	34096[a]	0.0	150253[a]	209463[a]	0.0	0.0	0.5
P61	0.3	18620[a]	103317[a]	0.3	170088[b]	42482[a]	13540[a]	93101[ab]	1.0	45511[a]	126931[b]	0.0	0.0	0.0
P62	0.5	33829[a]	79622[b]	0.0	24661[a]	134757[b]	43172[a]	56770[a]	0.1	81566[a]	60678[a]	0.3	0.3	0.0
P63	0.1	36548[b]	141222[a]	0.0	381310[b]	96141[a]	9963[a]	13571[a]	0.0	166990[a]	200386[a]	0.0	0.0	0.2
P64	0.0	23518[a]	19580[a]	0.0	389[a]	51736[b]	3509[a]	2829[a]	0.0	32099[b]	9090[a]	0.0	1.0	0.0
P65	0.6	55316[a]	175610[a]	0.4	345676[b]	44971[a]	18663[a]	1259[ab]	0.9	41565[a]	258767[b]	0.0	0.1	0.0
P66	1.0	3311[a]	21660[b]	0.0	8624[a]	4562[a]	63800[b]	278[a]	0.0	9139[a]	26773[b]	0.3	0.4	0.0
P67	0.0	78398[a]	170540[a]	0.0	74392[a]	218198[bc]	136415[ab]	388534[c]	1.0	148063[a]	157630[a]	0.2	0.3	0.0
P68	0.8	96171[a]	164258[a]	0.0	85653[a]	310094[b]	7860[a]	25224[a]	0.1	227832[b]	78125[a]	0.1	0.4	0.0
P69	0.6	256290[a]	306927[a]	0.0	169547[a]	394974[bc]	271981[ab]	753397[c]	0.7	294567[a]	299839[a]	0.4	0.3	0.0

Table 13. Univariate results for each dependent variable (negative mode) (continue).

	Category*			Class*					Origin*					
	p	T	HT	p	W	A	EA	UA	p	JA	JT	Category*Class	Category*Origin	Class*Origin
P70	0.1	187100^{a}	326784^{a}	0.0	276612^{a}	460621^{b}	107982^{a}	0^{a}	0.1	356606^{a}	246176^{a}	0.3	0.4	0.5
P71	0.0	212275^{b}	99358^{a}	0.0	92511^{b}	191461^{c}	44236^{a}	12745^{abc}	0.7	65785^{a}	173561^{b}	0.0	0.0	0.0
P72	0.1	118713^{a}	215000^{b}	0.0	213896^{a}	231025^{a}	113541^{b}	135441^{ab}	1.0	189936^{a}	203015^{a}	0.5	0.4	0.0
P73	0.4	38023^{a}	190840^{b}	0.0	122710^{b}	295311^{c}	21254^{a}	0^{a}	0.3	170513^{a}	153236^{a}	0.2	0.1	0.0
P74	0.0	242792^{b}	126023^{a}	0.1	158201^{a}	178867^{a}	87057^{b}	80625^{ab}	0.1	138280^{a}	157885^{a}	0.4	0.0	0.0
P75	0.0	57758^{a}	51941^{a}	0.0	35486^{a}	85061^{b}	33957^{a}	18112^{a}	0.5	41755^{a}	63841^{b}	0.0	0.2	0.0
P76	0.0	38392^{b}	7256^{a}	0.0	7660^{a}	16061^{b}	11994^{ab}	47009^{c}	0.2	10202^{a}	16059^{a}	0.0	0.2	0.2
P77	0.3	14066^{a}	38375^{b}	1.0	44986^{b}	2084^{a}	36882^{ab}	30606^{ab}	0.9	24662^{a}	42403^{b}	0.0	0.0	0.0
P78	0.1	42741^{a}	33954^{a}	0.0	35899^{b}	46956^{b}	21178^{a}	0^{a}	0.1	31383^{a}	39688^{a}	0.1	0.3	0.4
P79	0.1	344700^{a}	460255^{a}	0.3	306538^{a}	593924^{b}	382546^{a}	529337^{ab}	0.6	439786^{a}	436691^{a}	0.0	0.7	0.6
P80	0.0	427611^{b}	226152^{a}	0.1	316906^{a}	257416^{a}	210308^{a}	105077^{a}	0.9	250521^{a}	278045^{a}	0.0	0.3	0.7
P81	0.8	323988^{a}	395082^{a}	0.0	331906^{c}	570859^{d}	200408^{b}	0^{a}	0.0	359364^{a}	402673^{a}	0.0	0.1	0.2
P82	0.0	174862^{b}	83411^{a}	0.0	45483^{a}	160384^{c}	102669^{b}	61601^{a}	0.0	72070^{a}	128367^{b}	0.0	0.7	0.0
P83	0.0	66296^{b}	39553^{a}	0.0	27179^{a}	49796^{a}	67372^{a}	46414^{ab}	0.1	39245^{a}	49827^{a}	0.0	0.8	0.2
P84	0.0	82847^{b}	53244^{a}	0.0	59003^{c}	74118^{d}	41968^{b}	0^{a}	0.5	54002^{a}	63566^{a}	0.0	0.3	0.0
P85	0.0	442628^{a}	912641^{b}	0.0	693392^{a}	1075483^{b}	565588^{a}	1033312^{ab}	0.0	751172^{a}	891503^{a}	0.0	0.2	0.0
P86	1.0	396660^{a}	329072^{a}	0.0	376231^{a}	207432^{c}	501493^{b}	456429^{ab}	0.0	284399^{a}	396965^{b}	0.0	0.0	0.0
P87	0.0	372025^{b}	50524^{a}	0.0	11746^{c}	202037^{b}	124861^{a}	159548^{ab}	0.2	43929^{a}	176786^{b}	0.0	0.5	0.0
P88	0.5	54129^{a}	63796^{a}	0.2	62397^{a}	75078^{a}	33340^{a}	83346^{a}	0.3	37931^{a}	84896^{a}	0.0	0.3	0.1
P89	0.7	524809^{a}	692367^{b}	0.1	550102^{a}	721919^{b}	736315^{b}	750299^{ab}	0.0	581577^{a}	735674^{b}	0.0	0.2	0.0
P90	0.0	223234^{b}	36405^{a}	0.0	111505^{b}	57533^{a}	38720^{a}	0^{ab}	1.0	66688^{a}	77190^{a}	0.7	1.0	0.1
P91	0.2	122515^{b}	77750^{a}	0.4	91182^{a}	86408^{a}	58144^{a}	188279^{b}	0.0	115137^{b}	58740^{a}	0.7	0.0	0.0
P92	0.0	100523^{b}	25637^{a}	0.0	40003^{a}	32933^{a}	53326^{a}	35397^{a}	0.0	31295^{a}	48175^{a}	0.0	0.1	0.0
P93	0.0	57939^{b}	34590^{a}	0.0	37807^{a}	29827^{a}	49709^{b}	84205^{c}	0.0	45212^{b}	33156^{a}	0.3	0.0	0.0
P94	0.1	37025^{a}	30735^{a}	0.1	27441^{a}	37285^{a}	29930^{a}	35273^{a}	0.3	25363^{a}	38213^{b}	0.2	0.2	0.4
P95	0.0	19926^{a}	16079^{a}	0.0	4307^{a}	11901^{a}	43673^{b}	44974^{b}	0.2	19182^{a}	14551^{a}	0.0	0.5	0.1
P96	0.0	28750^{a}	31683^{a}	0.0	41151^{a}	25697^{a}	22288^{a}	31723^{a}	0.2	27787^{a}	34310^{a}	0.2	0.2	0.0
P97	1.0	47321^{a}	46524^{a}	0.0	16853^{c}	53238^{a}	82067^{b}	89490a^{b}	1.0	47986^{a}	45424^{a}	0.9	0.8	0.0
P98	0.8	141480^{a}	146685^{a}	0.8	127525^{a}	157258^{a}	147604^{a}	203679^{a}	0.9	157311^{a}	134592^{a}	1.0	0.8	0.1
P99	0.2	49557^{a}	130617^{b}	0.0	72072^{b}	137566^{a}	144343^{a}	168538^{ab}	0.2	85207^{a}	143747^{b}	0.6	0.2	0.3
P100	0.0	61414^{b}	31170^{a}	0.0	0^{b}	65948^{a}	46465^{a}	70678^{a}	0.9	34117^{a}	39639^{a}	0.0	1.0	0.4
P101	0.0	155435^{a}	269171^{b}	0.0	91625^{c}	326714^{a}	357019^{ab}	437652^{b}	0.5	273579^{b}	222522^{a}	0.0	0.7	0.0
P102	0.0	142543^{b}	66159^{a}	0.0	56442^{a}	103996^{c}	85561^{b}	69663^{ab}	0.0	65967^{a}	94843^{b}	0.0	0.1	0.9
P103	0.2	19482^{a}	36856^{b}	0.0	12648^{a}	71154^{b}	10180^{a}	0^{a}	0.3	32666^{a}	34376^{a}	0.0	0.1	0.0
P104	0.7	15646^{a}	14303^{a}	0.0	17493^{a}	11297^{b}	14974^{a}	14956^{ab}	0.1	13502^{a}	15570^{a}	1.0	0.7	0.4

*HT: 100% agave Tequila, T: Tequila, W: Silver Tequila, A: Aged Tequila, EA: Extra-aged Tequila, UA: Ultra-aged Tequila.

The samples ionized in negative mode, shown in Table 14, that has some peaks with different groups according to the Tequila category are in total 59 peaks, which represents more than 47.9% of the total peaks. According to origin are 60 peaks, which represent the 48.8% of the total peaks. The Tequila class that has the most different groups are all of them. Those variations are obtained by the Fisher's LSD analysis, and that mean that those peaks that are grouping differently one another, so that allow to classify each Tequila category by category and origin. In the case of the classes, the majority of them have their means in different groups, so it is possible to separate each Tequila by its class.

Table 14. Univariate results for each dependent variable (positive mode).

	Category*			Class*					Origin*					
	p	T	HT	p	W	A	EA	UA	p	JA	JT	Category*Class	Category*Origin	Class*Origin
P1	0.0	0^{a}	1561^{b}	0.0	0^{a}	3434^{b}	0^{a}	0^{a}	0.3	1065^{a}	1463^{a}	0.0	1.0	0.1
P2	0.3	39539^{a}	35220^{a}	0.0	47682^{c}	25111^{a}	33266^{ab}	45005^{bc}	0.5	35843^{a}	36184^{a}	0.1	1.0	0.4
P3	0.1	15400^{a}	18914^{a}	0.0	10870^{a}	19973^{b}	30780^{c}	0^{a}	0.8	19754^{a}	16880^{a}	0.2	0.3	0.0
P4	0.0	64632^{b}	29935^{a}	0.0	4300^{a}	65701^{c}	22742^{b}	145268^{d}	0.1	45493^{b}	27880^{a}	0.0	0.8	0.1
P5	0.1	25986^{a}	18622^{a}	0.4	21132^{a}	21705^{a}	14377^{a}	24966^{a}	0.0	26750^{b}	13704^{a}	0.0	1.0	0.4
P6	0.8	6393^{a}	7946^{a}	0.0	6208^{a}	9151^{ab}	6039^{a}	16948^{b}	0.6	7768^{a}	7556^{a}	0.3	0.0	0.0
P7	0.2	5421^{a}	4422^{a}	0.0	8365^{c}	3619^{b}	590^{a}	1013^{ab}	0.0	5329^{a}	3937^{a}	0.6	0.9	0.5
P8	0.5	706^{a}	1631^{a}	0.1	1703^{a}	1819^{a}	504^{a}	116^{a}	0.6	831^{a}	2043^{b}	0.1	0.1	0.1
P9	0.4	6633^{a}	8988^{a}	0.0	552a	15688^{b}	1993^{a}	677^{ab}	0.2	11745^{a}	5587^{a}	0.1	0.7	0.0
P10	0.9	713^{a}	800^{a}	0.2	1444^{c}	151^{a}	871^{bc}	0^{ab}	1.0	435^{a}	1109^{b}	0.0	0.0	0.0
P11	0.4	4860^{b}	1234^{a}	0.0	3952^{b}	1135^{a}	0^{a}	593^{ab}	0.0	3378^{b}	539^{a}	0.5	0.1	0.0
P12	0.2	2013^{a}	1714^{a}	0.0	2383^{b}	1368^{a}	1631^{ab}	481a^{b}	0.4	1576^{a}	1948^{a}	0.9	0.0	0.0
P13	0.0	6988^{a}	12036^{b}	0.1	10378^{a}	12438^{ab}	8519^{a}	20471^{b}	0.3	11263^{a}	10952^{a}	0.6	0.0	0.0
P14	0.1	1288^{b}	831^{a}	0.0	614^{a}	683^{a}	1257^{b}	4216^{c}	0.0	708^{a}	1108^{b}	0.0	0.0	0.0
P15	0.0	964^{a}	494^{a}	0.0	864^{bc}	48^{a}	1095^{c}	0^{ab}	0.3	471^{a}	683^{a}	0.6	0.0	0.0
P16	0.0	4286^{b}	1624^{a}	0.0	2980^{a}	322^{b}	3510^{a}	3082^{a}	0.0	1640^{a}	2560^{a}	0.0	0.0	0.0
P17	0.5	479^{a}	1823^{b}	0.0	220^{a}	1810^{b}	3599^{c}	608a^{b}	0.1	1285^{a}	1842^{a}	0.6	0.8	0.0
P18	0.0	10038^{b}	3143^{a}	0.0	3657^{a}	2356^{a}	6479^{b}	20343^{c}	0.0	6423^{b}	2560^{a}	0.1	0.0	0.3
P19	0.4	4689^{b}	1877^{a}	0.0	839^{a}	1219^{a}	4147^{b}	19276^{c}	0.0	3908^{b}	995^{a}	0.8	0.1	0.0
P20	0.3	4781^{a}	5448^{a}	0.0	1712^{a}	1239^{a}	16832^{b}	13261^{b}	0.0	2397^{a}	8043^{b}	0.2	0.6	0.0
P21	0.0	2987^{b}	1110^{a}	0.0	2620^{c}	324a	1618^{bc}	206a^{b}	0.0	1562^{a}	1361^{a}	0.3	0.0	0.1
P22	0.3	1654^{a}	1353^{a}	0.0	140^{a}	1030^{b}	3153^{c}	7430^{d}	0.2	1566^{a}	1263^{b}	0.7	0.5	0.0

Table 14. Univariate results for each dependent variable (positive mode) (continue).

	Category			Class					Origin					
	p	T	HT	p	W	A	EA	UA	p	JA	JT	Category*Class	Category*Origin	Class*Origin
P23	0.7	806^{a}	2993^{b}	0.0	566^{a}	739^{a}	9017^{c}	2726^{b}	0.0	859^{a}	4194^{b}	0.4	0.7	0.0
P24	0.3	3672^{b}	2085^{a}	0.0	426^{a}	70^{a}	6816^{b}	18360^{c}	0.9	3297^{b}	1526^{a}	1.0	0.2	0.2
P25	0.4	956^{a}	2593^{a}	0.0	1140^{ab}	0^{a}	768^{c}	4320^{bc}	0.0	1203^{a}	3299^{b}	0.7	0.9	0.0
P26	0.0	5722^{b}	1933^{a}	0.0	1193^{a}	2262^{ab}	2875^{b}	19342^{c}	0.1	3512^{b}	1820^{a}	0.0	0.3	0.0
P27	0.3	12709^{a}	19882^{b}	0.0	990b	10712^{c}	54141^{a}	59077^{a}	0.0	18434^{a}	18664^{a}	0.2	0.9	0.0
P28	0.2	2475^{a}	3833^{a}	0.1	2010^{a}	3411^{ab}	5708^{a}	8236^{a}	0.0	5869^{b}	1457^{a}	0.8	0.2	0.1
P29	0.7	6815^{a}	13633^{a}	0.0	1901^{a}	13622^{b}	27028^{c}	16615^{abc}	0.1	17583^{b}	7530^{a}	0.7	0.7	0.0
P30	0.5	2778^{a}	13322^{b}	0.0	3959^{a}	3206^{a}	38614^{b}	3640^{a}	0.0	6061^{a}	16299^{b}	0.6	0.7	0.0
P31	0.8	428^{a}	2015^{b}	0.0	673^{a}	0^{a}	6265^{b}	2138^{a}	0.2	1186^{a}	2217^{a}	0.6	0.6	0.1
P33	0.1	6214^{a}	8167^{a}	0.4	7855^{a}	9282^{a}	5021^{a}	9251^{a}	0.2	4951^{a}	10457^{b}	0.3	0.0	0.5
P34	0.3	8900^{a}	16687^{a}	0.0	485b	8772^{c}	46463^{a}	40261^{a}	0.0	12538^{a}	17758^{a}	0.2	0.9	0.0
P35	0.0	268^{a}	3724^{b}	0.1	95^{b}	4661^{a}	5726^{a}	1339^{ab}	0.0	1036^{a}	4985^{b}	0.0	1.0	0.0
P36	0.3	249^{a}	698^{a}	0.0	714^{a}	22^{c}	1332^{b}	1246^{ab}	0.1	293^{a}	914^{b}	0.1	0.0	0.0
P37	0.1	1960^{b}	987^{a}	0.0	932^{b}	0^{a}	2877^{c}	4923^{d}	0.2	1295^{a}	1048^{a}	0.4	0.0	0.3
P38	0.4	10873^{a}	7501^{a}	0.0	13877^{b}	1103^{a}	5947^{a}	33905^{c}	0.8	6514^{a}	9622^{a}	0.1	0.0	0.1
P39	0.0	4421^{b}	2548^{a}	0.0	1765^{a}	1450^{a}	6417^{b}	7518^{b}	0.7	3865^{b}	1994^{a}	0.1	0.4	0.9
P40	0.7	28239^{a}	17597^{a}	0.0	44533^{b}	2973^{a}	5181^{a}	22174^{ab}	0.1	20363^{a}	18828^{a}	0.1	0.2	0.9
P41	0.6	1098^{a}	5007^{b}	0.0	0^{a}	1738^{a}	15461^{b}	5489^{a}	0.0	1906^{a}	6490^{b}	0.6	1.0	0.0
P42	0.9	3647^{a}	5438^{a}	0.0	2218^{a}	440^{a}	16797^{c}	10502^{b}	0.2	6051^{a}	4229^{a}	0.7	0.6	0.8
P43	0.3	3086^{a}	3775^{a}	0.0	1632^{a}	1576^{a}	9329^{b}	10433^{b}	0.7	3905^{a}	3409^{a}	0.7	0.2	0.7
P44	0.1	3537^{a}	7225^{b}	0.0	1337^{a}	835a	24771^{c}	6287^{b}	0.0	1631^{a}	11101^{b}	0.2	0.2	0.0
P45	0.7	343^{a}	2345^{b}	0.0	558^{a}	179^{a}	7509^{b}	877^{a}	0.0	1264^{a}	2633^{b}	0.7	0.3	0.0
P46	0.5	720^{a}	2811^{a}	0.6	4132^{a}	0^{a}	3682^{a}	2024^{ab}	0.4	705^{a}	4019^{b}	0.2	0.1	0.1
P47	0.7	3681^{a}	3399^{a}	0.0	675^{a}	949^{a}	9755^{b}	18403^{c}	0.1	5312^{b}	1723^{a}	1.0	0.9	0.0
P48	0.0	356^{a}	3025^{b}	0.2	3638^{a}	690b	3878^{a}	1781^{ab}	0.2	1606^{a}	3390^{b}	0.3	0.6	0.0
P49	0.5	1677^{a}	2774^{a}	0.0	3210^{bc}	898a	4646^{c}	468^{ab}	0.4	1374^{a}	3682^{b}	0.4	0.0	0.4
P50	0.2	1327^{a}	3442^{a}	0.0	2224^{a}	1258^{a}	6974^{b}	5694^{ab}	0.7	2639^{a}	3432^{a}	0.8	0.4	0.1
P51	0.0	6335^{a}	26025^{a}	0.3	2378^{a}	49817^{b}	8850^{a}	29170^{ab}	0.1	4921^{a}	38589^{b}	0.0	1.0	0.0
P52	0.0	74288^{b}	9152^{a}	0.0	16950^{b}	38027^{c}	3631^{a}	1232a^{b}	0.3	1124^{a}	39869^{b}	0.0	0.0	0.0
P53	0.3	247^{a}	12304^{b}	0.4	15893^{a}	1051^{b}	16873^{a}	1233^{ab}	0.5	4016^{a}	15693^{b}	0.1	0.1	0.1
P54	0.8	3201^{a}	3225^{a}	0.1	5763^{a}	386^{b}	3570^{a}	4038^{ab}	0.1	1748^{a}	4587^{b}	0.5	0.1	0.0
P55	0.0	5974^{b}	1884^{a}	0.0	5180^{c}	556a	1645^{ab}	4082^{bc}	0.0	2596^{a}	2683^{a}	0.0	0.0	0.2
P56	0.2	10613^{a}	7136^{a}	0.0	16230^{b}	850a	5893^{a}	3901^{ab}	0.0	10291^{b}	5448^{a}	0.4	1.0	0.0
P57	0.3	5221^{a}	75009^{a}	0.2	143824^{b}	21415^{a}	3651^{a}	2028^{ab}	0.6	9742^{a}	110690^{b}	0.0	0.0	0.0
P58	0.1	57646^{a}	429195^{b}	0.0	457777^{a}	494150^{a}	25243^{b}	59784^{ab}	0.6	200074^{a}	509254^{b}	0.7	0.1	0.1
P59	0.9	934396^{a}	562966^{a}	0.0	1344339^{b}	301381^{a}	95553^{a}	26700^{a}	0.1	695287^{a}	572750^{a}	0.1	0.8	0.1
P60	0.2	1095^{a}	4488^{a}	0.1	10275^{b}	0^{a}	179^{a}	373^{ab}	0.8	2021^{a}	5566^{a}	0.3	1.0	0.9

Table 14. Univariate results for each dependent variable (positive mode) (continue).

	Category			Class					Origin					
	p	T	HT	p	W	A	EA	UA	p	JA	JT	Category*Class	Category*Origin	Class*Origin
P61	0.5	1388^{a}	13424^{a}	0.8	27839^{b}	0^{a}	2897^{ab}	6500^{ab}	0.8	1591^{a}	20113^{a}	0.2	0.2	0.1
P62	0.5	15760^{a}	24905^{a}	0.0	50404^{b}	11349^{a}	1119^{a}	2472^{ab}	0.1	9888^{a}	35584^{b}	0.9	0.6	0.2
P63	0.8	17002^{a}	18474^{a}	0.0	44684^{b}	3488^{a}	1214^{a}	2442^{a}	0.5	7322^{a}	28304^{b}	0.1	0.0	0.0
P64	0.0	101092^{a}	75521^{a}	0.0	202355^{b}	2437^{a}	14648^{a}	31111^{a}	0.0	85555^{a}	75335^{a}	0.0	0.0	0.0
P65	0.2	14631^{a}	29902^{b}	0.0	24829^{a}	38675^{b}	11956^{a}	24216^{ab}	0.9	23442^{a}	30446^{a}	0.1	0.1	0.0
P66	0.8	99783^{b}	42790^{a}	0.0	99329^{c}	39181^{b}	5296^{a}	23421^{ab}	0.0	51181^{a}	55352^{a}	0.0	0.0	0.0
P67	0.1	79309^{b}	33723^{a}	0.0	88562^{b}	17398^{a}	9836^{a}	19851^{ab}	0.0	38824^{a}	45268^{a}	0.1	0.0	0.0
P68	0.0	15953^{a}	37296^{b}	0.0	41343^{a}	28796^{a}	24474^{a}	52042^{a}	0.1	46757^{b}	20888^{a}	0.6	0.0	0.0
P69	0.3	12493^{a}	56660^{a}	0.2	79675^{b}	48586^{ab}	3031^{a}	8204^{ab}	0.6	20295^{a}	74654^{b}	0.2	0.1	0.1
P70	0.0	39658^{a}	29146^{a}	0.0	33173^{b}	18597^{a}	53205^{c}	2567^{a}	0.0	18964^{a}	42354^{b}	0.4	0.1	0.0
P71	0.4	90^{a}	37124^{b}	0.6	39191^{a}	13725^{a}	47928^{a}	4541^{a}	0.9	20005^{a}	40086^{a}	0.2	0.1	0.0
P72	0.7	18378^{a}	10777^{a}	0.0	10600^{ab}	5023^{a}	17221^{b}	69420^{c}	0.9	13731^{a}	10749^{a}	0.7	0.5	0.0
P73	0.7	1498^{a}	15072^{b}	0.8	12724^{a}	12788^{a}	13991^{a}	0^{a}	0.1	14752^{a}	10520^{a}	0.1	0.2	0.0
P74	0.0	4479^{a}	27048^{b}	0.0	12872^{a}	41287^{b}	9229^{a}	20478^{ab}	0.0	14944^{a}	30226^{b}	0.0	0.9	0.0
P75	0.0	38205^{a}	29608^{a}	0.0	23190^{a}	40123^{c}	26321^{ab}	51343^{bc}	0.8	33265^{a}	29283^{a}	0.0	0.6	0.0
P76	0.0	6244^{a}	34980^{b}	0.0	13418^{a}	61936^{b}	7697^{a}	1050^{a}	0.9	29842^{a}	29488^{a}	0.4	0.4	0.0
P77	0.4	38510^{a}	41736^{a}	0.0	27425^{a}	76796^{b}	9992^{a}	8575^{a}	0.0	34707^{a}	47111^{a}	0.0	0.0	0.0
P78	0.3	6653^{b}	3613^{a}	0.0	7949^{b}	1615^{a}	2433^{a}	2508^{a}	0.0	4440^{a}	3931^{a}	0.0	0.0	0.0
P79	0.4	21124^{a}	16672^{a}	0.0	23092^{a}	3693^{b}	26027^{a}	48391^{c}	0.0	22435^{b}	12910^{a}	0.0	0.0	0.0
P80	0.7	77027^{a}	83458^{a}	0.1	111046^{b}	56680^{a}	62868^{a}	166747^{b}	0.7	73984^{a}	89959^{a}	0.0	0.0	0.0
P81	0.7	185639^{a}	194452^{a}	0.1	171444^{a}	234502^{b}	188820^{ab}	13753^{c}	0.0	168997^{a}	214940^{b}	0.0	0.1	0.0
P82	0.6	2783^{a}	8974^{a}	0.2	8695^{ab}	11156^{b}	1180^{a}	5751^{ab}	0.7	8475^{a}	7226^{a}	0.4	0.4	0.0
P83	0.7	27528^{a}	21785^{a}	0.0	40002^{c}	19340^{b}	2796^{a}	6715^{ab}	0.1	21813^{a}	23810^{a}	0.0	0.0	0.7
P84	0.9	13026^{a}	17274^{a}	0.5	22041^{b}	11722^{a}	16918^{ab}	6017^{a}	0.0	8995^{a}	23445^{b}	0.2	0.1	0.0
P85	0.0	8719^{a}	34183^{b}	0.0	57469^{b}	12660^{a}	11670^{a}	24323^{ab}	0.3	31704^{a}	27391^{a}	0.1	0.2	0.5
P86	0.0	97519^{b}	33436^{a}	0.0	72741^{c}	39656^{b}	10841^{a}	34168^{abc}	0.0	34102^{a}	55705^{b}	0.0	0.1	0.1
P87	0.4	4152^{a}	57924^{a}	0.7	105742^{b}	8403^{a}	23040^{a}	15402^{ab}	0.7	10334^{a}	82911^{b}	0.1	0.1	0.0
P88	0.9	78148^{a}	66442^{a}	0.0	100312^{c}	38932^{a}	71016^{b}	33934^{a}	0.0	56512^{a}	79844^{b}	0.8	0.5	0.1
P89	0.9	76895^{a}	92210^{a}	0.0	86784^{a}	107945^{b}	68509^{a}	54733^{a}	0.0	75827^{a}	101952^{b}	0.9	0.4	0.0
P90	0.0	47491^{a}	9559^{b}	0.0	51896^{a}	139329^{b}	62441^{a}	57261^{a}	0.0	69333^{a}	103059^{b}	1.0	0.3	0.1
P91	0.3	165513^{b}	87248^{a}	0.0	218213^{b}	47086^{a}	10363^{a}	31852^{a}	0.0	99678^{a}	103658^{a}	0.0	0.0	0.7
P92	0.0	245661^{a}	396482^{b}	0.0	248716^{a}	513664^{c}	337133^{b}	304324^{ab}	0.0	313328^{a}	419833^{b}	0.0	0.0	0.0
P93	0.0	165793^{b}	126515^{a}	0.0	129865^{a}	129978^{a}	145321^{a}	141944^{a}	0.8	137411^{a}	130425^{a}	0.1	0.3	0.5
P94	0.1	17006^{b}	7630^{a}	0.0	20864^{b}	1527^{a}	4532^{a}	1784^{a}	0.0	6906^{a}	11651^{b}	0.0	0.0	0.9
P95	0.4	5687^{a}	30066^{a}	0.6	54671^{b}	7838^{a}	9907^{a}	5359^{ab}	0.5	6390^{a}	43344^{b}	0.2	0.1	0.0
P96	0.1	583^{a}	1439^{b}	0.5	1517^{a}	1239^{a}	1169^{ab}	0^{b}	0.8	1072^{a}	1474^{a}	0.0	0.7	0.8
P97	0.6	6555^{b}	4009^{a}	0.0	7253^{b}	2482^{a}	3852^{a}	516^{a}	0.0	5572^{b}	3467^{a}	0.2	0.0	0.0

Table 14. Univariate results for each dependent variable (positive mode) (continue).

	Category			Class					Origin					
	p	T	HT	p	W	A	EA	UA	p	JA	JT	Category*Class	Category*Origin	Class*Origin
P98	0.0	3320^{a}	3696^{a}	0.0	1830^{b}	4523^{a}	5078^{a}	3916^{ab}	0.0	3184^{a}	4037^{b}	0.1	0.0	0.1
P99	1.0	9695^{a}	7771^{a}	0.0	15797^{b}	3796^{a}	3916^{a}	0^{a}	0.2	6485^{a}	9652^{a}	0.9	0.2	0.9
P100	0.2	2879^{a}	5324^{a}	0.8	5808^{a}	3480^{a}	5334^{a}	6649^{a}	0.8	3572^{a}	6078^{b}	0.1	0.1	0.2
P101	0.5	6580^{a}	28829^{a}	0.4	59781^{b}	3284^{a}	6007^{a}	453^{ab}	0.6	4657^{a}	43329^{b}	0.1	0.1	0.1
P102	0.0	13113^{b}	4108^{a}	0.4	5823^{a}	5183^{a}	5222^{a}	14538^{b}	0.1	6149^{a}	5429^{a}	0.2	0.1	0.0
P103	0.0	3943^{a}	75679^{b}	0.0	157872^{b}	3372^{a}	11407^{a}	3769^{a}	0.8	50708^{a}	73246^{a}	0.0	0.0	0.0
P104	0.7	95141^{a}	80719^{a}	0.0	114620^{b}	71543^{a}	48366^{a}	99697^{ab}	0.2	77359^{a}	88990^{a}	0.2	0.0	0.0
P105	0.0	49764^{a}	50904^{a}	0.0	74258^{b}	41853^{a}	31382^{a}	19294^{a}	0.0	50447^{a}	50921^{a}	0.6	0.0	0.0
P106	0.1	13810^{b}	86753^{a}	0.0	176891^{b}	64437^{a}	25699^{a}	31598^{a}	0.0	103057^{a}	89953^{a}	0.0	0.0	0.4
P107	0.0	216091^{b}	133361^{a}	0.0	96991^{b}	173670^{a}	156630^{a}	368007^{c}	0.0	140568^{a}	156215^{a}	0.2	0.0	0.5
P108	0.1	153122^{a}	208206^{a}	0.0	173286^{a}	235801^{ab}	140758^{a}	410732^{b}	0.4	193512^{a}	202178^{a}	0.1	0.9	0.0
P109	0.0	45577^{a}	33745^{a}	0.0	65893^{b}	23334^{a}	13001^{a}	0a	0.0	36086^{a}	35798^{a}	0.0	0.0	0.0
P110	0.1	57561^{a}	74520^{a}	0.0	98426^{b}	55796^{a}	59331^{a}	29043^{a}	0.0	52444^{a}	88962^{b}	0.5	0.0	0.0
P111	0.8	124809^{a}	195402^{a}	0.0	136403^{a}	275813^{b}	111777a	130062^{ab}	0.5	184104^{a}	180681^{a}	0.5	0.1	0.2
P112	0.0	189157^{a}	527910^{b}	0.0	172789^{a}	766838^{c}	400574^{ab}	760088^{bc}	0.4	554911^{a}	381855^{a}	0.1	0.7	0.0
P113	0.0	96672^{b}	54308^{a}	0.0	43115^{a}	87812^{b}	52110^{ab}	56203^{ab}	0.4	61594^{a}	62673^{a}	0.0	0.4	0.0
P114	0.0	20003^{a}	55236^{b}	0.5	64161^{a}	26773^{b}	62050^{a}	33564^{ab}	0.1	31335^{a}	64846^{b}	0.0	0.4	0.0
P115	0.0	542131^{b}	120951^{a}	0.0	166928^{b}	332287^{c}	35385^{a}	167111^{ab}	0.1	17489^{a}	221285^{a}	0.0	0.8	0.0
P116	0.0	149191^{a}	319175^{b}	0.0	220728^{a}	410484^{b}	183469^{a}	354858^{ab}	0.3	262304^{a}	311275^{a}	0.0	0.5	0.0
P117	0.0	119407^{b}	53116^{a}	0.0	122219^{b}	24856^{a}	49139^{a}	0^{a}	0.5	47524^{a}	81984^{a}	0.6	0.0	0.6
P118	0.0	74823^{a}	121717^{a}	0.0	162477^{b}	66955^{a}	97232^{a}	174176^{ab}	0.9	115098^{a}	111116^{a}	1.0	0.2	0.0
P119	0.0	341807^{b}	210180^{a}	0.0	271317^{b}	278391^{b}	132777^{a}	39247^{a}	0.0	284041^{b}	188604^{a}	0.0	1.0	0.0
P120	0.0	167105^{b}	63020^{a}	0.0	73268^{a}	131064^{b}	29776^{a}	0^{a}	0.0	72241^{a}	91631^{a}	0.0	0.0	0.1
P121	0.0	10118^{a}	32065^{b}	0.0	13827^{ab}	54736^{c}	7912^{a}	22904^{b}	0.8	27997^{a}	28003^{a}	0.5	0.2	0.0
P122	0.0	20898^{a}	33945^{a}	0.0	41909^{a}	13576^{b}	38971^{a}	62607^{a}	0.1	43531^{b}	20384^{a}	0.1	0.0	0.0
P123	0.6	83561^{b}	47540^{a}	0.0	125038^{b}	5411^{a}	26531^{a}	0.0^{a}	0.0	46153^{a}	61693^{a}	0.0	0.0	0.2

*HT: 100% agave Tequila, T: Tequila, W: Silver Tequila, A: Aged Tequila, EA: Extra-aged Tequila, UA: Ultra-aged Tequila.

5.3 General Discriminant Analysis: Squared Mahalanobis distances.

Squared Mahalanobis distances are plot in the Cooman's graphic, which determines the similarity between two variables depending on the distance to T in the case of the X axis and HT for the Y axis. **The Tequila category is different the 100% agave from Tequila**, because they are grouped not in the center, as variables with similarities are, but away from each other on their own side of the graphic, as shown in Figure 4 and 6. The same happens to Tequila classes; neither of the four groups are in the center in Figure 5 and 7. They are also separated into groups without mixing with the other. For Tequila origin is the same; neither Tequila from Los Altos, Jalisco are Tequila in the middle with Tequila from Tequila, Jalisco, as shown in Figure 6 and 8.

It can be observed that both positive and negative variables can be separated from each other by the Squared Mahalanobis distances, and therefore allow identification of Tequila based on these. This analysis has already been used to discriminate between Tequila's barrel origin and maturation time. Martín-del-Campo et al. (2019) used Cooman's plot of Mahalanobis distances ($p < 0.0001$) to successfully classify according to barrel origin (Allier, Limousin, Tronçais, and Centre de la France); and between maturation time (2-8 weeks and 10-32 weeks).

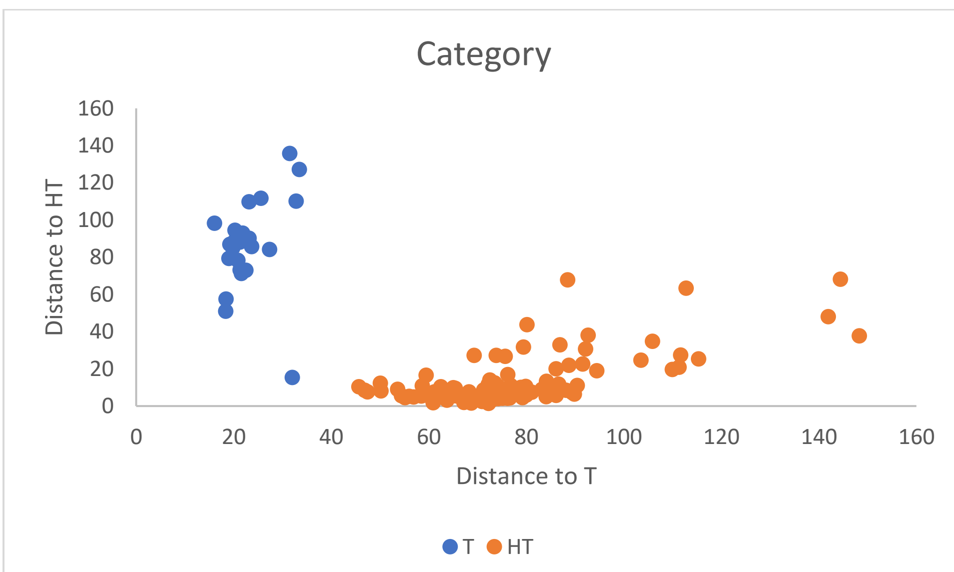

Figure 4. Cooman's graphic of Mahalanobis distances for Tequila categories (negative mode).

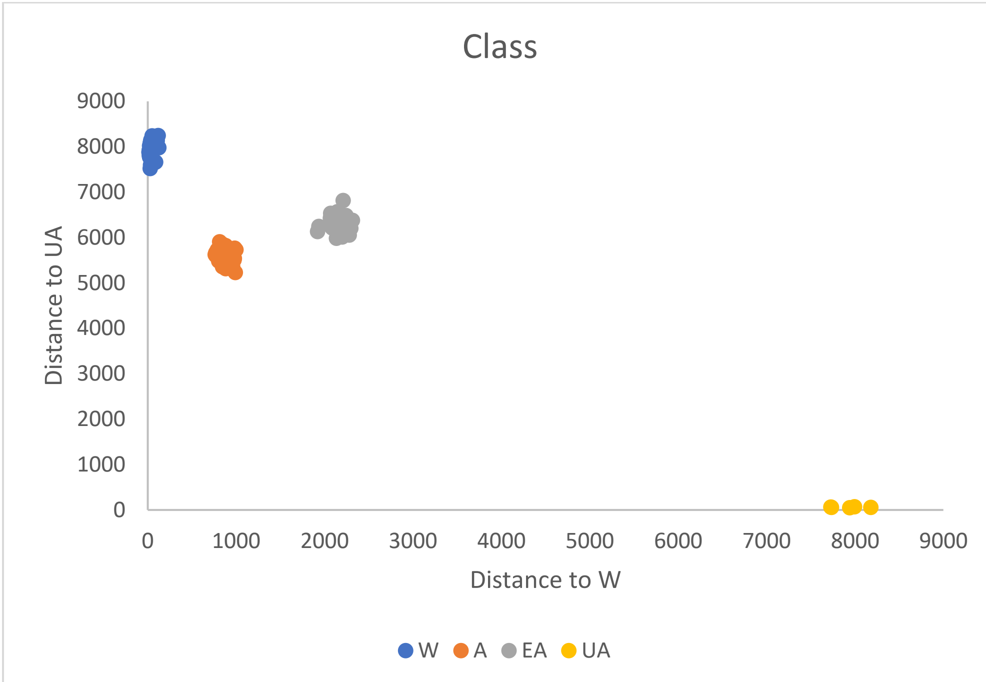

Figure 5. Cooman's plot of Mahalanobis for Tequila classes (negative mode).

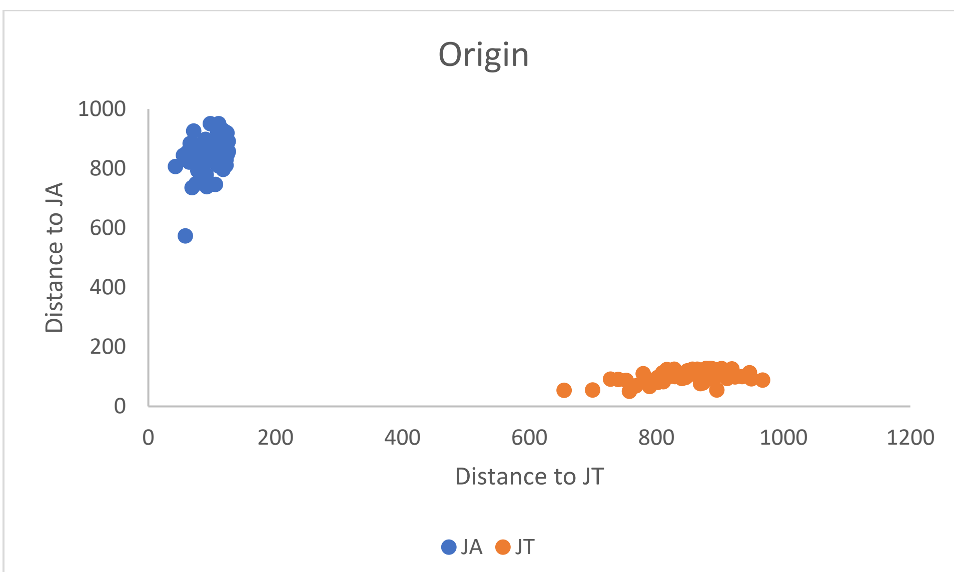

Figure 6. Cooman's plot of Mahalanobis for Tequila origins (negative mode).

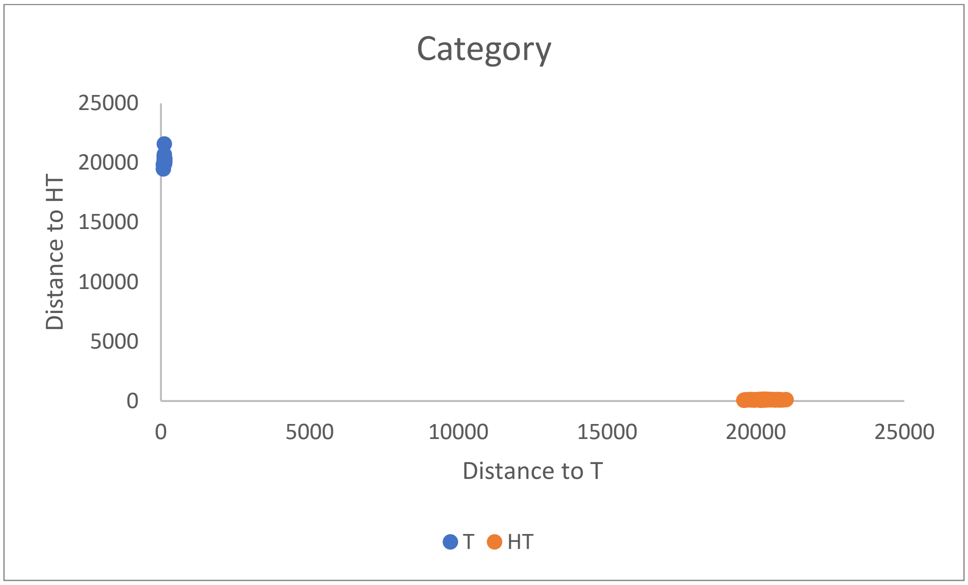

Figure 7. Cooman's graphic of Mahalanobis for Tequila categories (positive mode).

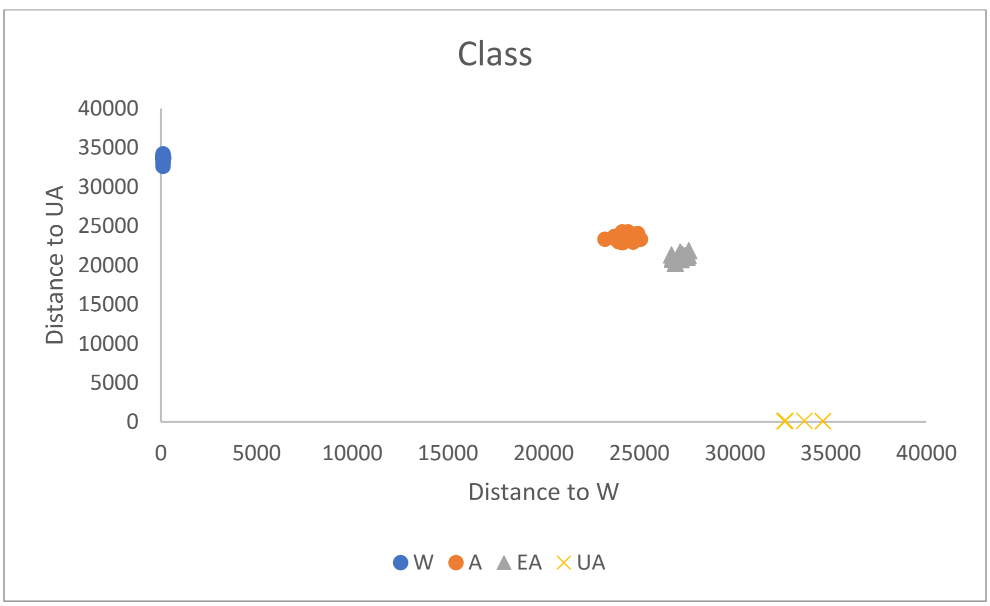

Figure 8. Cooman's plot of Mahalanobis for Tequila classes (positive mode).

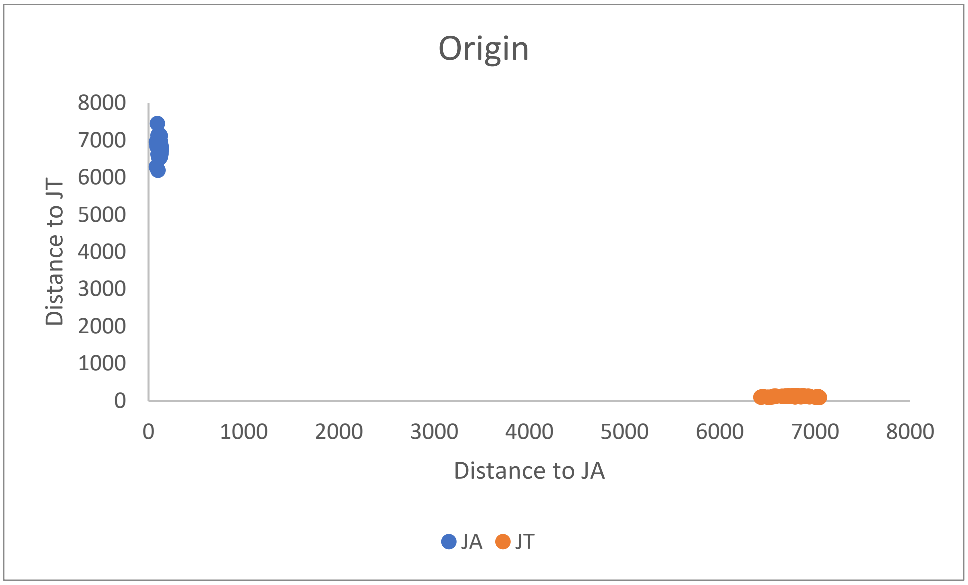

Figure 9. Cooman's plot of Mahalanobis for Tequila origins (positive mode).

5.4 General Discriminant Analysis: Forward Stepwise.

The forward stepwise for the negative mode was selected to minimize the peaks for the analysis (p inclusion 0.05, p exclusion 0.05), and keep the most statistically significant to discriminate between category, class, origin, and code. In Table 13 is shown that Tequila categories include 15 peaks in the analysis; in Table 14 Tequila classes include 49 peaks; in Table 15 Tequila origins include 31 peaks; and Tequila by code include 75 peaks, as shown in Table 16. The number of peaks is bigger in the code of Tequila because it is a mixture between the other three variables: category, class, and origin. The class of Tequila is the one, besides the codes, that includes more peaks that are statistically significant.

The forward stepwise for the positive mode was selected to minimize the peaks for the analysis (p inclusion 0.05, p exclusion 0.05), and keep the most statistically significant to discriminate between category, class, origin, and code. In Table 17 is shown that Tequila categories include 30 peaks in the analysis; in Table 18 Tequila classes include 38 peaks; in Table 19 Tequila origins include 30 peaks; and Tequila by code include 80 peaks, as shown in Table 20. The number of peaks is bigger in the code of Tequila because it is a mixture of the other three variables: category, class, and origin. The class of Tequila is the one, besides the codes, that includes more peaks that are statistically significant.

Table 15. Peaks selected by Forward Stepwise of the General Discriminant Analysis for the variable category (negative mode).

No.	Peak	Retention Time (min)	No.	Peak	Retention Time (min)
1	"P60"	22.60	9	"P11"	3.48
2	"P7"	1.74	10	"P39"	13.89
3	"P100"	40.72	11	"P91"	37.87
4	"P49"	18.24	12	"P15"	5.37
5	"P16"	5.80	13	"P80"	30.99
6	"P77"	29.83	14	"P98"	40.33
7	"P2"	0.89	15	"P18"	6.55
8	"P9"	2.84			

Table 16. Peaks selected by Forward Stepwise of the General Discriminant Analysis for the variable class (negative mode).

No.	Peak	Retention Time (min)	No.	Peak	Retention Time (min)
1	"P46"	16.96	26	"P68"	25.86
2	"P103"	41.52	27	"P45"	16.13
3	"P23"	9.27	28	"P13"	4.01
4	"P37"	13.35	29	"P43"	15.36
5	"P101"	41.23	30	"P48"	17.74
6	"P1"	0.71	31	"P17"	6.10
7	"P25"	10.38	32	"P77"	29.83
8	"P24"	9.65	33	"P8"	2.40
9	"P38"	13.48	34	"P20"	8.23
10	"P67"	25.43	35	"P66"	24.46
11	"P89"	36.07	36	"P65"	24.24
12	"P63"	23.61	37	"P34"	12.62
13	"P47"	17.30	38	"P36"	13.15
14	"P71"	27.80	39	"P51"	19.47
15	"P6"	1.25	40	"P74"	28.63
16	"P79"	30.86	41	"P59"	22.37

Table 16. Peaks selected by Forward Stepwise of the General Discriminant Analysis for the variable class (negative mode) (continue).

No.	Peak	Retention Time (min)	No.	Peak	Retention Time (min)
17	"P75"	29.04	42	"P30"	12.03
18	"P9"	2.83	43	"P31"	12.12
19	"P83"	32.25	44	"P26"	10.76
20	"P2"	0.89	45	"P14"	4.99
21	"P18"	6.55	46	"P27"	11.00
22	"P16"	5.80	47	"P84"	32.70
23	"P12"	3.80	48	"P19"	6.80
24	"P54"	20.43	49	"P40"	14.30
25	"P3"	0.92			

Table 17. Peaks selected by Forward Stepwise of the General Discriminant Analysis for the variable origin (negative mode).

No.	Peak	Retention Time (min)	No.	Peak	Retention Time (min)
1	"P58"	21.82	17	"P47"	17.30
2	"P68"	25.86	18	"P73"	28.26
3	"P89"	36.07	19	"P39"	13.89
4	"P44"	15.74	20	"P93"	38.41
5	"P43"	15.36	21	"P90"	36.51
6	"P65"	24.24	22	"P61"	22.95
7	"P35"	12.85	23	"P79"	30.89
8	"P15"	5.37	24	"P23"	9.27
9	"P13"	4.01	25	"P72"	27.94
10	"P83"	32.25	26	"P92"	38.22
11	"P2"	0.89	27	"P49"	18.24
12	"P70"	26.74	28	"P66"	24.46
13	"P81"	31.43	29	"P6"	1.25
14	"P32"	12.38	30	"P87"	34.77
15	"P71"	27.80	31	"P101"	41.23
16	"P57"	21.50			

Table 18. Peaks selected by Forward Stepwise of the General Discriminant Analysis for the variable code (negative mode).

No.	Peak	Retention Time (min)	No.	Peak	Retention Time (min)
1	"P2"	0.89	39	"P34"	12.62
2	"P33"	12.46	40	"P38"	13.48
3	"P65"	24.24	41	"P13"	4.0112.
4	"P51"	19.47	42	"P44"	15.74
5	"P9"	2.84	43	"P48"	17.74
6	"P15"	5.37	44	"P56"	21.27
7	"P16"	5.80	45	"P75"	29.04
8	"P22"	8.83	46	"P99"	40.53
9	"P61"	22.95	47	"P78"	30.23
10	"P1"	0.71	48	"P43"	15.36
11	"P67"	25.43	49	"P82"	32.16
12	"P72"	27.94	50	"P69"	26.38
13	"P14"	4.99	51	"P42"	15.11
14	"P39"	13.89	52	"P46"	16.96
15	"P63"	23.61	53	"P92"	38.22
16	"P54"	20.43	54	"P87"	34.77
17	"P41"	14.63	55	"P28"	11.50
18	"P68"	25.86	56	"P84"	32.70
19	"P64"	23.83	57	"P58"	21.82
20	"P57"	21.5	58	"P62"	23.33
21	"P3"	0.92	59	"P52"	19.75
22	"P29"	11.77	60	"P55"	20.75
23	"P32"	12.38	61	"P49"	18.24
24	"P23"	9.27	62	"P100"	40.72
25	"P25"	10.38	63	"P103"	41.52
26	"P31"	12.12	64	"P36"	13.15
27	"P35"	12.85	65	"P37"	13.35
28	"P40"	14.30	66	"P77"	29.83
29	"P21"	8.42	67	"P50"	18.52
30	"P30"	12.03	68	"P102"	41.37

Table 18. Peaks selected by Forward Stepwise of the General Discriminant Analysis for the variable code (negative mode) (continue).

No.	Peak	Retention Time (min)	No.	Peak	Retention Time (min)
31	"P12"	3.80	69	"P11"	3.49
32	"P66"	24.46	70	"P10"	3.00
33	"P45"	16.13	71	"P86"	33.83
34	"P24"	9.65	72	"P53"	20.07
35	"P74"	28.63	73	"P60"	22.60
36	"P19"	6.80	74	"P101"	41.23
37	"P17"	6.10	75	"P83"	32.25
38	"P26"	10.76			

Table 19. Peaks selected by Forward Stepwise of the General Discriminant Analysis for the variable category (positive mode).

No.	Peak	Retention Time (min)	No.	Peak	Retention Time (min)
1	"P75"	25.99	16	"P114"	40.02
2	"P25"	8.47	17	"P29"	9.52
3	"P99"	34.38	18	"P47"	14.15
4	"P68"	23.23	19	"P1"	0.67
5	"P32"	10.42	20	"P14"	5.80
6	"P52"	15.81	21	"P46"	13.96
7	"P93"	32.16	22	"P23"	8.15
8	"P16"	6.43	23	"P113"	39.85
9	"P117"	40.72	24	"P48"	14.50
10	"P66"	22.77	25	"P71"	24.59
11	"P101"	35.21	26	"P18"	6.97
12	"P50"	15.09	27	"P2"	0.71
13	"P90"	31.81	28	"P60"	20.40
14	"P20"	7.38	29	"P40"	12.59
15	"P103"	35.46	30	"P12"	4.11

Table 20. Peaks selected by Forward Stepwise of the General Discriminant Analysis for the variable class (positive mode).

No.	Peak	Retention Time (min)	No.	Peak	Retention Time (min)
1	"P32"	10.42	20	"P2"	0.71
2	"P119"	41.28	21	"P101"	35.21
3	"P23"	8.15	22	"P37"	11.80
4	"P91"	31.81	23	"P38"	12.14
5	"P103"	35.46	24	"P105"	36.17
6	"P5"	1.04	25	"P93"	32.16
7	"P70"	24.04	26	"P41"	12.79
8	"P29"	9.52	27	"P15"	6.10
9	"P71"	24.59	28	"P69"	23.82
10	"P92"	31.90	29	"P17"	6.74
11	"P4"	0.97	30	"P18"	6.97
12	"P49"	14.86	31	"P66"	22.77
13	"P68"	23.23	32	"P50"	15.09
14	"P40"	12.59	33	"P100"	35.23
15	"P108"	36.90	34	"P44"	13.39
16	"P86"	30.23	35	"P35"	11.39
17	"P74"	25.72	36	"P6"	1.10
18	"P42"	12.94	37	"P24"	8.33
19	"P13"	4.47	38	"P3"	0.87

Table 21. Peaks selected by Forward Stepwise of the General Discriminant Analysis for the variable origin (positive mode).

No.	Peak	Retention Time (min)	No.	Peak	Retention Time (min)
1	"P46"	13.96	16	"P51"	15.39
2	"P110"	38.45	17	"P6"	1.10
3	"P88"	30.76	18	"P69"	23.82
4	"P83"	29.78	19	"P89"	30.94
5	"P86"	30.23	20	"P58"	18.34
6	"P35"	11.39	21	"P56"	17.18
7	"P52"	15.81	22	"P1"	0.67
8	"P19"	7.01	23	"P4"	0.97

Table 21. Peaks selected by Forward Stepwise of the General Discriminant Analysis for the variable origin (positive mode) (continue).

No.	Peak	Retention Time (min)	No.	Peak	Retention Time (min)
9	"P74"	25.72	24	"P78"	27.00
10	"P81"	28.24	25	"P34"	10.95
11	"P48"	14.50	26	"P23"	8.15
12	"P119"	41.28	27	"P117"	40.72
13	"P60"	20.40	28	"P65"	22.42
14	"P64"	22.00	29	"P93"	32.16
15	"P13"	4.47	30	"P50"	15.09

Table 22. Peaks selected by Forward Stepwise of the General Discriminant Analysis for the variable code (positive mode).

No.	Peak	Retention Time (min)	No.	Peak	Retention Time (min)
1	"P101"	35.21	41	"P53"	16.07
2	"P103"	35.46	42	"P39"	12.37
3	"P23"	8.15	43	"P5"	1.04
4	"P106"	36.49	44	"P69"	23.82
5	"P66"	22.77	45	"P15"	6.10
6	"P57"	17.56	46	"P75"	25.99
7	"P32"	10.42	47	"P72"	25.14
8	"P33"	10.75	48	"P76"	26.30
9	"P34"	10.95	49	"P42"	12.94
10	"P9"	2.00	50	"P17"	6.74
11	"P105"	36.17	51	"P49"	14.86
12	"P13"	4.48	52	"P16"	6.43
13	"P51"	15.39	53	"P45"	13.54
14	"P41"	12.79	54	"P87"	30.48
15	"P25"	8.47	55	"P20"	7.38
16	"P52"	15.81	56	"P68"	23.23

Table 22. Peaks selected by Forward Stepwise of the General Discriminant Analysis for the variable code (positive mode) (continue).

No.	Peak	Retention Time (min)	No.	Peak	Retention Time (min)
17	"P73"	25.31	57	"P29"	9.52
18	"P92"	31.90	58	"P4"	0.97
19	"P35"	11.39	59	"P119"	41.28
20	"P38"	12.14	60	"P6"	1.10
21	"P90"	31.36	61	"P3"	0.87
22	"P27"	8.80	62	"P118"	40.80
23	"P18"	6.97	63	"P107"	36.79
24	"P24"	8.33	64	"P96"	33.84
25	"P40"	12.59	65	"P10"	2.90
26	"P11"	3.87	66	"P83"	29.78
27	"P104"	36.06	67	"P89"	30.94
28	"P2"	0.71	68	"P14"	5.80
29	"P82"	29.21	69	"P31"	9.92
30	"P67"	23.07	70	"P56"	17.18
31	"P12"	4.11	71	"P21"	7.53
32	"P71"	24.59	72	"P109"	38.25
33	"P28"	9.08	73	"P54"	16.39
34	"P70"	24.04	74	"P30"	9.73
35	"P47"	14.15	75	"P55"	16.71
36	"P86"	30.23	76	"P37"	11.80
37	"P93"	32.16	77	"P116"	40.63
38	"P50"	15.09	78	"P7"	1.45
39	"P44"	13.39	79	"P43"	13.05
40	"P36"	11.62	80	"P77"	26.52

5.4 Principal Component Analysis.

The PCA analysis was carried out to separate the peaks in each variable of the Tequila, also to differentiate the Tequila by code; and projections of cases and variables were made for each of them. However, the separation could not be possible due to the complex graphics that showed all the peaks and Tequila codes mixed.

However, some other authors could explain their data with this category of analysis in other distillates, like in the case of whisky that Stupak et al (2018) could classify 191 whisky samples, with an ethyl-acetate extraction, of bourbon cask from bourbon/wine casks used for its maturation. Another example, was the analysis of rum by Belmonte et al (2020), the PCA of twenty-four commercial rums from ten different countries, with 89% of variance, clearly separate the rums by its origin country.

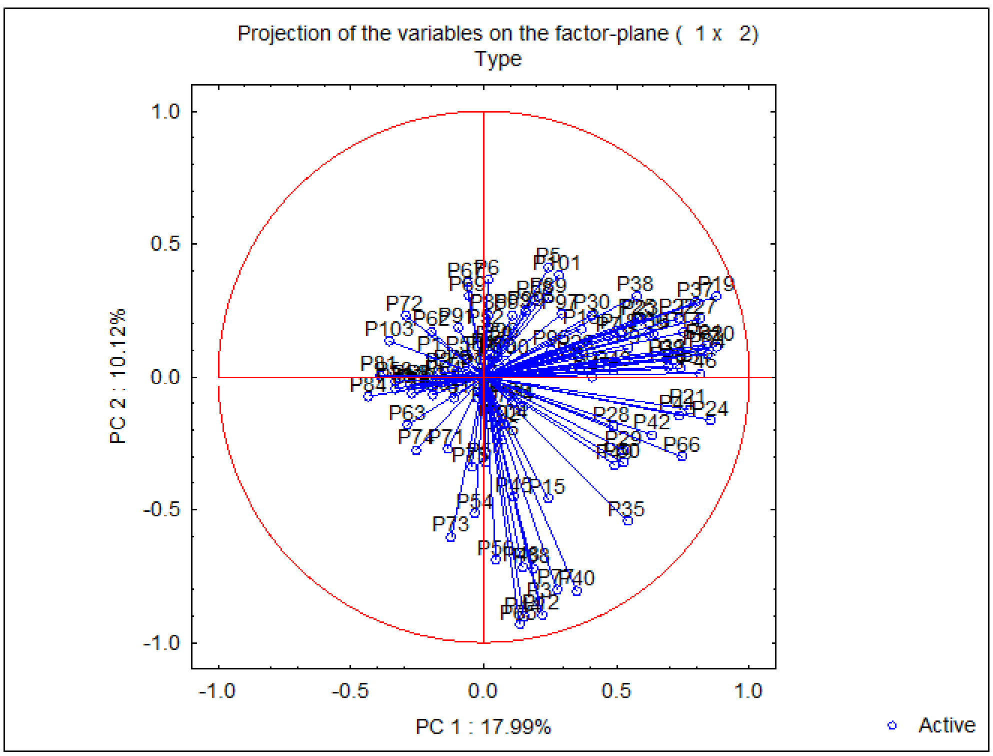

Figure 10a. Projection of the variables by Principal Component Analysis for negative mode.

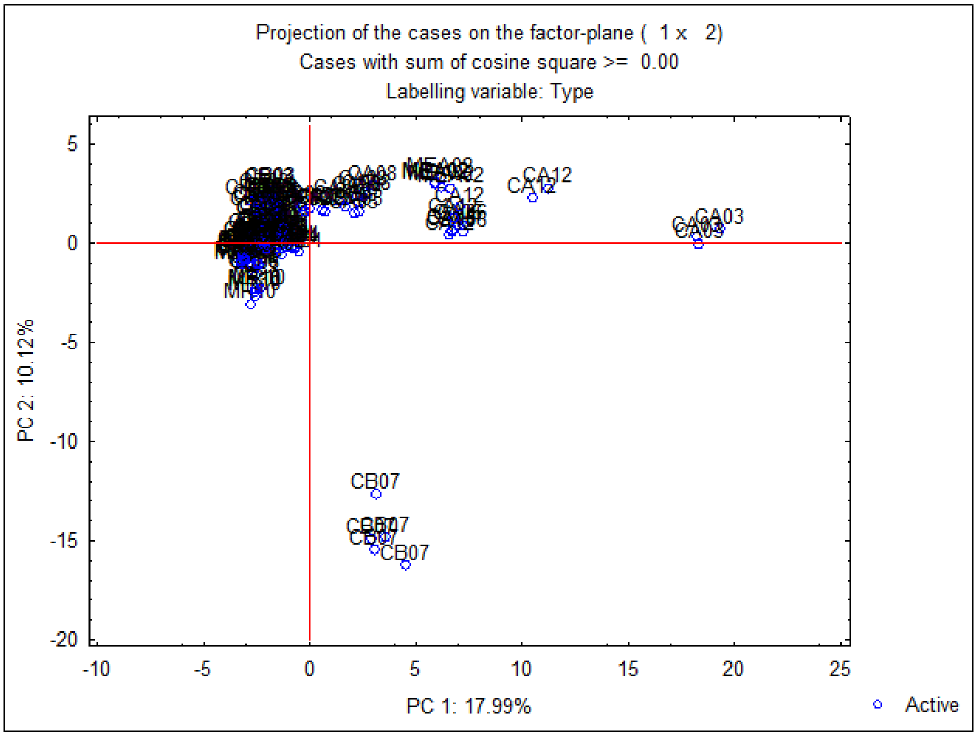

Figure 10b. Projection of the cases by Principal Component Analysis for negative mode.

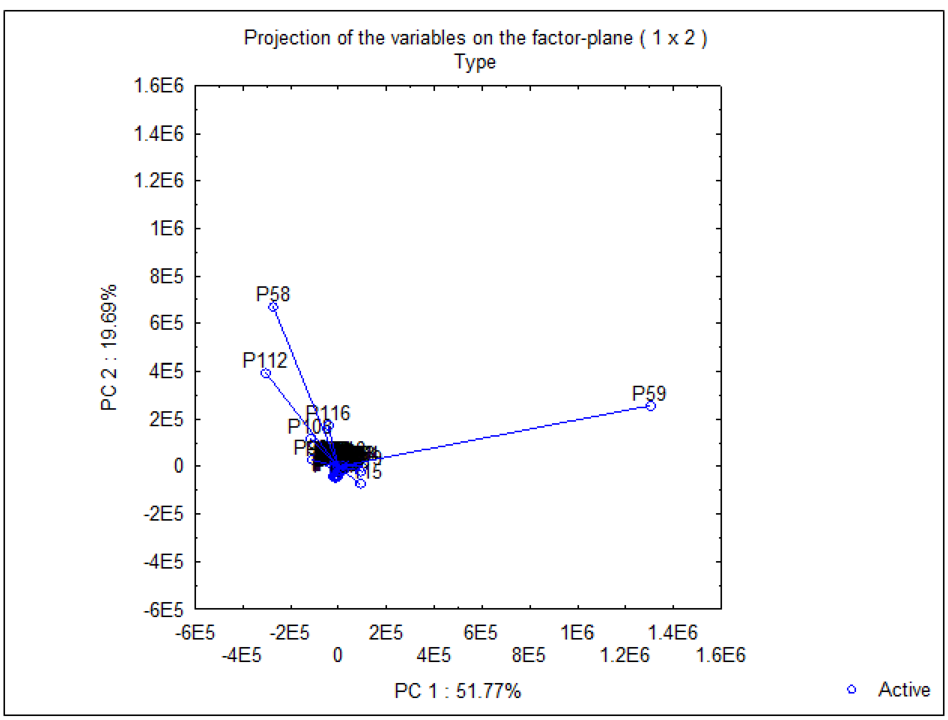

Figure 11a. Projection of the variables by Principal Component Analysis for positive mode.

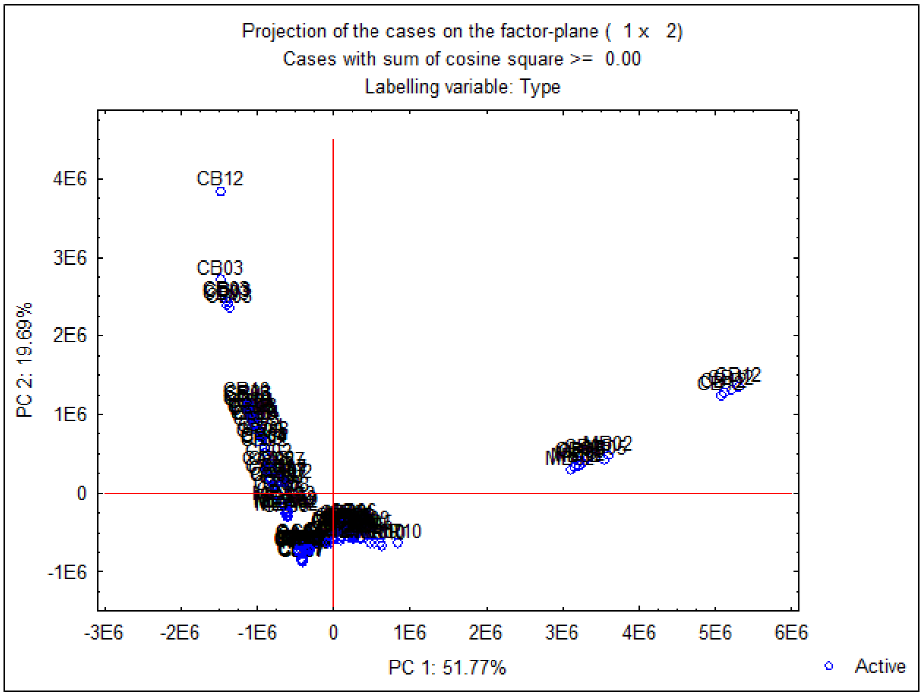

Figure 11b. Projection of the cases by Principal Component Analysis for positive mode.

6. Conclusions.

Ionizing the samples in positive and negative is what integrates a more complete data, since in negative 104 peaks were obtained, while in positive 123, but as observed in the forward stepwise of the General Discriminant Analysis (GDA), the significant peaks are different between each of them.

The ANOVA allowed the determination of the variables that influence the classification of tequila, of which all were significant, both category, class, and origin. Although Principal Component Analysis was not the best analysis for the purpose of tequila classification, the GDA showed favorable results both in its analysis of Fisher's LSD and the distances of Mahalanobis that were useful in its graphical version as the Cooman's plot to observe the grouping of the different variables and their correct classification.

The general objective, identify Tequila by class and category, through a mathematical statistical model, was achieve, due to the General Discriminant Analysis, which classify the Tequila not only by class and category, but also by origin.

Further studies are aimed at verifying the method used in Tequila; but now in other agave distillates originating in México, such as sotol, bacanora, raicilla, and mezcal.

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