UNIVERSITAT ROVIRA I VIRGILI

DISTILLATION STRATEGIES: A KEY FACTOR TO OBTAIN SPIRITS WITH SPECIFIC ORGANOLEPTIC CHARACTERISTICS

Pau Matias-Guiu Martí

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Distillation strategies: a key factor to obtain spirits with specific organoleptic characteristics

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DOCTORAL THESIS
2018

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Distillation strategies: a key factor to obtain spirits with specific organoleptic characteristics

Doctoral thesis

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Grup d'Investigació en Tecnologia dels Aliments

UNIVERSITAT ROVIRA i VIRGILI

Tarragona

2018

UNIVERSITAT
ROVIRA i VIRGILI

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I STATE that the present study, entitled “Distillation strategies: a key factor to obtain spirits with specific organoleptic characteristics”, presented by Pau Matias-Guiu Martí for the award of the degree of Doctor, has been carried out under my supervision at the Chemical Engineering Department of this university

Tarragona, 7th May 2018

Doctoral Thesis Supervisors

Dr. Francisco López Bonillo

Dr. Juan José Rodríguez Bencomo

Acknowledgements

First of all, I would like to thank my supervisors, Dr. Francisco López Bonillo and Dr. Juan José Rodríguez Bencomo, for all the support, trust and especially for the time they have dedicated me, and thank Martí Franquès grant and Universitat Rovira i Virgili for making me feel accomplished as a researcher. I have sincerely learned more in these three years than I had learned during bachelor's and master's degree period. Likewise, particular thanks to Drs. Ricardo Pérez and Ignacio Orriols for their time during the development of the thesis and writing of articles. And also special gratitude to the members of the thesis committee, Dra. Ana Isabel Briones, Dr. Francesc Medina and Dr. Ignacio Orriols, for willing to evaluate this thesis.

However, this thesis would not have been possible without the following people, friends and family:

A en Paco, del que sempre recordaré les reunions al despatx oficial del grup, compartit amb les orenetes, on començàvem parlant de ciència i s'acabava parlant d'injustícies i d'altres metafísiques existencials; i els tasts improvisats al despatx secundari, on hem assaborit aiguardents de tota mena, amb ampolles sempre amunt i avall.

A Juanjo, porque sin Juanjo no hay Lab, y sin Lab no hay Juanjo; porque sin Juanjo tampoco hay datos ni software ni reparaciones ni nada. Por las puntas de micropipeta y por todas las risotadas entre viales y patrones internos, pues los catalanes hacen cosas, mas los canarios arios más.

Habéis sido unos tutores y jefes inmejorables. Me habéis dado mucha libertad y me habéis ayudado dentro y fuera de la tesis. Os estaré siempre agradecido.

Als engranatges del Departament d'Enginyeria Química de l'Escola Técnica Superior d'Enginyeria Química i de la Facultat d'Enologia. Molt especialment a la Núria Juanpere, puix està allí sempre.

To all my people from GITA Lab and surroundings, ordered alphabetically. A Andrea y sus ruiditos tras la estantería, a la sinyoreta Anna y els seus concentrats de garrofa; al maratoniano Antonio y sus ascensos; al destil·lador de destil·ladors Carles y les seves fustes; al curador de chuletones y otros mil proyectos Eduardo; al radical Jordi y sus confesiones cafeteras; to the always smiling Jun and her chopsticks; a Laura y sus horas metida en la campana; a l'home H i màster of de yúnivers Llorenç; a Mateo y su «Miriam del Refugio, lucha contra el mal, Miriam de Refugio, nadie la ganará»; al balón de oro Ricardo el niño bolla y su buti-barco; to the spiritual Lord Sir Mr. Richard; a la paciente Susana que pide y pide pues pido, al gran docent i millor hooligan Victor; and to Wael, the team mascot, who sings and sings like a goldfinch, but ¡bum!

Als de sempre, que són tota la Cinta Sound, vetusts i nouvinguts, pablos i pabludos, i a la omnipresent mare del grup.

Molt especialment a la meva família. Al Javier, que s'ha esforçat moltíssim en que jo expandís la meva curiositat per tot el que m'envolta. A la Mamà, que m'ha encomanat ser bona persona i cuidar dels demés, puix encara em cuida a mi. A la Laurete, la noia esvelta adolescent, amb la que cada cop em sento més germans. I a la resta de la família, estiguin a la terra com en el cel. Moltíssimes gràcies a tots.

Y, cómo no, a familia más spod paszki. A ti, malutki Llull, muchísimas gracias por aguantarme y dejarte aguantar; somos un gran equipo; el mejor; sí, el mejor. A l'acabat de néixer Vito, que has trigat molt a sortir i encara falta molt perquè aprenguis a llegir aquestes paraules; ja veuràs què bé ens ho passarem. I a en Lolo, la foca més famèlica del mediterrani. Us estimo.

Tanmateix, les gràcies no s'haurien d'escriure i encara menys amb un teclat. Les gràcies, les de veritat, s'han de donar a l'instant, d'imprevist i, per damunt de tot, amb sinceritat (o si més no fer-ho veure). I la sinceritat és de tu a tu; avui, de jo a tu. Les gràcies escrites, en canvi, les que ara estic teclejant, vulgui o no, són... formals. I la formalitat, vulgui o no, sempre falseja. «Com més falsari, més protocol·lari» a casa sempre s'ha dit, o «com més fal·laciós, més cerimoniós». Tot i així, donada la tradició doctoranda, avui he fet una excepció, es clar, per confirmar la regla. Més tard, quan trobi el bon moment, ja us les donaré en persona (o si més no fer-ho veure).

Resum

El disseny d’estratègies de destil·lació per obtenir begudes espirituoses amb perfils aromàtics específics segueix sent un desafiament per als productors que utilitzen mètodes tradicionals (destil·lació discontínua). En el cas la destil·lació industrial (destil·lació contínua), s’ha progressat en termes de separació i purificació de compostos volàtils, productes que més tard es poden diluir o barrejar novament. Tot i així, la legislació de la majoria d’aiguardents tendeix a exigir l’ús de mètodes tradicionals. Per tant, el sector requereix l’actualització dels processos tradicionals amb tecnologies més avançades.

L’objectiu principal d’aquesta tesi ha estat descriure i optimitzar la destil·lació i l’evolució dels compostos volàtils dels aiguardents joves durant i després del procés per obtenir productes amb característiques aromàtiques específiques. Aquest objectiu principal s’ha dividit en quatre objectius parcials: i) descriure l'efecte de la variació controlada del reflux intern d'una columna discontínua amb un condensador parcial intern (CDCP) sobre la destil·lació dels congèneres; ii) desenvolupar i testejar un model de regressió per a un sistema CDCP, per predir i optimitzar el procés; iii) explorar les interaccions d'aroma entre compostos volàtils dels aiguardents per així correlacionar la seva concentració química amb l'anàlisi sensorial; i iv) estudiar l'evolució d'un aiguardent embotellat durant un any per conèixer els efectes de la temperatura, el pH i la llum.

Per dur a terme els subsegüents estudis, s’han utilitzat tres tipus de sistemes de destil·lació: un CDCP d’acer inoxidable de 50 L amb rebliment de coure, un alambí de coure d’estil *Charentais* de 20 L, i un CDCP de vidre de 2 L amb rebliment de coure. La cromatografia de gasos amb un detector d’ionització de flama (GC-FID) s’ha utilitzat per a l’anàlisi de la composició de begudes fermentades i els seus aiguardents. Les anàlisis sensorials es van dur a terme en una sala de tast, tant l'entrenament dels assessors com l'avaluació de les mostres. L’anàlisi de variància (ANOVA), la metodologia de superfície de resposta (RSM), l’optimització multi-objectiu mitjançant funcions de desitjabilitat, l’anàlisi de components principals (PCA) i les xarxes neuronals artificials (ANN) han sigut les metodologies utilitzades per a l’anàlisi de dades.

La principal conclusió d’aquesta tesi és la gran capacitat que presenten els

sistemes CDCP per produir aiguardents molt diferenciats a partir de la mateixa matèria primera, amb marcades característiques químiques i organolèptiques. Això s'aconsegueix establint unes estratègies de destil·lació òptimes. Així, pel que fa a les condicions operatives del procés de destil·lació, una reducció dràstica de la rectificació interna després d'una alta rectificació durant les fraccions de cap augmenta els nivells de compostos terpènics, alcohols i èsters (aromes agradables) i disminueix els nivells de compostos del cap (olors desagradables) en les fraccions del cor. D'altra banda, un pH baix afavoreix la reactivitat del linalol i l'acetaldehid per formar α-terpineol i acetal, respectivament, i afavoreix la formació d'èsters etílics per esterificació. Tanmateix, majors volums de fraccions de cap redueixen els principals compostos de cap a costa de reduir els èsters etílics C_4 - C_{10} i el linalol i augmentar els compostos de cua. Pel que fa als efectes d'interacció d'aromes, l'hexanoat d'etil (aroma fruital) mostra un efecte d'emmascarament sensorial sobre el linalol (aroma floral) i acetat d'etil (aroma a cola); l'acetaldehid i l'acetat d'etil augmenten la percepció afruitada a baixos nivells de l'hexanoat d'etil; i alts nivells d'acetat d'etil mostren un efecte d'emmascarament sobre el descriptor afruitat a nivells elevats de l'hexanoat d'etil. Pel que fa a les condicions d'emmagatzematge després de la destil·lació, els valors de pH baix redueixen els compostos rellevants associats a aromes afruitats i terpènics (èsters etílics i linalol) i picants (acetaldehid) durant set mesos d'emmagatzematge; tot i així, aquest efecte és poc significatiu després d'un any. Altrament, mantenir els aiguardents de fruita a altes temperatures redueix considerablement la concentració de la gran majoria de compostos.

En últim terme, les metodologies utilitzades en aquesta tesi doctoral permeten l'avaluació i optimització del procés de producció d'aiguardents d'una manera sistemàtica, senzilla i econòmica. Per això, els resultats obtinguts poden ser útils i aplicables a la indústria dels aiguardents.

Resumen

El diseño de estrategias de destilación para obtener bebidas espirituosas con perfiles aromáticos específicos sigue siendo un desafío para los productores que utilizan métodos tradicionales (destilación discontinua). En el caso la destilación industrial (destilación continua), se ha progresado en términos de separación y purificación de compuestos volátiles, productos que más tarde se pueden diluir o mezclar nuevamente. Sin embargo, la legislación sobre la mayoría de aguardientes suele exigir el uso de métodos tradicionales. Por tanto, el sector requiere la actualización de los procesos tradicionales con tecnologías más avanzadas.

El objetivo principal de esta tesis ha sido describir y optimizar la destilación y la evolución de los compuestos volátiles de los aguardientes jóvenes durante y después del proceso para obtener productos con características aromáticas específicas. Este objetivo principal se ha dividido en cuatro objetivos parciales: i) describir el efecto de la variación controlada del reflujo interno de una columna discontinua con un condensador parcial interno (CDCP) sobre la destilación de los congéneres; ii) desarrollar y testear un modelo de regresión para un sistema CDCP, para predecir y optimizar el proceso; iii) explorar las interacciones de aroma entre compuestos volátiles de los aguardientes para correlacionar su concentración química con el análisis sensorial; y iv) estudiar la evolución de un aguardiente embotellado durante un año para conocer los efectos de la temperatura, el pH y la luz.

Para llevar a cabo los estudios experimentales, se han utilizado tres tipos de sistemas de destilación: un CDCP de acero inoxidable de 50 L con relleno de cobre, un alambique de cobre de estilo *Charentais* de 20 L, y un CDCP de vidrio de 2 L con relleno de cobre. Se ha utilizado cromatografía de gases con un detector de ionización de llama (GC-FID) para el análisis de la composición de bebidas fermentadas y sus aguardientes. Los análisis sensoriales se llevaron a cabo en una sala de cata, tanto el entrenamiento de los asesores como la evaluación de las muestras. El análisis de varianza (ANOVA), la metodología de superficie de respuesta (RSM), la optimización multi-objetivo mediante funciones de deseabilidad, el análisis de componentes principales (PCA) y las redes neuronales artificiales (ANN) han sido las metodologías utilizadas para el análisis de

datos.

La principal conclusión de esta tesis es la gran capacidad que presentan los sistemas CDCP para producir aguardientes muy diferenciados a partir de la misma materia prima, con marcadas características químicas y organolépticas. Ello se consigue estableciendo unas estrategias de destilación óptimas. Así, en cuanto a las condiciones operativas del proceso de destilación, una reducción drástica de la rectificación interna tras una alta rectificación durante las fracciones de cabeza aumenta los niveles de compuestos terpénicos, alcoholes y ésteres (aromas agradables) y disminuye los niveles de compuestos de la cabeza (aromas desagradables) en las fracciones del corazón. Por otra parte, un pH bajo favorece la reactividad del linalol y el acetaldehído para formar α-terpineol y acetal, respectivamente, y favorece la formación de ésteres etílicos por esterificación. Asimismo, mayores volúmenes de fracciones de cabeza reducen los principales compuestos de cabeza a costa de reducir los ésteres etílicos C_4 - C_{10} y el linalol y aumentar los compuestos de cola. En cuanto a los efectos de interacción de aromas, el hexanoato de etilo (aroma frutal) muestra un efecto de enmascaramiento sensorial sobre el linalol (aroma floral) y el acetato de etilo (aroma a pegamento); el acetaldehído y el acetato de etilo aumentan la percepción afrutada a bajos niveles de hexanoato de etilo; y altos niveles de acetato de etilo produce un efecto de enmascaramiento sobre el descriptor afrutado a niveles elevados de hexanoato de etilo. En cuanto a las condiciones de almacenamiento después de la destilación, valores de pH bajo reducen los compuestos relevantes asociados a sabores afrutados y terpénicos (ésteres etílicos y linalol) y picantes (acetaldehído) durante siete meses de almacenamiento; sin embargo, este efecto es poco significativo tras un año. En cambio, mantener los aguardientes de fruta a altas temperaturas reduce considerablemente la concentración de la gran mayoría de compuestos.

Finalmente, las metodologías utilizadas en esta tesis doctoral permiten la evaluación y optimización del proceso de producción de aguardientes de una manera sistemática, sencilla y económica. Por ello, los resultados obtenidos pueden ser útiles y aplicables a la industria de los aguardientes.

Summary

The design of distillation strategies to obtain spirits with specific aroma profiles remains a challenge for producers who use traditional methods (batch distillation). In the case of industrial distillation (continuous distillation), progress has been achieve in terms of separation and purification of volatile compounds, products which can later be diluted or mixed again. However, the legislation of most spirits tends to demand the use of traditional methods. Therefore, spirit industry requires the updating of traditional processes with more advanced technologies.

The main objective of this thesis has been to describe and optimize the distillation and evolution of the volatile compounds of young spirits during and after the process to obtain products with specific aromatic characteristics. This main objective has been divided into four partial objectives: i) to describe the effect of the controlled variation of the internal reflux of a batch column with a partial internal condenser (BCPC) on the distillation of the congeners; ii) develop and test a regression model for a BCPC system, to predict and optimize the process; iii) explore aroma interactions between volatile compounds of wine spirits to correlate their chemical concentration with sensory analysis; and iv) study the evolution of a bottled spirit during a year, to understand the effect of temperature, light and pH.

To carry out the subsequent studies, three types of distillation systems were used: a 50 L stainless steel BCPC with copper packed bed, a 20 L copper Alembic *Charentais* style, and a 2 L glass BCPC with copper packed bed. For the analysis of the composition of fermented beverages and their spirits, gas chromatography with a flame ionization detector (GC-FID) has been used. Sensory analyzes were carried out in a tasting room, both assessors training sessions and studies evaluations. Analysis of variance (ANOVA), response surface methodology (RSM), multi-objective optimization by desirability functions, principal component analysis (PCA) and artificial neural networks (ANN) were the methodologies used for data analysis.

The main conclusion of this thesis is that BCPC systems show a great capacity to produce clearly differentiated spirits from the same raw material, with marked chemical and organoleptic characteristics. This goal is achieved by establishing optimal distillation

strategies. With regard to the operating conditions of the distillation process, a drastic reduction of the internal rectification after a high rectification during head fractions increases the levels of terpenic compounds, alcohols and esters (pleasant aromas) and decreases the levels of head compounds (unpleasant aromas) in heart fractions. On the other hand, low pH favors the decomposition of linalool and acetaldehyde to form α-terpineol and acetal, respectively, and favors the formation of ethyl esters by esterification. In addition, larger volumes of head fractions reduce head compounds at the expense of reducing C_4-C_{10} ethyl esters and linalool, and increasing tail compounds. With regard to aroma interaction effects, ethyl hexanoate (fruity aroma) shows a sensory masking effect on linalool (flowery aroma) and ethyl acetate (glue-like aroma); acetaldehyde and ethyl acetate increase fruity perception at low levels of ethyl hexanoate; and high levels of ethyl acetate show a masking effect on the fruity descriptor at high levels of ethyl hexanoate. With regard to the storage conditions after distillation, low pH reduces the relevant compounds associated with fruity and terpenic aromas (ethyl esters and linalool) and pungent (acetaldehyde) for seven months; however, this effect is barely significant after one year. Furthermore, storing fruit spirits at high temperatures considerably reduces the concentration of most compounds.

Finally, the methodologies used in this doctoral thesis allow the evaluation and optimization of the production process of spirits in a systematic, simple and economical way. Hence, results can be useful and applicable to spirits industry.

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Chapter 1

INTRODUCTION

1.1. Introduction

Since the discovery of the distillation process of fermented beverages, spirit drinks have spread and become an indispensable element of most gastronomic cultures around the world. Its goal is to obtain a flavor quality, sterile and heady drink by concentering ethanol (major volatile compound) and other volatile compounds (congeners) from alcoholic fermented materials of any kind of agricultural origin, since almost all raw materials with carbohydrates can be fermented by yeasts through different previous processes. This fact allows a wide variety of differentiated spirit beverage, which many are highly appreciated within food product field.

Numerous equipment and techniques for spirits production have been described and studied in the course of history, from simple distillation to fractional distillation, steam distillation and vacuum distillation. Each methodology may enhance or decrease the extraction of different congeners and consequently, contribute to the organoleptic characteristics of the product. Likewise, each equipment can be operated using different distillation procedures to emphasize (or not) its strengths with respect to the others. All this knowledge allows producers to generate a wide range of commercial product [1].

Even with so many centuries of study, there are still many unknowns to be resolved on distillation. Big industries usually focus on improving ethanol yield and energy efficiency, to minimize costs. However, there are still challenges such as developing methods to predict congeners' distillation kinetics and formulating distillation strategies to obtain spirit beverages with specific organoleptic characteristics.

1.2. But what's so important about distillation strategies?

The fact that currently more than 700 aroma compounds have been identified in wine and more than 1000 volatile substances in spirit drinks [2–5] can help us to get an idea of the difficulty of obtaining a balanced product by distillation, from the point of view of the aroma.

Like most food products, organoleptic quality of a spirit depends on the aroma compounds composition, which sensory impact and aroma attributes answer to the concentration level and aroma threshold of each compound. Furthermore, each type of spirit has its own targets. For example, vodka is expected to have neutral aroma, mescal to have baked flavors and Pisco to have aromas reminiscent of wine. And if we focus on the production of Pisco, for example, producers may want to enhance the aromas from grapes, intensify the aromas from fermentation or produce a profitable product from a deteriorated wine. In addition, each company also requires differentiating its products from the rest of the market. Therefore, distillation strategies are focused on obtaining a reproducible operational methodology to accentuate or distinguish the desired organoleptic characteristics of the spirit drink, all taking into account the efficiency of the process and its corresponding legislation.

Nowadays, standards and specifications for production, definition and presentation of the different categories of spirit drinks are stablished by regional, national and international legislations. In the case of European Legislation, a spirit drink should be produced by the distillation of and alcoholic fermentation obtained exclusively from agricultural raw materials [6]. From this framework, producers have infinite possibilities to produce the spirit that may convince consumers. But how? The study of the capabilities of the distillation process and product design generates an important knowledge that allows producers to find the correct and fastest strategy.

1.3. An overview of distillation techniques

Alembic can be considered the first apparatus designed for the production of distilled beverages (**Figure 1.1**), although there is evidence of more primitive devices [7]. Constructed with copper to improve heat transfer, this simple distillation device consists of a boiler where the raw material is heated, a header chamber at the top where the vapors rectify, and an outlet coupled to a refrigeration system to condensate the vapors. The condensate could later be redistilled, macerated or aged to modify its aroma. Although this technology may seem obsolete, it is still a very popular methodology as it allows obtaining complex spirits that preserve the typical flavor and in consequence, genuineness of the product. However, the supplied power to the boiler is the only controlled variable that can modify the process (apart from raw material characteristics), since condensation of vapors in the header chamber (rectification) depends on the heat exchange produced by surrounding air that is at room temperature.

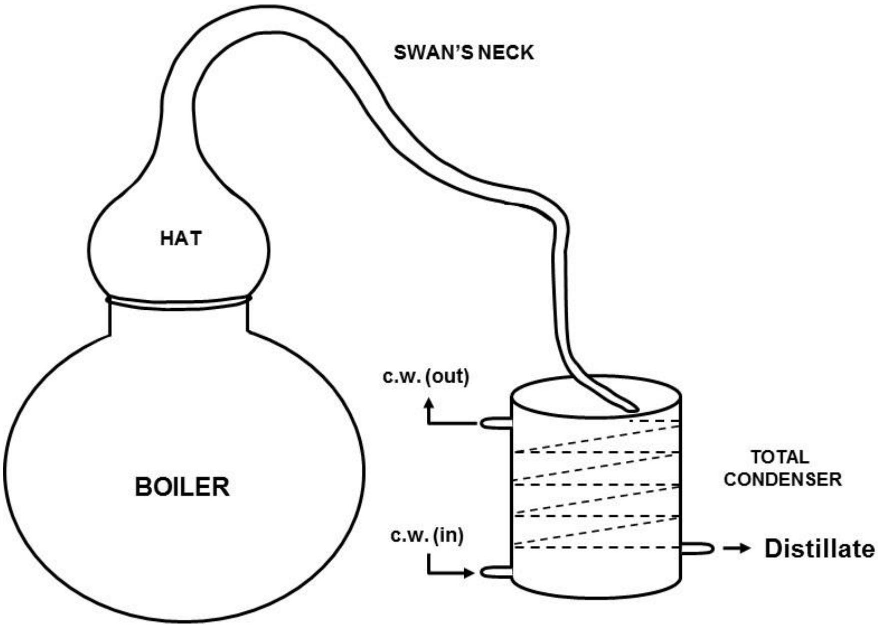

Figure 1.1. Sketch of a cooper Charentais *alembic, where c.w. refers to cooling water stream*

After the discovery of simple distillation, the increase of the rectification ability of the alembic was aimed to increase the alcoholic strength, aside from re-distillation and distillation in series which have low productivity. To achieve this goal, similar equipment to modern fractional column were developed during the 19th century and, with it, the producers could obtain more rectification and a greater control of the distillation.

Fractional distillation equipment consist in a boiler coupled to a distillation column which is typically a large vertical cylinder filled with trays or a packing material to increase the surface of condensation. Therefore, as vapors condensate through the column, a temperature gradient is generated which, in turns, generate different stages of liquid-vapor equilibrium. Then, the longer the column, the greater separation of the compounds according to their physicochemical characteristics. To increase the temperature gradient without increasing the dimensions of the column, a fraction of the product called reflux is reintroduced in the column from the top to control the rectification. In addition, this type of equipment is very versatile, and currently can operate at different pressures or include outlets at intervals to extract different fractions with compounds that have specific physicochemical characteristics.

Nowadays in spirit industry, fractional distillation at a continuous steady state is commonly used. This process achieves an extraordinary yield, with a product composition close to the azeotrope of the mixture ethanol-water and barely without presence of congeners; regularly called “rectified spirit”. However, with the exception of some kinds of beverages (such as vodka or some macerated spirits), spirit drinks are meant to maintain the positive organoleptic characteristics of raw material provided by congeners. Moreover, a minimum content of volatiles substances (other than ethyl alcohol and methanol) is usually required in most spirits legislations to avoid excessive rectification and to guarantee the genuineness of traditional practices [6]. Currently in spirits industry, simple distillation (French style) is also used to produce higher quality spirits, although, as explained, the process leaves little leeway to product design and optimization. It should be noted that modern equipment still maintain copper parts, given the ability of copper to remove certain volatile organosulfur compounds during distillation. Steam distillation is also used for the production of distilled spirits, but especially for the production of pomace brandies and other semi-solid raw materials [8–10].

Batch columns (German style) represent an intermediate technique between simple distillation and industrial continuous columns, which can vary the internal rectification during process by varying the reflux rate. However, classic external reflux has a slow response on the system. Another way to increase rectification is coupling a partial condenser to the outlet of the column (commonly named as “dephlegmator” or “overhead condenser”) that allows a better control of congeners’ rectification. Inspired by this last technique, an innovative experimental system propose to locate the partial condenser inside the top part of the column (**Figure 1.2**), obtaining a much faster control of the internal reflux rate during the distillation process [11]. This system has been compared with traditional alembic by distilling wine and other fermented agricultural raw materials (kiwi, pear and grape pomace) showing significant compositional differences [11–14]. Although packed columns increase distillation time and are difficult to control [11,15], rectification rate can be quickly modified and in consequence the congeners composition, since minor volatile compounds concentration is related to ethanol concentration and distillation moment [16,17]. The study of distillation strategies of the present doctoral thesis is focused on this last distillation equipment, the batch column with internal partial condenser.

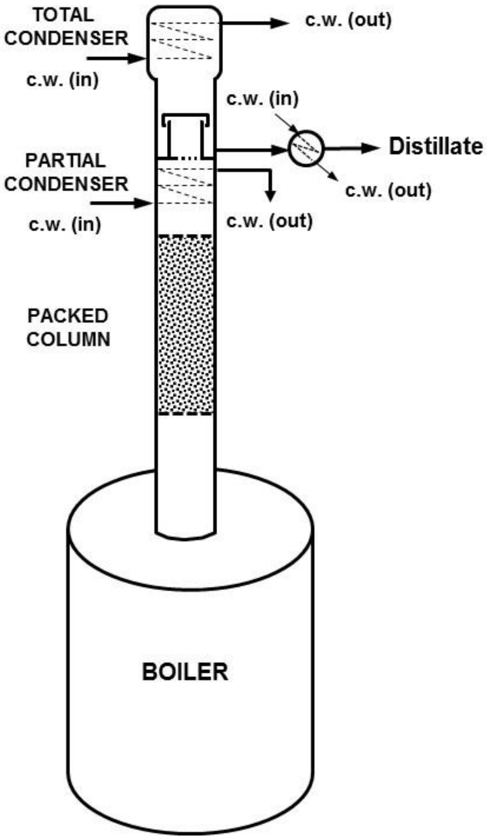

Figure 1.2. Sketch of a batch column with an internal partial condenser, where c.w. refers to cooling water stream

1.4. Batch distillation kinetics

During batch distillation, ethanol shows a decreasing concentration over time due to exhaustion in the boiler, although this behavior can be attenuated by controlling the internal rectification. Being ethanol more volatile than water (both are the major compounds in spirit production process), ethanol shows high levels at the beginning and low levels at the end. In the same way, congeners can be associated to certain fractions of the distillation where their concentration is higher, according to their physicochemical characteristics such as vapor pressure and solubility in ethanol and water [18].

Traditionally, batch distillation output is separated into three consecutive fractions: head, heart and tail. Heart fraction is the main product of the process, while head and tail fractions can be redistilled or used for a purpose other than food [1]. A theoretical example of the distillation behavior of ethanol and congeners is shown in

Figure 1.3. Consequently, distillation strategies focus on concentrating congeners with positive organoleptic characteristics in the heart fraction, and congeners with negative aroma (off-flavors) in head and tail fractions, all with an acceptable alcoholic yield. Based on this premise, the degree of control of each distillation system determines the ability or possibilities of finding optimal strategies, depending to the raw material composition and the knowledge of producers.

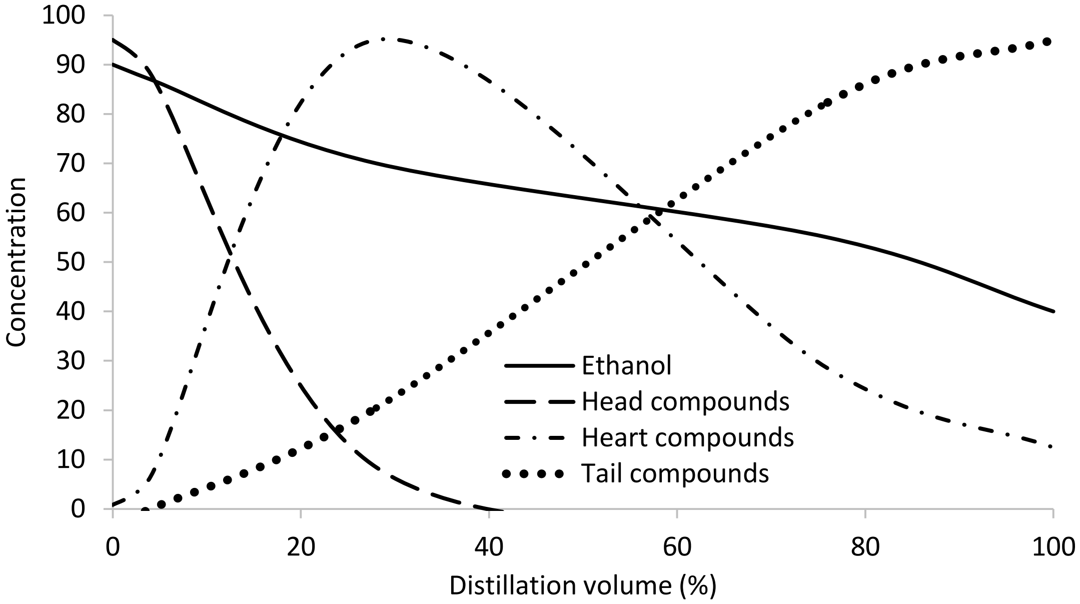

Figure 1.3. Representation of the output levels of ethanol (% v / v) and congeners classes (without specific units) during head and heart fractions of a batch column distillation. Data was adapted from a preliminary study.

1.5. Young wine spirits

The definition of a wine spirit according to the European Legislation is as follows [6]:

(i) Produced exclusively by the distillation at less than 86 % vol. of wine or wine fortified for distillation or by the re-distillation of a wine distillate at less than 86 % vol.

(ii) Containing a quantity of volatile substances equal to or exceeding 125 grams per hectoliter of 100 % vol. alcohol.

(iii) Having a maximum methanol content of 200 grams per hectoliter of 100 % vol. alcohol.

This open description allows for countless production models, since it does not specify distillation techniques or pre and post treatments, such as aging or maceration. A young wine spirit is considered a wine spirit without any post treatment other than its dilution with water to obtain the desired alcohol by volume (typically from 35 to 45 % vol.), except for some styles of wine spirits were water cannot be added such as Peruvian Pisco [19]. Young wine spirit can be later used for production of spirits aged in wood (such as brandy), liqueurs (such as mistelle), fortified wines (such as vermouth) or cocktails (such as Pisco sour), as well as for direct consumption. Peruvian Pisco and some Chilean Piscos are the best known young wine spirits [1], although there are other young wine spirit all over the world often named as "white brandies" [20].

1.5.1. Congeners of young wine spirits

The aroma composition of spirits comes from the interaction between the volatile compounds and consumer's olfactory system, which will determine the acceptance of the product. In young wine spirits, the origins of aroma compounds are the grapes, winemaking (mostly alcoholic fermentation) and distillation process. In addition, spirits compounds are associated to head, heart and/or tail fractions, and many have similar chemical structure. In order to standardize the concentration range, it is common to quantify congeners in grams per hectoliter of pure ethanol ($g \cdot hL^{-1}$ a.a.), since dilution factor usually varies between spirits producers, or in milligrams per liter of ethanol at 40 % vol. ($mg \cdot L^{-1}$ 40 % v/v), since the commercial standard spirits have and alcohol by volume of 40 %. **Table 1.1** gives a summary of all studied congeners during the present thesis, which are presented in the following sections according to their chemical structure.

1.5.1.1. Aldehydes

Acetaldehyde (ethanal) is the major aldehyde from alcoholic fermentation and it may be found in high levels in wine spirits. Since acetaldehyde has a low vapor pressure and high solubility in ethanol, the highest concentration is found in first distillation fractions. Considered as an off-flavor, often defined as pungent odor, acetaldehyde is one of the main reasons why it is important to perform an optimal head-cut to avoid its aroma predominance in heart fraction. Acetaldehyde reacts with ethanol to form acetal (1,1-diethoxyethane) in acid media [21], with an equilibrium concentration ratio of 10:1 approx. This reaction has a positive effect, since the aroma of the acetal is not so unpleasant [3].

The presence of furfural (furan-2-carbaldehyde) is associated with the reactions of Maillard between amino acids and residual sugars, which are favored in hot acid media as happens during distillation [22–24]. Therefore, distillation time, pH and heating power influence its formation, and its almond-like and burnt aroma is a clear indicator of a bad performance of the tail cut. Furfural and other compounds from the Maillard reaction can also originate from winemaking [4,25], but normally at pleasant and much lower levels.

Table 1.1. Brief summary of properties and information of the studied volatile compounds during the thesis projects [a].

Compound name	CAS number	Chemical structure	Flavor quality	Associated fraction	Main formation origin
Water	7732-18-5	Water	No odor	Heart/Tail	Raw fruit
Ethanol	64-17-5	Alcohol	Alcoholic	Head/Heart	Fermentation
Acetaldehyde [b]	75-07-0	Aldehyde	Pungent, sweet	Head	Fermentation
Acetal	105-57-7	Aldehyde	Fruity, sherry-like	Head	Fermentation
Methyl acetate	79-20-9	Ester	Solvent-like	Head	Fermentation
Isoamyl acetate	123-92-2	Ester	Fruity, banana	Heart	Fermentation
Ethyl acetate	141-78-6	Ester	Solvent-like	Head	Fermentation
Ethyl butyrate	105-54-4	Ester	Fruity, floral	Head	Fermentation
Ethyl hexanoate	123-66-0	Ester	Fruity, apple	Head/Heart	Fermentation
Ethyl octanoate	106-32-1	Ester	Fruity, pineapple	Head/Heart	Fermentation
Ethyl decanoate	110-38-3	Ester	Floral, brandy-like	Head/Heart	Fermentation
Ethyl carbamate [b]	51-79-6	Ester	No odor	Head/Heart	Distillation
Ethyl lactate	687-47-8	Ester	Lactic	Tail	Fermentation
Furfural	98-01-1	Aldehyde	Almond, burnt	Tail	Distillation
β-phenylethanol	60-12-8	Alcohol	Rose-like	Tail	Fermentation
Limonene	138-86-3	Terpene	Terpenic, lemon	Heart	Raw fruit
Linalool	78-70-6	Terpenol	Terpenic, Muscat	Heart	Raw fruit
α-terpineol	98-55-5	Terpenol	Terpenic	Heart	Raw fruit
β-citronellol	106-22-9	Terpenol	Terpenic	Heart	Raw fruit
Geraniol	106-24-1	Terpenol	Terpenic, geranium	Heart	Raw fruit
Nerol	106-24-1	Terpenol	Terpenic	Heart	Raw fruit
2-butanol	78-92-2	Alcohol	Alcoholic	Heart	Fermentation
1-propanol	71-23-8	Alcohol	Fusel-like	Heart	Fermentation
2-methyl-1-propanol	78-83-1	Alcohol	Alcoholic	Heart	Fermentation
1-butanol	71-36-3	Alcohol	Alcoholic	Heart	Fermentation
2-methyl-1-butanol	137-32-6	Alcohol	Fusel-like	Heart	Fermentation
3-methyl-1-butanol	123-51-3	Alcohol	Fusel-like	Heart	Fermentation
1-hexanol	111-27-3	Alcohol	Mown grass	Heart	Winemaking
Methanol [b]	67-56-1	Alcohol	Alcoholic	Head/Tail	Winemaking
Acetic acid	64-19-7	Fatty acid	Vinegar-like	Heart/Tail	Fermentation

[a] This information is generic and is based on the existing consensus [3–5]. [b] Toxic compounds which levels are regulated in some countries [6,36,37].

1.5.1.2. Aliphatic and aromatic alcohols

Higher alcohols (1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol 3-methyl-1-butanol and β-phenylethanol) are produced during wine fermentation, as metabolites from the degradation of amino acids. Except β-phenylethanol which tends to distill during last fractions and has a positive rose-like flavor, higher alcohols tend to distill during heart fractions and are called "fusel alcohols" due to their fusel-like off-flavor, although its aroma can be positive in low concentrations [3].

Methanol, 1-butanol and 2-butanol do not contribute to the aroma of wine spirits, due to their high odor thresholds. Methanol is formed as a result of the enzymatic degradation of pectin of grapes, petiole of other parts of *Vitis vinifera* plant. Methanol is potentially toxic and its legal limit for wine spirits is 200 g/hL a.a. according to wine spirit definition in the European legislation. However, this concentration limit is higher for other types of spirits made from other raw materials [6]. Methanol is considered a head compound, although its relative concentration (g/hL a.a) is greater both in head and in tail fractions [26,27]. 1-butanol and 2-butanol are formed during the fermentation of must sugars and are characteristic substances of some fermented beverages as wine. Furthermore, 1-hexanol is formed during pre-fermentative steps of winemaking, being a typical compound of wine and pomace spirits that can be a varietal marker for assessment of wine origin [28], although it may be found in other spirit beverages. Its aroma is easily recognizable by its green character reminiscent of grape marc [3].

1.5.1.3. Monoterpenes

The synthesis of terpenic compounds occurs during grape ripening and they are found in wine both in free form and as glycoside precursors [29]. White wine varieties of *Vitis vinifera* Muscat, Malvasia and Gewurztraminer are expected to have high levels of monoterpenes, although it is detected in many other varieties [4]. Among terpenic compounds, it is common to consider linalool (3,7-dimethylocta-1,6-dien-3-ol) the most relevant for its high levels and low odor threshold, followed by geraniol ((2E)-3,7-dimethyl-2,6-octadien-1-ol). Isomeric with geraniol is nerol ((Z)-3,7-dimethyl-2,6-octadien-1-ol). On the other hand, α-terpineol (2-(4-methyl-1-cyclohex-3-enyl)propan-2-ol) can be detected with high levels in spirits made from terpenic grapes, however its higher odor threshold reduces a lot its flavor relevance [30]. Limonene (1-methyl-4-(1-methylethenyl)cyclohexene) and β-citronellol (3,7-dimethyloct-6-en-1-ol) have also been studied in the present thesis, of which there is little information reported in literature [31].

Monoterpenes play a very important role in the aromatic profile of wine spirits, especially with aromatic wine spirits [29]. Therefore, terpenic compounds are intended to be distilled during heart fraction, although their vapor pressures and water solubility vary considerably among them. Their aroma is usually included in an overall descriptor called "terpenic", given their similar aroma that reminds the smell of Muscat and Malvasia wine, citrus notes, as well as flower-like odor such as rose and geranium. These compounds tend to react under distillation conditions (hot acid media) to form more stable compounds [16,32–35].

1.5.1.4. Esters and fatty acids

The origin of esters and fatty acids is mostly microbiological, produced during alcoholic fermentation by yeasts and other microorganisms. In addition, the ester formation is favored through the chemical esterification of carboxylic acids and ethanol, given the high levels of ethanol in spirits (>30% v/v), or other minor alcohols such as methanol and amyl alcohols [3].

The positive fruity odor in wine spirits mostly comes from short chain ethyl esters (C_4-C_{10}) and isoamyl acetate (3-methylbutyl acetate). On the other hand, ethyl acetate and methyl acetate provides a glue-like odor, which is one of the most relevant off-flavors in spirits drinks. These esters present very high volatility and show a high solubility in ethanol; thus tend to distil during the first fractions. As general rule, the smaller the molecule is, the greater its volatility. For this reason, to optimize ester aroma, the distillation strategies focus on separating the ethyl acetate and methyl acetate during the head fraction and keeping the rest of the esters in the heart fraction, since differences between their volatilities may allow it. Apart from ethyl acetate that is the major ester, the rest are usually found in low concentrations but above their respective odor thresholds [3,18]. Within the group of short chain esters present in alcoholic beverages, ethyl carbamate (without smell) has gained relevance in recent decades by being a carcinogen compound of Group 2A according to IARC [38]. Its presence in spirits is much more relevant than in other fermented beverages because its formation from cyanate or urea is favored by copper (II), in addition to the high levels of ethanol of spirits [39].

Short-chain fatty acids are in equilibrium with the esters, where acetic acid is the most relevant due to its high concentration, vinegar-like off-flavor odor and potential to form ethyl acetate which has a lower odor threshold. Fatty acids have low vapor pressure and high solubility in water, which presumably should be distilled during tail fractions. However, acids are also present in head and heart fractions due to their reversible equilibrium with esters, and vice versa. Wine spirits also contain longer-chain esters and acids, although they are more abundant in other types of raw materials (such as pear and pomace brandies) [3,40].

1.5.2. Wine spirits analysis

Being a food product, wine spirits should be chemical and sensory analyzed. Densimetry (measured by pycnometer, electronic density meter using frequency oscillator or hydrostatic balances) is the official and standardized method to determine the ratio of the two major compounds, ethanol and water, of spirits [41]. Regarding the analysis of congeners, given their high volatility, it is common to use gas chromatography with mass spectrometry (GC-MS) or flame ionization (GC-FID) detectors [30,31,42–44], although other methodologies are also used such as liquid chromatography [40] or colorimetry [27]. Thanks to novel analytical techniques (such as headspace solid phase micro-extraction method followed by GC-MS analysis [45]), more and more accurate compositional results can be obtained.

Even so, the relationship between compound concentration and consumer's perception stills unclear. The ratio between chemical concentration and odor threshold concentration, named odor activity value (OAV), is a common method used to predict the odor intensities of volatile compounds from chemical analysis. OAV is a measurement linearly proportional to concentration, however the relationship between odor intensity and concentration responds to a sigmoidal function [46]. In any case, apart from the odor-concentration correlation, it is also necessary to take into account the odor interactive effects among the compounds, such as enhancement or suppression of odors [47–49], and the olfactory fatigue of strong-smelling agents, such as ethanol in spirits [50]. Therefore, given the difficulty of predicting consumer perception, it is important to verify chemical analyses with sensory analyses and vice versa.

Moreover, after distillation, spirits need a period of time to stabilize its aroma compounds before put them on the market, a fact to take into account when performing chemical and sensory analyses. A clear example is Peruvian Pisco, which must rest a minimum of 3 months in inert recipients after distillation according to Peruvian regulation [19], in order to improve organoleptic properties. A similar procedure is carry out for cachaça and rum not aged in oak [1] and for blanc Armagnac [20]. However, scientific information on this topic is scarce and the existing one focuses on other types of no-aged spirits [51–53].

1.6. Modeling and statistical analysis

To describe or predict processes which theoretical models are unknown or more laborious than what is required, heuristic or designed experimentation is used and analyzed by the principle of causality, usually called as “cause and effect”. In statistical language, the “causes” (i.e. operational variables of distillation process such as heating power, cooling system, device components or raw material characteristics) are called factor, predictor, input, independent or exploratory variables. On the other hand, the “effects” (i.e. variables that define the product such as congeners’ concentration, professional sensory evaluation or consumer acceptability) are called response, output or dependent variables. Therefore, the aim of using advanced modeling and statistical techniques in distillation strategies studies is to understand or predict the effect of known factors on the responses that defines the spirit drink of study. Causality logic can also be applied between responses (i.e. the effect of the composition on the acceptability of the product).

Design of experiments (DOE) is a widely used technique to plan an experimental procedure which aims to model the variation of one or more quantitative variables (responses) under some controlled conditions (factors). DOE are optimized to be analyzed with regression models and the best known is the full factorial design (FFD), which studies the effect of all possible combinations of 2 or more factors at 2 levels. **Figure 1.4** shows a DOE with 2 factors, where squared symbols represent the experimental points of a FFD. The main idea of DOE is to test the effect of controlled factors at a specific range using their limit values, in order to predict the responses at all possible intermediate levels. Thus, for FFD in **Figure 1.4**, 1 and -1 are the limits of both factors. In addition, the experiments must be run randomly and replicated to ensure validity, reliability, and replicability. Modeling is usually carried out with polynomic regressions. In the case of FFD, linear equation with interactions between factors is used. Over time, different designs have been proposed to include more data points while minimizing the number of experiments. One of the most complete DOE is the central composite design (CCD), which consist in a 2-level fractional factorial design with a center point and axial points. These additional data points allow 3 to 5 levels per factor, where usually only the central point is replicated to minimize experimentation. All points in **Figure 1.4** configure a CCD example with 2 factors, where each symbol is an experimental point. Should be noted that axial points locations are similar to FFD points but with a 45 degrees rotation, a design characteristic called “rotatability”. Other common design characteristic for axial points locations are “orthogonality” and “face centered”. As can be appreciated, the arrangement of the experiments allows a subsequent polynomic regression with linear, quadratic and interaction coefficients that may predict a response at all intermediate levels. This modeling methodology is usually called response surface methodology (RSM), since allows the creation of very visual tools such as surface and contour plots. Furthermore, more complex DOE can be constructed and considered, depending on the type of study and level of accuracy [54].

Nonlinear regression and other statistical analyses may be used for modeling specific DOE designs. Artificial neural networks (ANN) is a widely used tool to build nonlinear mathematical models, inspired by how biological brains work. ANN has become very popular since does not require a prior specification of suitable fitting functions, unlike most methodologies [55]. In relation to DOE, some studies have shown that ANN models may have better predictions of CCD responses than RSM [56–58].

Furthermore, DOE are usually designed for modeling and optimize more than one response. For example, during spirits evaluation, the concentration of each compound is a numerical response. Therefore, a multiple criteria decision in needed since, normally, no single solution exist that optimizes all responses simultaneously. In these cases, multi-objective optimization can be used to explore designs with several responses. Especially for CCD, desirability functions are widely used. This technique consist in transforming each response model into a range of acceptability values between 0 (undesirable) and 1 (very desirable). Then, to obtain an overall desirability that relate several responses, the geometric mean of the individual desirability values is calculated. With this data processing, optimization proceeds on determining the factor values that maximize an overall desirability [59,60].

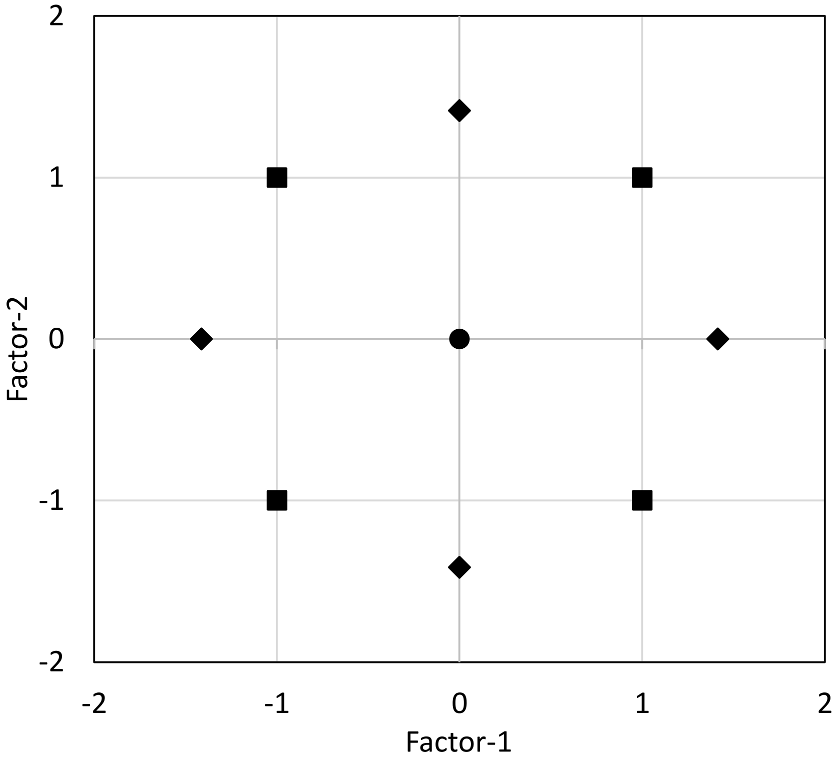

Figure 1.4. Example of a 2-factor Design of Experiments with full fractional design points (■), central point (●) and axial points (♦).

1.7. Scientific and technological objectives

The main objective of this thesis has been to describe and optimize the distillation and evolution of wine congeners in young spirits during and after the process to obtain products with selected organoleptic characteristics.

This main goal have been divided into four partial objectives.

1. Describe the effect of the controlled variation of the internal reflux of a batch column with an internal partial condenser (BCPC) on congeners' distillation to:
    a. Obtain products with high levels of terpenic compounds.
    b. Compare BCPC capabilities with traditional alembic distillation.

2. Develop and test a regression model for a BCPC to predict and optimize the effects of:
    a. The internal reflux during distillation first fractions (head).
    b. The pH of the initial fermented beverage in the boiler.
    c. The volumes of the distilled fractions (head and heart).

3. Explore the aroma interactions between four relevant compounds of wine spirits to correlate chemical concentration and sensory analysis.

4. Study the evolution of a bottled spirit during one year to:
    a. Report the effects of different temperature, light and pH levels on the composition of a bottled spirit.
    b. Identify the storing practices that can improve or deteriorate the spirit after its production.

1.8. Novelty of the project

In industrial distillation (continuous distillation), much progress has been achieved in terms of separation and purification of volatile compounds, which can later be diluted or mixed again. However, most spirits legislation does not encourage such practices, since tends to demand traditional methods. In addition, obtaining a multi-compound product from a single operation reduce process and transportation of reactants, a fact that promotes the "local food movement" so socially extended. For both reasons, the improvement of traditional processes with advanced operational and statistical technologies is needed.

Furthermore, the study of operational strategies for batch distillation can be applied to many other fields where aromas are distilled, since it undoubtedly helps to better understand the distillation of minor compounds. In addition, advanced statistical techniques were used for this project, which had barely been applied before for the study of spirits production. Besides, information in scientific literature on the evolution of volatile compounds of wine spirits and the aroma interaction between them is close to nil. Unfortunately, these and many other issues related to post-production of spirits have been little investigated. In the same way that wine research has been very extensive during the last decades, knowledge about wine byproducts must also be updated.

Finally, the methodologies used in this project are relatively simple to apply and available to all businesses. Therefore, the improvement of regional and traditional techniques such as batch distillation, which may be displaced from global market for marketing reasons, offers more opportunities for small and medium-sized enterprises to stand out and compete with the beverage canons.

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Chapter 2

FLORAL AROMA IMPROVEMENT OF MUSCAT SPIRIT BY PACKED COLUMN DISTILLATION WITH VARIABLE INTERNAL REFLUX

A study published in: Food Chemistry 2016, 213, pp. 40-48

2.1. Abstract

The organoleptic quality of wine distillates depends on raw materials and the distillation process. Previous work has shown that rectification columns in batch distillation with fixed reflux rate are useful to obtain distillates or distillate fractions with enhanced organoleptic characteristics. This study explores variable reflux rate operating strategies to increase the levels of terpenic compounds in specific distillate fractions to emphasize its floral aroma. Based on chemical and sensory analyses, two distillate heart sub-fractions obtained with the best operating strategy found, were compared with a distillate obtained in a traditional alembic. Results have shown that a drastic reduction of the reflux rate at an early stage of the heart cut produced a distillate heart sub-fraction with a higher concentration of terpenic compounds and lower levels of negative aroma compounds. Therefore, this sub-fraction presented a much more noticeable floral aroma than the distillate obtained with a traditional alembic.

2.2. Introduction

Wine spirit is an alcoholic beverage obtained from the distillation of fermented grape musts. The quality of the distillate depends on both the raw materials and the distillation process used.

The grape variety used to produce the wine could provide varietal compounds, such as terpenes and terpenols in the case of Muscat or Malvasía grape varieties, that give wine floral aroma characteristics [1]. *Pisco* is one of the most relevant terpenic spirit, so distillers aim to preserve the floral and fruity aromas, a factor traditionally associated with the variety and quality of grapes [2]. However, large chemical composition differences between aromatic *Piscos* have been observed [3]. Among floral and fruity aroma compounds, linalool is the most relevant compound in *Pisco* [4], although its characteristic aroma is also related to the sensory perception of other molecules [5]. In addition, terpenic compounds present high reactivity in catalyzed and hot acid media [6–8] and tend to distil in early fractions of the distillation [5], thus these compounds cannot be easily concentrated in the heart cut (commercial distillate fraction). Muscat distillates such as *Pisco* contain other non-terpenic compounds with important sensory attributes [9], whose distillation behaviors vary throughout the process [10].

The traditional distillation with a copper *Charentais* alembic (French Style) allows limited intervention during the distillation process (only the heating power in the boiler can be manipulated) to modify the composition of the distillate. A more flexible system is the batch distillation column (German Style) in which the reflux rate can be varied in a wide range. However, none of these systems allows a rapid variation of the internal reflux of the system during distillation. An interesting alternative is the use of a boiler coupled with a rectification column, equipped with an internal partial condenser that allows rapid control of the reflux rate of the column by manipulating the cooling flow rate [11].

Several studies have compared the spirits obtained by classical alembics and columns with an internal partial condenser. Kiwi and pear fermented juices and grape pomace have been tested with both methods of distillation [12–14] and showed that column distillates presented better fruit and floral characteristics and less solvent-like and toxic compounds (head compounds). In addition, García-Llobodanin et al. (2011) [11] found differences between both methods. The partial reflux column system produced heart fractions of distillate with high levels of esters and higher alcohols, although they observed a lack of reproducibility of the distillation. No previous studies have tested specific variable reflux policies focused on concentrating or removing specific positive or negative compounds in certain distillate fractions.

Therefore, the aim of this study was to develop variable reflux strategies to concentrate terpenic compounds in the heart fraction of the distillate. Hence, non-aromatic wine was doped with several terpenic compounds to study the extraction/distillation kinetics. Moreover, using chemical and sensory analyses, Muscat wine (non- doped) spirits obtained with the optimum column strategy and with a traditional alembic were compared.

2.3. Material and methods

2.3.1. Wines.

Experiments were performed at the Department of Chemical Engineering of the Rovira I Virgili University. Two white wines were used: a *Vitis vinifera* Macabeo produced in the experimental cellar "Mas dels Frares" of the University (Tarragona, Spain), and a *Vitis vinifera* Muscat kindly donated by Dalmau Hermanos y Cía. Suc. S.A. (Tarragona, Spain). The basic oenological parameters of Macabeo and Muscat wines were: alcohol degree 10.8 and 12.6 % (v/v), pH 3.31 and 3.32, and glucose + fructose concentration < 0.10 and 0.43 g/L, respectively. Since Macabeo wine contains very low amounts of terpenic compounds, it was doped with six representative terpenic compounds; limonene, linalool, α-terpineol, β-citronellol, geraniol and nerol, all of them of food grade quality (Sigma-Aldrich; Saint Louis, USA). The doses were 4 mg/L for the three most volatile compounds (limonene, linalool, α-terpineol) and 6 mg/L for the others, according to their volatility and the results of preliminary tests. These levels are much higher than those usually found in Muscat wine; the aim was to enhance the sensitivity of the chemical analysis to clearly observe the impact of the different strategies on the evolution of the terpenic compounds during distillations. Physical-chemical characteristics and terpenic compound levels of the wines (doped Macabeo and Muscat) before distillation are shown in **Table 2.1**.

Table 2.1. Terpenic compounds proprieties and their concentrations in the wines before distillation

Compound	Molar mass^a (g/mol)	Boiling point^a (ºC)	log $K_{o/w}$^{a}	Doped Macabeo wine^b	Non-doped Muscat wine^b
Limonene	136	176	4.57	4.12 ±0.07	n.d.
Linalool	154	197	2.97	4.03 ±0.06	2.10 ±0.00
α-Terpineol	154	220	2.98	4.23 ±0.20	2.52 ±0.14
β-Citronellol	156	224	3.91	6.07 ±0.19	1.60 ±0.08
Geraniol	154	230	3.56	6.13 ±0.13	0.306 ±0.028
Nerol	154	225	3.47	6.11 ±0.11	0.117 ±0.002

^{a}Data extracted from EPI Suite database [28]. ^{b}Concentrations are expressed in mg/L.

2.3.2. Distillation systems

Column distillation system assays were performed in a distillation boiler (50 L) heated with two electrical resistances and coupled with a stainless steel distillation column with a copper mesh. The distillation column was equipped with a total condenser (on the top) and a partial condenser with variable flow (controlled with a peristaltic pump) to control the internal reflux of the column. In addition, the system was equipped with several temperature sensors (in the boiler, at different levels of the distillation column and in the partial cooling water system). Details of the distillation column have been previously described in García-Llobodanin et al. (2011) [11]. The process was controlled with Lab-view software (LabVIEW 8.6.1, National Instruments). Before experimentation, the peristaltic pump of the partial condenser was calibrated between 0 and 200 mL/min.

Traditional distillation system assays were performed in a 20 L copper *Charentais* alembic heated by an electrical hotplate.

2.3.3. Distillation processes

First, column distillation assays were performed with doped Macabeo wine, in order to determine the behavior of the terpenic compounds and other relevant compounds. Then, based on the obtained results, column and alembic distillation assays were performed with Muscat wine.

For column distillation, 25 L of wine (Macabeo or Muscat) were placed in the boiler. In case of Macabeo wine, terpenic compounds were added 12 hours before distillation. Total condenser's cooling flow rate was constant at 1.7 L/min. Partial condenser's cooling flow rate ranged from 0 to 180 mL/min and was modified during distillation according to two different strategies (STR-1 and STR-2) detailed in **Table 2.2.** Electrical resistances operated at a constant power of 2400 W until the temperature below the partial condenser raised to 72 ºC; then the power was reduced and kept constant at 960 W. The first 200 mL of distillate were collected in 50 mL fractions and the rest in 100 mL fractions until 3500 mL of distillate. Temperatures were monitored and recorded every 16 s at different points of the distillation systems.

For traditional distillation, 12.5 L of Muscat wine and 5 g of pumice stones were placed in the copper *Charentais* alembic boiler. Total condenser cooling flow rate was constant at 1.8 L/min. Electrical hotplate operated at a constant power of 2900 W until the first drop, then the power was reduced and kept constant at 2333 W (both values were calculated without considering heat loss). As in column distillations, first 200 mL of distillate were collected in 50 mL fractions and the rest in 100 mL fractions until 2600 mL of distillate.

Table 2.2. Doped Macabeo distillation kinetics of both strategies[a]

Fraction name	Distillation accumulated volume (mL)	Strategy 1 (STR-1)					Strategy 2 (STR-2)				
		P.C.[b] flow rate (mL/min)	Alcohol content (% v/v)	P.C.[b] temperature (ºC)	P.C.[b] removed heat (kJ/min)	**Σ**Head compounds[c] (g/hLa.a.)	P.C.[b] flow rate (mL/min)	Alcohol content (% v/v)	P.C.[b] temperature (ºC)	P.C.[b] removed heat (kJ/min)	**Σ**Head compounds[c] (g/hLa.a.)
F1	100	100	83.3±0.2	75.3±0.2	17.5±2.4	646±86	180	83.1±0.5	74.3±2.1	28.3±7.2	834±96
F2	200	100	87.0±0.3	74.2±0.3	18.3±0.7	651±82	120	89.3±0.6	74.2±0.1	20.8±0.1	1077±301
F3	500	50	87.0±0.9	75.2±0.0	9.46±0.39	226±10	100	90.5±0.4	74.5±0.1	19.3±0.3	250±52
F4	800	75	88.8±0.4	74.7±0.3	14.0±0.2	143±24	25	79.3±1.5	79.4±2.1	4.34±0.23	58.2±1.4
F5	1100	100	90.5±0.1	74.4±0.2	18.8±0.7	97.5±16.0	15	70.4±1.5	84.3±1.3	2.15±0.31	46.2±0.6
F6	1400	50	84.8±0.4	76.0±0.4	9.59±0.35	41.1±0.7	50	82.0±0.6	77.2±0.1	9.70±0.13	42.9±2.1
F7	1700	25	69.9±0.9	83.8±0.0	4.69±0.11	37.8±0.1	15	66.6±0.3	84.9±1.3	2.16±0.17	43.2±2.7
F8	2000	50	74.7±0.4	80.5±0.3	9.92±0.25	37.3±0.6	25	66.8±1.4	85.9±0.3	4.60±0.01	39.1±1.6
F9	2300	25	61.8±0.6	87.5±0.4	4.84±0.10	32.5±9.5	50	74.0±1.1	81.0±0.1	10.1±0.0	49.5±1.8
F10	2600	75	76.0±2.8	79.0±1.8	15.1±0.3	50.4±0.8	75	82.5±2.4	77.1±1.0	15.2±0.0	63.6±15.5
F11	2900	50	59.0±1.8	87.5±0.8	10.6±0.4	49.8±3.8	25	53.4±3.0	89.4±0.1	5.03±0.04	45.8±2.1
F12	3200	25	38.8±1.5	91.7±0.3	5.39±0.17	51.1±11.3	75	70.6±0.9	81.9±0.6	15.7±0.0	61.3±9.2
F13	3500	0	20.4±1.0	92.1±0.3	0	63.6±3.0	0	23.6±3.8	91.5±1.1	0	60.8±6.5

[a]Mean and standard deviations were calculated with two replicates. [b]P.C: Partial Condenser. [c]**Σ** Acetaldehyde, methyl acetate, acetal, ethyl acetate and methanol.

Distillation assays were performed in duplicate for doped Macabeo wine and in triplicate for Muscat wine. Head cuts were decided by sensorial analysis.

2.3.4. Chemical analysis of wines and distilled fractions

Analyses of wine ethanol content and distillation residues were determined by ebulliometry (electronic ebulliometer, GAB instruments), wine glucose + fructose concentration by enzymatic bioanalysis (R-Biopharm AG**)** and wine pH with a pH-meter (Crison Basic 20). For distilled fractions, ethanol content of each sample was analyzed with an electronic density meter (Anton Paar DSA 5000M).

Volatile compounds of wines before distillation and distillation residues were extracted with dichloromethane and analyzed by gas chromatography, using a methodology adapted from Ferreira, Lopez, Escudero, & Cacho (1998) [15]. For the liquid-liquid extraction, in a 12-mL glass tube, 10 mL of wine were added with 2.5 g of ammonium sulfate and 0.5 mL of dichloromethane. As internal standard, 50 µL of 2-octanol (400 mg/L) solution was added. The extraction was carried out for 1 h in an orbital shaker at 110 rpm. Extractions were done in duplicate.

In the case of distilled fractions, first four samples were grouped in 100 mL fractions, and the rest in 300 mL fractions. For all fractions, 50 µL of the internal standard solution were added to 1 mL of each sample (previously adjusted to 40% v/v of alcohol). In addition, Muscat heart fractions of column and alembic distillations were extracted with dichloromethane to obtain enough instrumental sensibility for terpenic compounds using a methodology adapted from Guichard, Lemesle, Ledauphin, Barillier, & Picoche (2003) and Lukić et al. (2010) [16,17]. For the liquid-liquid extraction, 100 mL of distillate (adjusted to 40% v/v of alcohol), 200 mL of water, 30 g of sodium chloride and 10 mL of dichloromethane were added in a 500 mL separating funnel. As internal standard, 100 µL of 2-octanol (3900 mg/L) solution was used, and the extraction was carried out for 1 h in an orbital shaker at 110 rpm. Dichloromethane extracts were concentrated with a 20 cm Dufton column in a bain-marie at a constant temperature of 50 ºC, until the extracted became 0.5 mL. Extractions were done in duplicate.

2.3.5. Chromatographic analysis

Chromatographic analysis was carried out by using a gas chromatograph equipped with a flame ionization detector (GC-FID) (Agilent 6890) and an automatic sampler (Agilent 7683). The capillary chromatographic column was a polar column MetaWAX (60 of length, 0.25 mm ID and 0.5 µm of phase thickness) from Teknokroma (Barcelona, Spain). The temperatures of the injector and detector were 250 ºC and 260 ºC, respectively. Separations were performed using two different methods. Quantification was performed by interpolation into calibrations built with synthetic solutions doped with all the analytes at different levels. For liquid-liquid extractions, calibration curves were built by the extraction of synthetic solutions doped with the volatile compounds. Concentration ranges of the calibration solutions were selected according to typical levels in commercial spirits [18].

2.3.5.1. Chromatographic method for major and most volatile compounds

For the analysis of acetaldehyde, methyl acetate, acetal, ethyl acetate, methanol, ethyl butyrate, 1-propanol, 2-methyl-1-propanol, 1-butanol, ethyl hexanoate, 2-methyl-1-butanol, 3-methyl-1-butanol, 1-hexanol, ethyl lactate and ethyl octanoate, the injection (2 µL) was done in split mode (1:5) and was performed with an oven temperature program of: 40 ºC (5 min), 7 ºC/min up to 100 ºC (15 min), 3 ºC/min up to 140 ºC and 2 ºC/min up to 200 ºC (5 min). The carrier gas was helium with a column-head flow ramp of 0.5 mL/min (28 min) and 5 mL/min^2 up to 1.1 mL/min (67 min).

2.3.5.2. Chromatographic method for heavier and minor volatile compounds

For the analysis of 2-butanol, isoamyl acetate, limonene, acetoin, furfural, acetic acid, linalool, ethyl decanoate, α-terpineol, β-citronellol, nerol, geraniol and β-phenylethanol, the injection (2 µL) was done in splitless mode, with an oven temperature ramp of 40 ºC (7 min), 2 ºC/min up to 140 ºC and 6 ºC/min up to 220 ºC (20 min). The carrier gas was helium at a constant column-head flow of 1 mL/min.

2.3.6. Muscat spirits sensory analysis

Spirit aroma analysis was performed in a panel room with 18 experienced assessors. The assessors had previously attended a 30 min training session to set the tasting descriptors. Distilled Muscat heart cuts (column and alembic) were analyzed by the following orthonasal and retronasal attributes: floral, fruit, sweet, burn/smoke and pungent/solvent notes. In order to scale the tasting descriptors, the training session was done with a neutral Muscat distillate as base spirit (with low terpenic compounds concentrations, e.g. linalool less than 0.25 g/hL a.a.) by spiking each attribute in two different levels according to the concentration range of the spirit samples: food grade quality terpenic compounds, for floral notes; food grade quality isoamyl acetate (Sigma-Aldrich, Saint Louis) and commercial apple juice, for fruit notes; 1% of column tail fraction, for sweet notes; 3% of column tail fraction, for burn/smoke notes; and 3% of column head fraction, for pungent/solvent notes. Sensory analysis was performed with a 4 point-scale (0=not detected; 1=weakly detected-hardy recognizable; 2=clear-but not intense; 3=intense), processed by using the modified frequency expressed as MF(%)=[F(%)·I(%)]1/2, where I(%) is the average intensity expressed as percentage of the maximum intensity and F(%) is the detection frequency of an attribute in percentage [19].

2.3.7. Statistical analysis

For comparison of column distillation strategies and alembic distillation, ANOVA was applied to data and compared by Fisher's least significant difference (LSD) test at $p < 0.05$. For the sensory preference test, a Sign test between samples was done. Both statistical analyses were performed with the STATISTICA 7.0 statistical package. Principal component analysis (PCA) and ANOVA at $p < 0.1$ were applied for the 4 point-scale sensory analysis, using the Product Characterization tool of XLSTAT-sensory 2016 statistical ad-in for Microsoft Office.

2.4. Results and discussion

2.4.1. Distillation kinetics and fractions of Macabeo distillations

Table 2.2 shows, for both distillation strategies and for each fraction, the alcoholic content, the temperature under the partial condenser, the exchanged heat by the partial condenser (measured by the increment of outlet and inlet cooling flow temperatures) and the total contents of head compounds (acetaldehyde, methyl acetate, acetal, ethyl acetate and methanol). In an ideal system, the heat exchanged by the partial condenser will be directly related to the dew point of the gas-liquid mixture. For high cooling flow rates, the mixture above the condenser should be enriched in ethanol, the major light volatile compound. Given that batch distillation is a discontinuous process, the depletion of ethanol and other compounds (in the boiler) cause that at different distillation times the same cooling flow rate produces fractions with different compositions. In addition, as can be seen in **Table 2.2**, the temperature reading below the condenser was lower than expected, according to the equilibrium of the water/ethanol ratio of the mixture. This difference may be caused by the down-flowing condensed liquid in contact with the temperature sensor.

The total content of head compounds in each fraction (**Table 2.2**) helped to define the head/heart cut, avoiding high levels of head compounds in the heart fraction. Thus, the sum of the first three fractions (F1 - F3) was assigned to the head fraction (Head) and the next nine fractions (F4 - F12) to the heart fraction (Heart) (usual fraction used to elaborate spirits). Moreover, two heart fractions were separated (Heart-1: F4 - F7; and Heart-2: F8 - F12). This cut was established according to the levels of terpenic compounds throughout the distillation, between distillation fractions whose contents decreased to very low values in both strategies (F7 - F8), as can be seen in **Figure 2.1** (behavior discussed in the following sections). According to preliminary studies, the 13th fraction (F13) had an alcoholic content lower than 40% (v/v) and it was considered as a distillation tail.

2.4.2. Aroma compounds of doped Macabeo distillations

Table 2.3 shows the average concentrations of each volatile compound analyzed (terpenes, alcohols, esters, and head and tail compounds) on Head and Heart (Heart-1 +

Heart-2) described in the previous section. The behavior of compounds during distillation and their concentrations in the different fractions depended on the physical-chemical characteristics of the compounds such as boiling point, volatility and solubility. Thus, more volatile compounds (such as acetaldehyde, methyl acetate, acetal, ethyl acetate) were mainly distilled in the first fractions. However, some compounds with high boiling points were distilled by steam stripping effect due to their high solubility in ethanol (such as higher alcohols and terpenic compounds), especially in heart fractions.

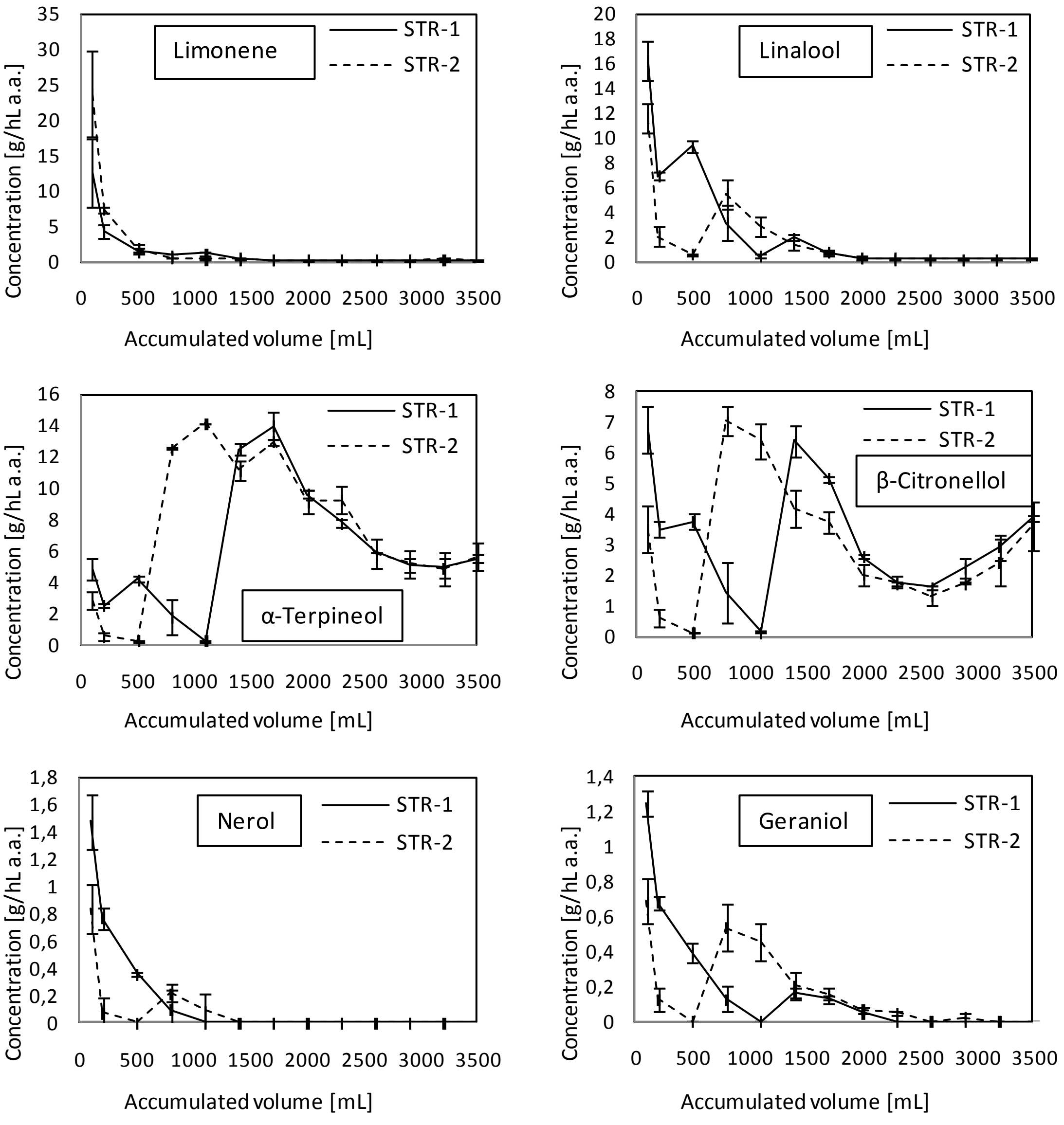

Figure 2.1. Limonene, linalool, α-terpineol, β-citronellol, nerol and geraniol average concentrations throughout the column distillation process performed with doped Macabeo wine. Standard deviation was calculated with two replicates.

On the other hand, compounds with high molecular weight and/or that are highly soluble in water tended to distil in the tail fraction (such as β-phenylethanol and ethyl lactate), where water percentage and temperature were higher. These behaviors can be more clearly observed in **Table 2.4**, which shows the distilled mass percentage in each fraction (Head and Heart (Heart-1 + Heart-2)) with respect to the total distilled for each compound and group of compounds.

2.4.2.1. Terpenic compounds of Macabeo distillation

Regarding the behavior of terpenic compounds, **Figure 2.1** shows the levels throughout the distillation of limonene, linalool, α-terpineol, β-citronellol, nerol and geraniol.

Limonene is the terpene that presents the highest vapor pressure and lowest water solubility values among the terpenic compounds studied (**Table 2.1**); therefore, it tended to distil at the beginning of the distillation. In addition, the high reactivity of this compound in acid medium and at high temperatures can transform or degrade limonene to other terpenic compounds during distillation [8]. Therefore, no tested strategy was able to concentrate this compound in the heart fractions.

Linalool is a monoterpene with an alcohol group and it has the second highest vapor pressure (**Table 2.1**), therefore, it tended to distil in the first fractions. **Figure 2.1** shows how a higher partial cooling flow rate at the beginning of the distillation (STR-2) can reduce the linalool extraction in the head fractions (fractions not used for spirits production). Later, linalool concentration in the heart fractions can be significantly increased with a drastic reduction of the cooling flow rate.

α-Terpineol, β-citronellol, geraniol and nerol present similar boiling points and vapor pressures (**Table 2.1**). Unlike the previous compounds, these compounds were mainly distilled in heart fractions for both strategies (**Figure 2.1**). STR-2 achieved lower concentrations in the first fractions (with a higher reflux rate) and higher concentrations in the following fractions (with a drastic reflux rate reduction). However, geraniol and nerol showed much lower levels and after the middle of the process, they were no longer quantifiable.

Table 2.3. Average concentrations (g/hL a.a.) of volatile compounds of both strategies (STR-1 and STR-2 of Macabeo distillations) in fractions (Head, Heart, Heart-1, Heart-2)[a]

Compound	Head		Heart		Heart-1		Heart-2	
	STR-1	STR-2	STR-1	STR-2	STR-1	STR-2	STR-1	STR-2
Terpenic compounds								
Limonene	4.31±1.37	7.23±0.71	0.630±0.147	0.416±0.012	0.885±0.188	0.482±0.001	0.355±0.102	0.359±0.021
Linalool	10.3±0.0 b	3.02±0.42 a	1.01±0.25	1.44±0.27	1.68±0.49	2.75±0.63	0.288±0.001 a	0.315±0.008 b
α-Terpineol	3.99±0.00 b	0.836±0.194 a	6.79±0.01 a	9.56±0.04 b	6.66±0.19 a	12.6±0.2 b	6.93±0.18	6.91±0.02
β-Citronellol	4.30±0.05 b	0.856±0.21 a	2.67±0.15 a	3.47±0.12 b	3.13±0.38 a	5.37±0.49 b	2.17±0.09	1.84±0.14
Nerol	0.658±0.031 b	0.174±0.056 a	0.014±0.019	0.036±0.021	0.026±0.037	0.078±0.046	d.-n.q.	n.d.
Geraniol	0.616±0.045 b	0.153±0.037 a	0.060±0.015	0.170±0.044	0.105±0.029	0.339±0.088	0.011±0.001	0.025±0.011
Esters								
Ethyl butyrate	1.14±0.00 a	1.26±0.02 b	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Isoamyl acetate	4.25±0.68	5.69±0.08	d.-n.q.	d.-n.q.	d.-n.q.	d.-n.q.	n.d.	n.d.
Ethyl hexanoate	3.30±0.04	2.45±1.19	d.-n.q.	d.-n.q.	d.-n.q.	d.-n.q.	n.d.	n.d.
Ethyl octanoate	2.77±0.20	2.69±0.32	d.-n.q.	0.097±0.031	d.-n.q.	0.209±0.062	d.-n.q.	d.-n.q.
Ethyl decanoate	1.04±0.10	0.897±0.140	0.056±0.038	0.109±0.037	0.027±0.038	0.133±0.029	0.087±0.039	0.089±0.043
Higher alcohols								
2-Butanol	d.-n.q.	0.044±0.063	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1-Propanol	17.2±0.4 b	12.4±1.0 a	14.3±0.0 a	16.2±0.5 b	15.5±0.2 a	18.4±0.5 b	12.9±0.2	14.3±0.5
2-Methyl-1-propanol	35.9±1.1 b	24.8±1.2 a	14.0±0.1 a	16.7±0.1 b	20.9±0.3 a	26.6±0.5 b	6.70±0.03	8.13±0.52
1-Butanol	0.355±0.013	0.427±0.485	0.132±0.038	0.200±0.008	0.255±0.075	0.432±0.008	d.-n.q.	d.-n.q.
2-Methyl-1-butanol	49.3±1.2 b	21.8±0.7 a	20.3±0.2 a	25.3±0.5 b	30.2±0.1 a	43.5±1.2 b	9.63±0.42	9.73±0.76
3-Methyl-1-butanol	234±5 b	96.4±1.2 a	121±1 a	152±2 b	167±1 a	247±7 b	71.4±3.6	71.5±4.7
1-Hexanol	1.10±0.04 b	0.225±0.03 a	0.508±0.014 a	0.672±0.028 b	0.759±0.022 a	1.23±0.05 b	0.239±0.005 b	0.188±0.013 a
Head compounds								
Acetaldehyde	188±30	278±80	17.9±4.9	5.74±0.12	34.1±9.2	10.9±0.7	0.491±0.46	1.32±0.22
Methyl acetate	0.739±0.099	1.03±0.59	0.365±0.516	0.577±0.272	0.344±0.486	0.123±0.174	0.388±0.548	0.969±0.371
Acetal	27.6±8.4	40.3±18	2.50±1.07	0.785±0.004	4.83±2.09	1.70±0.03	d.-n.q.	d.-n.q.
Ethyl acetate	146±1	167±9	11.0±0.2 b	8.16±0.41 a	14.5±0.1 b	7.94±0.11 a	7.30±0.40	8.34±0.68
Methanol	29.9±1.8	38.9±3.2	32.2±1.7	35.2±2.7	29.1±0.1 b	27.2±0.0 a	35.5±3.6	42.1±5.3
Tail compounds								
Ethyl lactate	d.-n.q.	0.061±0.086	2.70±0.29	2.84±0.57	0.786±0.091 a	1.81±0.29 b	4.75±0.5	3.73±0.85
Furfural	n.d.	d.-n.q.	0.848±0.041	0.877±0.312	0.146±0.059	0.151±0.107	1.61±0.15	1.50±0.51
β-Phenylethanol	0.300±0.031	0.204±0.099	2.73±0.10	2.41±0.29	0.681±0.013 a	1.37±0.02 b	4.93±0.21	3.31±0.60
Other compounds								
Acetic acid	10.5±3.2	4.21±0.81	3.57±0.26	4.10±0.43	1.57±0.01 a	3.12±0.00 b	5.72±0.55	4.94±0.84

[a] Different letters after standard deviation in the same row indicate a significant difference ($p<0.05$) with respect to both strategies (STR-1 and STR-2) at the same fraction. Means and standard deviations were calculated with two replicates.

Table 2.4. Distilled mass percentages (%) of volatile compounds of both strategies (STR-1 and STR-2 of Macabeo distillations) in fractions (Head, Heart, Heart-1, Heart-2)[a]

Compound[b]	Head		Heart		Heart-1		Heart-2	
	STR-1	STR-2	STR-1	STR-2	STR-1	STR-2	STR-1	STR-2
Limonene	60.1±1.9 a	79.9±1.8 b	39.9±1.9 b	20.1±1.8 a	29.1±1.9 b	10.8±0.9 a	10.7±0.0	9.3±1.0
Linalool	69.6±5.1 b	32.6±1.4 a	30.4±5.1 a	67.4±1.4 b	26.2±5.5 a	59.3±2.4 b	4.2±0.4 a	8.0±1.0 b
α-Terpineol	11.6±0.1 b	2.0±0.4 a	88.4±0.1 a	98.0±0.4 b	45.0±1.0 a	59.9±0.9 b	43.4±1.1 b	38.1±0.4 a
β-Citronellol	26.5±0.7 b	5.3±1.0 a	73.5±0.7 a	94.7±1.0 b	44.7±3.2 a	67.6±1.7 b	28.8±2.4	27.1±2.7
Nerol	92.1±11.2	54.1±7.4	7.9±11.2	45.9±7.4	7.9±11.2	45.9±7.4	d.-n.q.	n.d.
Geraniol	69.8±6.7 b	17.2±0.4 a	30.2±6.7 a	82.8±0.4 b	27.4±6.6 a	76.3±0.9 b	2.8±0.1	6.5±1.3
ΣTerpenic compounds	**32.5±1.7 b**	**15.7±1.2 a**	**67.5±1.7 a**	**84.3±1.2 b**	**39.1±2.7 a**	**55.9±0.0 b**	**28.4±1.0**	**28.4±1.2**
Ethyl butyrate	100	100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Isoamyl acetate	100	100	d.-n.q.	d.-n.q.	d.-n.q.	d.-n.q.	n.d.	n.d.
Ethyl hexanoate	100	100	d.-n.q.	d.-n.q.	d.-n.q.	d.-n.q.	n.d.	n.d.
Ethyl octanoate	100 b	86.6±2.2 a	d.-n.q.	13.4±2.2	d.-n.q.	13.4±2.2	d.-n.q.	d.-n.q.
Ethyl decanoate	81.6±9.5	65.8±4.0	18.4±9.5	34.2±4.0	4.2±6.0	19.4±0.2	14.1±3.5	14.8±3.8
Σ Esters	**98.0±1.4**	**93.6±1.2**	**2.0±1.4**	**6.4±1.2**	**0.5±0.7**	**4.9±0.7**	**1.5±0.7**	**1.5±0.5**
2-Butanol	d.-n.q.	100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1-Propanol	21.2±0.3 b	15.0±0.7 a	78.8±0.3 a	85.0±0.7 b	44.3±0.6	44.7±0.2	34.4±0.3 a	40.4±1.0 b
2-Methyl-1-propanol	36.3±0.7 b	25.4±1.1 a	63.7±0.7 a	74.6±1.1 b	49.1±0.7 a	55.0±0.9 b	14.6±0.0	19.6±2.0
1-Butanol	38.1±7.6	28.1±27.4	61.9±7.6	71.9±27.4	61.9±7.6	71.9±27.4	d.-n.q.	d.-n.q.
2-Methyl-1-butanol	35.2±0.2 b	16.5±0.3 a	64.8±0.2 a	83.5±0.3 b	50.0±0.6 a	66.2±1.7 b	14.8±0.4	17.3±2.0
3-Methyl-1-butanol	30.1±0.1 b	12.7±0.1 a	69.9±0.1 a	87.3±0.1 b	50.0±0.8 a	65.3±2.1 b	19.8±0.7	22.1±2.2
1-Hexanol	32.5±0.1 b	7.1±1.1 a	67.5±0.1 a	92.9±1.1 b	52.2±0.3 a	78.8±2.7 b	15.3±0.2	14.0±1.6
Σ Higher alcohols	**30.7±0.2 b**	**14.5±0.2 a**	**69.3±0.2 a**	**85.5±0.2 b**	**49.6±0.8 a**	**62.9±1.8 b**	**19.8±0.6**	**22.6±2.0**
Acetaldehyde	70.3±2.3 a	91.4±2.5 b	29.7±2.3 b	8.6±2.5 a	29.3±2.0 b	7.5±2.4 a	0.4±0.3	1.0±0.1
Methyl acetate	58.5±58.8	28.4±2.7	41.5±58.8	71.6±2.7	20.4±28.8	5.2±7.4	21.2±30.0	66.3±10.0
Acetal	71.6±2.6 a	91.4±3.6 b	28.4±2.6 b	8.6±3.6 a	28.4±2.6 b	8.6±3.6 a	d.-n.q.	d.-n.q.
Ethyl acetate	74.7±0.5 a	82.4±0.2 b	25.3±0.5 b	17.6±0.2 a	17.2±0.1 b	7.9±0.2 a	8.0±0.4 a	9.7±0.0 b
Methanol	17.2±0.0 a	20.2±0.3 b	82.8±0.0 b	79.8±0.3 a	38.9±2.0 b	28.6±1.7 a	43.9±2.0	51.2±1.4
Σ Head compounds	**57.8±1.1 a**	**70.2±3.3 b**	**42.2±1.1 b**	**29.8±3.3 a**	**28.3±1.3 b**	**13.1±2.2 a**	**14.0±2.4**	**16.7±1.2**
Ethyl lactate	d.-n.q.	0.4±0.6	100	99.6±0.6	15.1±0.1 a	29.5±0.8 b	84.9±0.1 b	70.1±0.2 a
Furfural	n.d.	d.-n.q.	100	100	9.0±4.0	7.5±3.1	91.0±4.0	92.5±3.1
β-Phenylethanol	2.4±0.2	1.9±0.7	97.6±0.2	98.1±0.7	12.6±0.8 a	26±3.2 b	85.0±0.6 b	72.2±2.5 a
Σ Tail compounds	**1.1±0.1**	**0.9±0.5**	**98.9±0.1**	**99.1±0.5**	**13.2±0.3 a**	**25.1±1.6 b**	**85.8±0.4 b**	**74.0±1.1 a**
Acetic acid	39.1±5.6 b	19.0±1.5 a	60.9±5.6 a	81.0±1.5 b	13.9±2.2 a	28.7±3.0 b	46.9±3.4	52.3±1.5

[a] Different letters after standard deviation in the same row indicate a significant difference ($p<0.05$) with respect to both strategies (STR-1 and STR-2) at the same fraction. Means and standard deviations were calculated with two replicates. [b] Compounds sums collect the compounds located above each sum row until next bold type text.

Iwai et al. (2014) and Ohta et al. (1991) [6,8] showed that limonene, geraniol and nerol are precursors of α-terpineol and other minor terpenic compounds in hydrothermal and catalyzed acid media. This would explain the low concentrations of limonene, geraniol and nerol; and the slow reduction of α-terpineol levels throughout the distillation. These tendencies are confirmed in **Table 2.3**. Except for limonene, the concentrations of terpenic compounds were significantly lower in STR-2 Head than in the STR-1. In STR-2, Heart and Heart-1 had significantly higher levels of α-terpineol and β-citronellol, in relation to STR-1. In addition, linalool levels in Heart-2 of STR-2 were also higher than in Heart-2 of STR-1. Besides, **Table 2.4** shows the extraction mass yields of these terpenic compounds and their sum. Most of the terpenic compounds were distilled in the Heart (67.5% in STR-1 and 84.3% in STR-2) and especially in Heart-1 (39.1% in STR-1 and 55.9% in STR-2). However, nerol and geraniol were concentrated in Heads due to their subsequent degradation during distillation. In turn, linalool showed significant differences between both strategies, presenting higher extraction mass yields in STR-2 Heart, Heart-1 and Heart-2. In summary, a drastic cooling flow rate reduction after high reflux levels favors the recovery of terpenic compounds in the heart fractions.

2.4.2.2. Other volatile compounds in Macabeo distillations

The chemical group of esters was responsible for the fruity aroma notes [18]. As can be seen in **Table 2.3**, the esters were presented mainly in the Head and its contents in Heart were very low for most of them. These compounds have a limited solubility in water and their hydrophobicity constants (log $K_{o/w}$) ranged between 1.85 (ethyl butyrate) and 4.79 (ethyl decanoate), so these were distilled in the first moments of the process by steam stripping. This behavior is consistent with that observed by Jouret et al. (1998) [10] and Peña y Lillo, Latrille, et al. (2005) [20]. The ester with the highest boiling point, ethyl decanoate, was the only detected in all heart fractions. In addition, ethyl octanoate was detected in the Heart of the distillation STR-2, although the amount detected was very low. In the case of ethyl decanoate, the distilled mass percentage (**Table 2.4**) with STR-2 was around 16% more than with STR-1.

The group of higher alcohols is characterized by an alcoholic and malty odor [21]. Their hydrophobicity constants (log $K_{o/w}$) ranged between 0.25 and 2.03 and their boiling points between 97.2 and 157.6 ºC (values for 1-propanol and 1-hexanol, respectively).

Consequently, the levels of higher alcohols were higher in the Head (due to their low boiling points) and in Heart-1 (due to their water solubility) (**Table 2.3**). Low levels in Heart-2 of all compounds could be due to their depletion in the boiler. Like terpenic compounds and esters, STR-2 produced lower higher alcohol levels in Head and higher levels in Heart and Heart-1 than STR-1, due to the cooling flow differences. **Table 2.4** illustrates that 62.9% of the higher alcohols were concentrated in STR-2 Heart-1 compared to 49.6 % in STR-1 Heart-1.

The most undesirable and toxic compounds in distillates have low boiling points and tend to concentrate in the first fractions. As shown in **Table 2.3**, significant differences between strategies were only observed for ethyl acetate (glue-solvent aroma) in Heart and Heart-1, and for methanol (the most relevant toxic compound) in Heart-1; both cases showed lower levels in STR-2 than in STR-1. Methanol is the only head compound which maintained a similar concentration through the distillation. Its contents were much lower than the legal limit of 200 g/hL a.a. for wine spirits [22]. In addition, tail fraction (F13) had higher relative methanol levels in all experiments due to ethanol depletion (data not shown), a behavior experimentally observed and simulated by Carvallo, Labbe, Pérez-Correa, Zaror, and Wisniak (2011) [23]. Acetaldehyde and ethyl acetate had high concentrations in the initial wine and both were concentrated in the Head, so the total distilled mass percentage of head compounds (**Table 2.4**) mostly refers to them. As can be seen, except for methyl acetate and methanol, head compounds were mostly extracted in the Head. Moreover, except in the case of methyl acetate, we have found significant differences between strategies in Head, Heart and Heart-1 in most head compounds and their sum, where STR-2 presented the best behavior with a lower extraction in Heart and Heart-1.

The tail compounds group has high boiling points and high water solubility, and they can be generated during the process. Therefore, these compounds were distilled at the end of the distillation (**Table 2.3**). STR-2 obtained higher concentration of ethyl lactate (possible formation from lactic acid) and β-phenylethanol (rose aroma) in Heart-1 due to the lower cooling flow rates. In addition, there was no furfural in the Head, since this compound is generated during distillation by Maillard reactions [24]. Distilled mass percentages (**Table 2.4**) were the same in both strategies, where most of tail compounds were extracted at the end of the Heart (85.8 and 74.0 % in Heart-2 for STR-1 and STR-2, respectively).

Finally, STR-2 showed higher distilled mass percentages (Heart and Heart-1) and concentration (Heart-1) values for acetic acid than STR-1. Acetic acid has a vinegar-like, pungent aroma and is a precursor of ethyl acetate (glue-solvent aroma). Although, its concentration should not be a problem with an undamaged initial wine.

2.4.3. Comparison of column and alembic Muscat distillations

According to the results obtained with the doped Macabeo wine, STR-2 was chosen as the most suitable distillation strategy for terpenic wines. Therefore, STR-2 and alembic were compared in non-doped Muscat wine distillations.

2.4.3.1. Chemical analysis of Muscat distillations

Table 2.5 shows alcohol content, levels of most relevant compounds and calculated odor activity values of Muscat distillation fractions: column Heart-1 and Heart-2, and Alembic Heart. Odor activity values (OAV) were calculated using the odor thresholds found in bibliography.

The compounds behavior of the column distillation was not affected by the change of the raw material. Otherwise, alembic distillations had much lower internal refluxes than column distillation process, since their rectification (with a constant power heat in the boiler) only depends on the environmental temperature and on the alembic-head design. Thus, lower contents and a uniform distribution of ethanol during alembic distillation avoid the fluctuations of other volatile compounds.

Alembic distillations had much lower head compounds content in the first fractions (data not shown) due to its low rectification. However, head compound levels decreased steadily throughout alembic distillation and this behavior increased acetaldehyde, acetal and ethyl acetate levels in the Alembic Heart (**Table 2.5**). The content of terpenic compounds and isoamyl acetate was higher in column Heart-1, since a low amount of these compounds was extracted in the first fractions (compared with alembic). In addition, total column heart cut (Heart-1 + Heart-2) also had higher terpenic compounds levels. In both processes, linalool was the only terpenic compound above its aroma threshold and was much higher in Heart-1 (**Table 2.5**). Distillation times were 4.29

± 0.54 and 7.41 ± 0.42 h for alembic and column distillations, respectively, since initial wine volume and reflux differences are remarkable. Thus, high distillation time increased furfural concentration in Heart-2 by Maillard reactions [24]. Ethyl ester compounds tended to distil with high refluxes (column first fractions), as had been observed with Macabeo wine experiments. However, higher ethyl ester levels were found in alembic heart cut, due to their low refluxes and distillation time. In addition, compounds levels were in line with the range observed in commercial *Piscos*, however, linalool and α-terpineol levels in column Heart-1 were around their maximum published values [3].

Table 2.5. Aroma threshold, concentration and odor activity value of the most relevant aroma compounds with respect to column Heart-1 and Heart-2 and Alembic Heart done with Muscat wine[a]

Compound	Aroma[b] threshold	Concentration (mg/L alc. 40% v/v) and odor activity values[c] (u.a.)		
		Heart-1	Heart-2	Alembic Heart
Linalool	1.00[d]	8.81 ±1.52 c (8.81)	2.74 ±0.30 a (2.74)	5.58 ±0.46 b (5.58)
α-Terpineol	300[d]	8.36 ±0.78 b (0.028)	7.80 ±0.59 b (0.026)	5.92 ±0.03 a (0.020)
β-Citronellol	1.00[d]	0.335 ±0.045 b (0.335)	0.195 ±0.039 a (0.195)	0.260 ±0.017 a (0.260)
Nerol	40.0[d]	0.387 ±0.055 b (0.010)	0.193 ±0.038 a (0.005)	0.356 ±0.017 b (0.009)
Geraniol	3.00[d]	1.06 ±0.15 b (0.352)	0.550 ±0.114 a (0.183)	1.15 ±0.07 b (0.384)
Acetaldehyde	19.2[e]	103 ±15 b (5.36)	22.5 ±4.7 a (1.17)	413 ±34 c (21.5)
Acetal	0.719[e]	26.0 ±14.5 a (36.2)	4.40 ±1.86 b (6.12)	40.1 ±4.5 b (55.7)
Ethyl acetate	50.0[f]	16.5 ±0.8 a (0.330)	11.6 ±2.1 a (0.232)	124 ±5 b (2.47)
Isoamyl acetate	0.245[e]	0.693 ±0.114 c (2.83)	0.025 ±0.004 a (0.100)	0.282 ±0.035 b (1.15)
Ethyl butyrate	0.0095[e]	n.q.	n.q.	n.q.
Ethyl hexanoate	0.030[e]	d. - n.q.	n.q.	4.90 ±0.36 (163)
Ethyl octanoate	0.147[g]	2.80 ±0.61 a (19.0)	d. - n.q.	10.9 ±0.8 b (74.5)
Ethyl decanoate	0.420[h]	2.15 ±1.21 a (5.13)	d. - n.q.	4.57 ±0.07 b (10.9)
Ethyl lactate	100[i]	n.q.	73.8 ±9.9 (0.738)	55.9 ±29.9 (0.559)
Furfural	20.4[f]	2.63 ±0.18 a (0.129)	8.27 ±0.72 b (0.406)	3.55 ±0.69 a (0.174)
β-Phenylethanol	2.60[e]	2.04 ±0.10 a (0.783)	3.69 ±0.40 a (1.42)	11.6 ±1.7 b (4.46)

[a]Different letters after standard deviation in the same row indicate a significant difference ($p<0.05$) in respect to heart fractions. Means and standard deviations were calculated with tree replicates. [b]Aroma thresholds are expressed in [mg/L] in wine spirit. [c]Odor activity values are shown in brackets expressed in units of aroma (u.a.). [d]Referenced in [29]. [e]Referenced in [30]. [f]Referenced in [31]. [g]Referenced in [32]. [h]Referenced in [33]. [i]Referenced in [18].

Moreover, terpenic compounds can be produced by bound monoterpenes (non-volatiles) present in wine during distillation, as boiler conditions favor acid hydrolysis of glycosides [25]. Therefore, mass balance deviation (data not shown) helps to estimate formed or degraded amounts of each compound, calculated for each compound as the percentage of the difference between the mass sum of the free compound in all the outputs (Head + Heart + Residue) and the mass of the free compound in the initial wine. Muscat wine is known for its high contents of bound and free terpenic compounds [26], not like Macabeo wine which had not detectable free terpenic compounds until it was doped. Therefore, Muscat should have a higher mass balance deviation of terpenes, since bound compounds were released. However, Muscat distillation STR-2 had a mass balance deviation of linalool and α-terpineol significantly lower than Macabeo STR-2, probably because of the wine concentration differences. Otherwise, Muscat residues had much more linalool (1.5 times) and α-terpineol (9.3 times) than Macabeo ones, which suggest a release of free terpenic compounds from the remaining precursors after distillation.

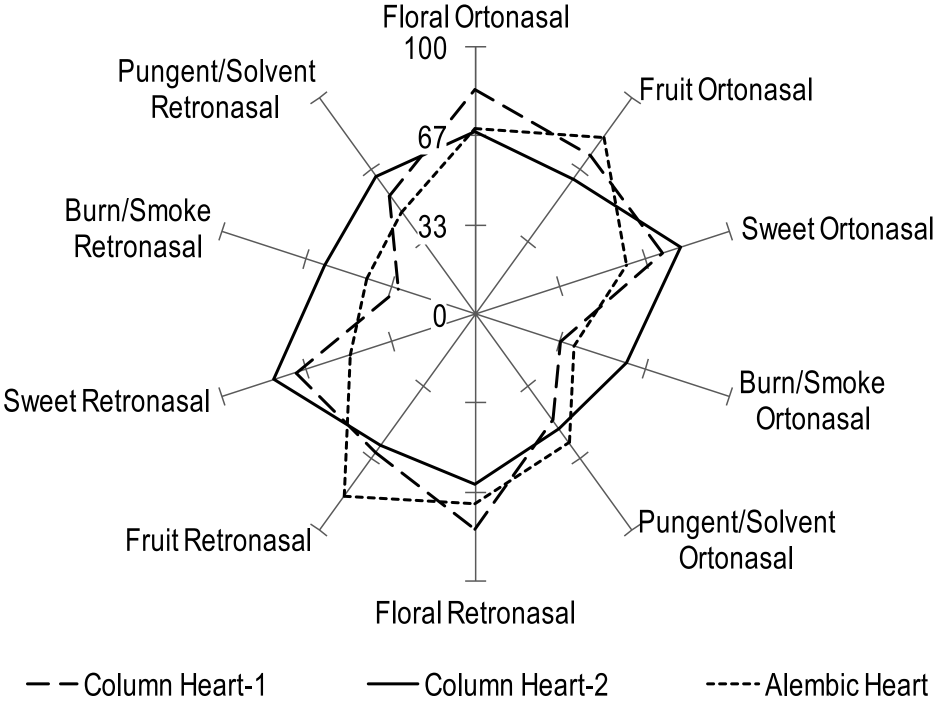

Figure 2.2. Graph of mean sensory modified frequency MF (%) ratings of studied Muscat wine spirits, obtained by a sensory descriptive analysis performed with 18 experienced assessors.

2.4.3.2. Sensory analysis of Muscat distillations

Modified frequency MF (%) ratings (**Figure 2.2**) showed higher ratings of floral notes in Heart-1 (terpenic compounds), of fruit notes in Alembic Heart (ester compounds), and of sweet and burn/smoke notes in Heart-2 (tail compounds). These results were consistent with the aroma descriptors associated to the chemical composition data. However, assessors found higher levels of retronasal pungent/solvent notes in Heart-1 and Heart-2, and higher sweet notes in Heart-1, than in Alembic Heart. Due to its lower degree of rectification throughout the process, Alembic Heart had a more complex and uniform aroma composition and some synergistic or antagonistic aroma interaction may have occurred [5,20,27].

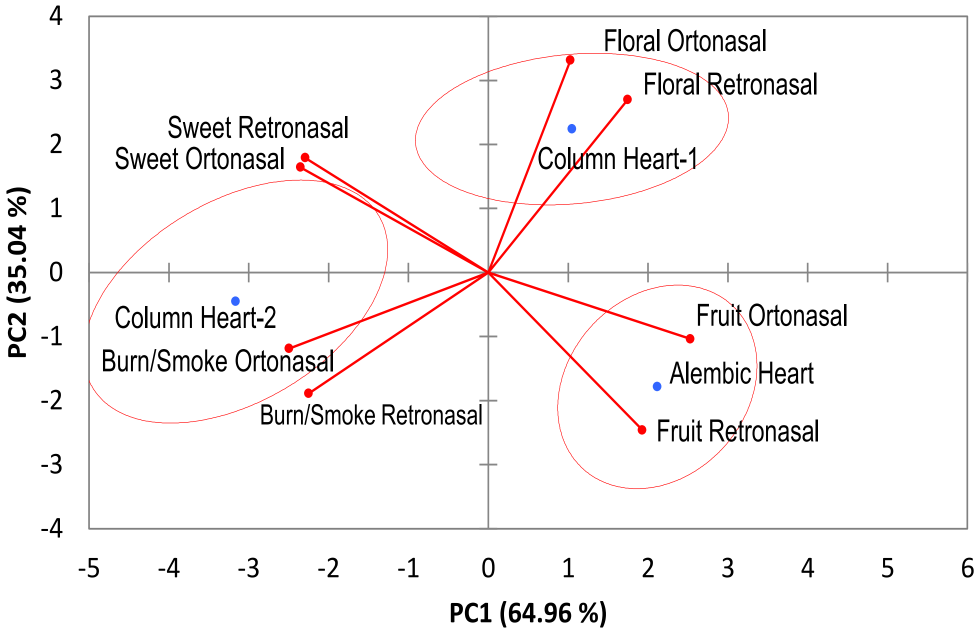

Figure 2.3. Biplot with 95% confidence ellipses for the sensory profiles obtained by PCA of the studied Muscat wine spirits with 18 experienced assessors.

In addition, ANOVA differenced the 3 samples as follows: Alembic Heart had significant high fruit notes and low sweet notes values; Heart-1 had significant high floral notes and low burn/smoke notes values; and Heart-2 had significant high sweet and burn/smoke notes and low fruit notes values. In order to emphasize the sensory differentiation between samplers, the tasting panel results were also analyzed by PCA product characterization (**Figure 2.3**) according to ANOVA analysis. 2 principal components (PC1 and PC2) covered the 100% of the variance. Pungent/solvent descriptor was not considered as had not significant differences. PC1 axis places fruit and floral notes descriptors against sweet and burn/smoke descriptors. Thus PC1 shows good separation between heart and tail compounds and indicates the quality of the tail-cut. Moreover, PC2 places the floral and sweet notes descriptors in the same quadrant. This behavior coincides with the MF ratings. Linalool's sweet-like aroma could be easily confused with the sweet notes descriptor [21]. Besides descriptors, the 3 analyzed spirits were clearly differentiated in the PCA biplot. Heart-1 presented high intensities of floral notes, Heart-2 presented high intensities of burn/smoke notes and Alembic Heart presented high intensities of fruit notes, confirming the explained MF ratings and chemical analyses. Sweet notes vector was placed between Heart-1 and Heart-2, according to the aroma confusion between linalool and tails sweet-like perception.

Finally, there were no significant differences in the sensory preference test (data not shown).

2.5. Conclusions

High internal refluxes of the distillation column at the first distillate fractions allowed a lower extraction of terpenic compounds in the head fraction. In addition, a drastic reduction of the internal reflux during distillation of the heart enhanced the recovery of terpenic compounds, producing a distillate rich in floral aromas. Furthermore, a drastic cooling flow reduction increased the presence of higher alcohols and esters, and decreased the head compounds in the heart fractions. These behaviors observed for negative and positive aroma compounds allowed to obtain a heart sub-fraction with high quality aroma characteristics and better characteristics than the classical alembic product. This study could help the industry to introduce new premium products with differentiated characteristics in the market.

Acknowledgments

J. J. R.-B. appreciates the fellowship from the program "Beatriu de Pinós" (2011 BP_B00141) with the support of the Secretariat d'Universitats I Recerca del Departament d'Economia i Coneixement de la Generalitat de Catalunya and the European Union. P. M.-G. appreciates the Ph.D. Grant "Martí Franquès" (2015PMF-PIPF-29) with the support of the Universitat Rovira i Virgili. Authors acknowledge Melis Akyildiz and

Selin Çankaya the collaboration on laboratory work during Muscat distillations in their Erasmus+ stage in our department.

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Chapter 3

AROMA PROFILE DESIGN OF WINE SPIRITS: MULTI-OBJECTIVE OPTIMIZATION USING RESPONSE SURFACE METHODOLOGY

A study published in: Food Chemistry 2018, 245, pp. 1087-1097

3.1. Abstract

Developing new distillation strategies can help the spirits industry to improve quality, safety and process efficiency. Batch stills equipped with a packed column and an internal partial condenser are an innovative experimental system, allowing a fast and flexible management of the rectification. In this study, the impact of four factors (heart-cut volume, head-cut volume, pH and cooling flow rate of the internal partial condenser during the head-cut fraction) on 18 major volatile compounds of Muscat spirits was optimized using Response Surface Methodology and Desirability Function approaches. Results have shown that high rectification at the beginning of the heart-cut enhances the overall positive aroma compounds of the product, reducing off-flavors compounds. In contrast, optimum levels of heart-cut volume, head-cut volume and pH factors varied depending on the process goal. Finally, three optimal operational conditions (head off-flavors reduction, flowery terpenic enhance and fruity ester enhance) were evaluated by chemical and sensory analysis.

3.2. Introduction

One of the main challenges of the food and beverage industry is to obtain unique products in an increasingly competitive market. In the case of spirits, new operating strategies can aid to improve quality, food safety and efficiency of the distillation process. Batch distillation is the most used technique to produce spirits, where the distillate is collected in three consecutive fractions: head-cut (waste), heart-cut (product) and tail-cut (waste), to obtain a product with minimum off-flavors and toxic compounds. Traditional systems, like copper *Charentais* alembics (French style), produce drinks with high levels of volatile compounds that enhance their genuineness, an important feature of distinctive alcoholic beverages. However, alembics allow limited control of the distillation process to improve the product. On the other hand, modern continuous columns are generally used to obtain spirits with a neutral aroma, intended for flavoring or ageing. In the spirits industry, batch columns (German style) represent an intermediate technique, which provides enhanced control of the rectification by varying the reflux rate; however, these systems are slow to respond, severely limiting the process flexibility. Many studies have reported the differences of the available spirits distillation systems [1–5].

An innovative experimental system is the batch packed column equipped with an internal partial condenser that allows fast and flexible control of the internal reflux rate [6]. This system has been compared with a traditional alembic by distilling wine and other fermented agricultural raw materials (kiwi, pear and grape pomace) showing significant differences [7–9]. In particular, high refluxes at early fractions removed acetaldehyde, ethyl acetate and acetal from the heart-cut, which allows obtaining a cleaner wine spirit [10,11]. Although packed columns increase distillation times and are difficult to control [6], the variable internal cooling flow rate can quickly adapt the rectification level; hence, the producer can modify the volatile composition of the spirit throughout the process by specific operational strategies [10].

The distillation of fermented beverages causes the reaction of several aroma compounds such as terpenes [12–16], esters [17], furfural [18–20] and aldehydes [21], which undergo transformations in a hot acid media. It has been reported that the juice pH alters the microbiological behavior during the fermentation, affecting the aroma composition of pear and melon spirits [22,23]. However, there is no information regarding the influence of pH during the distillation. Furthermore, the distillation behavior of each compound changes throughout the process [24].

The aim of this study was to determine operational conditions for a batch distillation column with an internal partial condenser to obtain different specific organoleptic characteristics for a Muscat wine spirit. Heart-Cut Volume (HTV), Head-Cut Volume (HDV), pH (adjusted with sulfuric acid, a common technique used in marc storage [25]) and Cooling Flow Rate of the internal partial condenser during the head-cut fraction (CFR) were considered to be the main operational factors. To carry out the head-cut multi-objective optimization (18 compounds), Response Surface Methodology (RSM) was applied by performing a Central Composite Design with face centered axial points [26]. For the multi-objective optimization, the Desirability Function Approach [26] was used for the most relevant volatile compounds of Muscat spirits. In addition, optimal strategies were sensory analyzed.

3.3. Material and methods

3.3.1. Central composite design (CCD)

Design of experiments is widely used to unveil the impact of defined input process variables (factors) on output process variables (responses). The CCD is a type of design of experiments consisting of a 2-level full factorial design (FFD) with a center point and axial points [27]. The FFD is a design that studies the effect of all possible combinations of 2 or more factors at 2 levels. Center point is an experimental run whose factor values are the average of the two levels of the FFD factors; they are usually replicated to estimate and improve the variance of the system. Axial points are experimental runs with the same factor values as the center point, except for one factor whose value is at a given distance (α) from the center point. Factor values are usually rescaled (coded): FFD points = ±1, center point = 0, and axial points = $\pm\alpha$ (one factor) and 0 (the other factors). Thereby, CCD results allow a statistical estimation of linear and quadratic effects on a given response with a reduced number of experiments.

For this study, a 3-level-3-factor CCD with face centered axial points ($\alpha = 1$) and six replicates of the center point was designed (20 runs). Factors were Head-Cut Volume (HDV), pH and Cooling Flow Rate (CFR) of the internal partial condenser of the column. To minimize the number of runs, the factor HTV (Heart-Cut Volume) was not considered; however, HTV was considered with the RSM, since 2 heart cuts were obtained from each experiment. Runs were ordered with a randomized 2-block design to enhance the reliability and validity of the statistical analysis of the factor effects. Center point runs were randomized with a 2-block distribution (10 random runs with 3 center point replicates for each block). A blocking variable was included to reduce the impact of possible nuisance variables throughout the experimentation period (2 month) [27]. **Table 3.1** shows all the experiments in the standard and real order of runs with coded and experimental values of the factors.

Table 3.1. Central Composite Design 3-level-3-factor with face centered Axial Points (AP) and 6 Center Points (CP). Uncoded values are shown in brackets.

Run number	Run order	Head-Cut Volume (mL)	Cooling Flow Rate[a] (mL/min)	pH
1	2	-1 (5.00)	-1 (30.0)	-1 (1.70)
2	1	+1 (20.0)	+1 (100)	-1 (1.70)
3	5	+1 (20.0)	-1 (30.0)	+1 (3.20)
4	3	-1 (5.00)	+1 (100)	+1 (3.20)
5	12	+1 (20.0)	-1 (30.0)	-1 (1.70)
6	9	-1 (5.00)	+1 (100)	-1 (1.70)
7	10	-1 (5.00)	-1 (30.0)	+1 (3.20)
8	8	+1 (20.0)	+1 (100)	+1 (3.20)
9 (AP)	13	0 (12.5)	0 (65.0)	-1 (1.70)
10 (AP)	17	0 (12.5)	0 (65.0)	+1 (3.20)
11 (AP)	16	0 (12.5)	-1 (30.0)	0 (2.45)
12 (AP)	15	0 (12.5)	+1 (100)	0 (2.45)
13 (AP)	18	-1 (5.00)	0 (65.0)	0 (2.45)
14 (AP)	19	+1 (20.0)	0 (65.0)	0 (2.45)
15 (CP)	6	0 (12.5)	0 (65.0)	0 (2.45)
16 (CP)	4	0 (12.5)	0 (65.0)	0 (2.45)
17 (CP)	11	0 (12.5)	0 (65.0)	0 (2.45)
18 (CP)	7	0 (12.5)	0 (65.0)	0 (2.45)
19 (CP)	20	0 (12.5)	0 (65.0)	0 (2.45)
20 (CP)	14	0 (12.5)	0 (65.0)	0 (2.45)

[a] The cooling flow rate was working just during the Head-cut distillation.

3.3.2. Wines

All experimental distillations were carried out at the Departament d'Enginyeria Química of the Universitat Rovira i Virgili. The CCD distillations used a *Vitis vinifera* "Muscat" wine (2015 vintage year), with an alcoholic strength by volume of 12.6 % (v/v) and a pH of 3.20, which was donated by Dalmau Hermanos y Cía. Suc. S.A. (Tarragona, Spain). The pH of the wine was adjusted before each assay with sulfuric acid solution 2.5 M (GAB system, Barcelona, Spain) at three levels (3.20, 2.45 and 1.70) according to the CCD method (**Table 3.1**). The pH levels were chosen with the intention of observing marked differences.

Optimal distillations used a *Vitis vinifera* "Muscat" (2016 vintage year) with an alcoholic strength by volume of 11.5 % (v/v) and a pH of 3.20, as well as a *Vitis vinifera* "Macabeo" (2016 vintage year) with an alcoholic strength by volume of 10.6 % (v/v) and a pH of 2.95. Both wines were donated by Cooperativa de Vila-rodona (Vila-rodona, Tarragona, Spain). Their alcoholic strengths by volume were adjusted to 12.6 % (v/v) with food grade ethanol of 95 % (v/v) (Droguería Boter SL, Badalona, Spain). According to the optimal conditions obtained with the study of Muscat wine (2015 vintage year), the wines' pH levels were adjusted before each assay with a sulfuric acid solution 2.5 M (GAB system, Barcelona, Spain) or sodium hydroxide solution 2.5 M (Sigma-Aldrich; Saint Louis, USA).

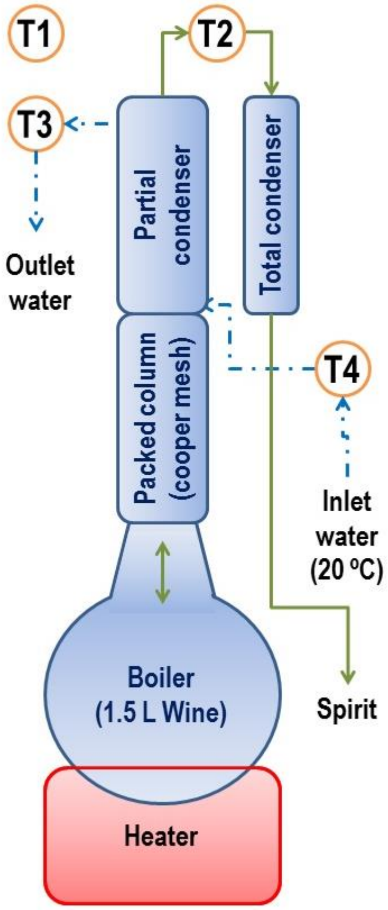

Figure 3.1. Sketch of the distillation device. T1 to 4 are temperature sensors. Expect for T1, distances and dimensions between device parts were maintained using an original drawing.

3.3.3. Distillation system

The distillation system (**Figure 3.1**) was scaled down from a 50 L pilot scale batch packed column [6] to a 1.5 L glass laboratory scale. A Florence flask (2 L) was coupled to a packed glass column (filled with a 1.1 g copper mesh) and a glass tubular heat exchanger (partial condenser). Both inner heat tubes had 8 mm of internal diameter and 80 mm of length. The system was isolated and introduced in a fume hood at constant recirculating air. The internal reflux was modified by changing the cooling water flow rate (at 20 ºC) of the partial condenser with a peristaltic pump (313S, Watson-Marlow Ltd., Falmouth, England). The boiler was heated with a heating mantle (Fibroman-C 1000 mL, JP Selecta S.A., Abrera, Spain). Distillate samples were collected in 20 mL test tubes. Test tubes were covered with perforated caps to minimize evaporation loss of the most volatile compounds. In addition, the system was equipped with four temperature sensors: two in the partial condenser system (shell outlet, T3; and shell inlet, T4), one after the partial condenser (outlet stream of the inner tube, T2) and half a meter away from the device (fume hood's room temperature, T1). Before the experiments, the peristaltic pump of the partial condenser was calibrated between 0 and 300 mL/min.

3.3.4. Distillation process

The 1.5 L of wine was placed in the Florence flask with 3 g of pumice stone. The electrical heating mantle operated at a constant power of 410 W during the first 33 min. Then power was reduced and kept constant at 205 W until the end of each assay. Power values were calculated without considering heat loss. Afterwards, to ensure reproducibility of the first fraction, 300 mL/min of CFR was kept constant during 7 min to achieve total reflux. Power and time values were defined after preliminary experiments (data not shown). Then, 40 min (33 + 7 min) after the onset of the process, CFR was decreased to 100, 65 or 30 mL/min during a HDV of 20, 12.5 or 5 mL according to the experimental design (**Table 3.1**). After the first sample (S1), the partial condenser was stopped and emptied (CFR = 0 mL/min). The next 13 samples (S2-S14) of 20 mL each were distilled without CFR. The last sample (S14) had an alcoholic strength by volume around 39 % (v/v). Optimal distillations were performed with CFR and HDV values according to the optimal conditions obtained with the Muscat wine (2015 vintage year).

3.3.5. Chemical analysis of wine and distilled fractions

Wine ethanol content was determined by ebulliometry (electronic ebulliometer, GAB instruments, Moja-Olèrdola, Spain), wine pH by a pH-meter (Crison Basic 20, L'Hospitalet de Llobregat, Spain) and ethanol content of all distillation samples (S1-S14) by an electronic density meter (Anton Paar DSA 5000M, Graz, Austria). Distilled samples were grouped in four fractions: head-cut (S1), heart-1 (S2-S7), heart-2 (S8-S13) and tail-cut (S14). A 50 µL of the internal standard solution (400 mg/L of 2-octanol, Sigma-Aldrich, Saint Louis, USA) were added to 1 mL of each fraction (previously adjusted to an alcoholic strength by volume of 40 % v/v). All analyses were performed 21 days after each distillation.

3.3.6. Chromatographic analysis

Chromatographic analyses were performed by using a gas chromatograph equipped with a flame ionization detector (GC-FID) (Agilent 6890, Agilent Technologies, Waldbron, Germany), an autosampler (Agilent 7683, Agilent Technologies, Waldbron, Germany) and a capillary polar column (MetaWAX, 60 of length, 0.25 mm ID and 0.5 µm of phase thickness) from Teknokroma (Barcelona, Spain). Injection (2 µL) was done in split mode (1:5). Injector and detector temperatures were at 250 ºC and 260 ºC, respectively. Oven temperature program was: 40 ºC (5 min), 7 ºC/min up to 100 ºC (15 min), 3 ºC/min up to 140 ºC and 2 ºC/min up to 200 ºC (5 min). Column-head flow was initially set at 0.5 mL/min (28 min) and increased with a rate of 5 mL/min^2 up to 1.1 mL/min (67 min) using helium as carrier gas. Quantifications were carried out by interpolation into calibrations built with a synthetic hydro-alcoholic solution (40 % v/v of ethanol) doped with all compounds at different levels. Reagents' CAS, supplier companies and Kovats retention indices are shown in **Table S3.1**. Detection and quantification limits were determined by Signal-to-Noise ratios (S/N) of 3 and 10, respectively.

3.3.7. Response surface methodology (RSM)

RSM aims to screen, model and optimize an experimental design by studying the relationships between two or more independent variables (factors) and a response (compound concentration). In addition to factors of CCD (HDV, pH and CFR of **Table 3.1**), the volume of the heart-cut fraction was added to the model as a fourth factor. For that, the composition of heart-1 (120 mL) plus a percentage of heart-2 (0 to 100 % of 120 mL) was considered as a model response. The 0, 50 and 100 % of heart-2 (HTV) were the three levels chosen for the RSM. It should be noted that the response variation produced by HTV is not necessarily linear, since the concentration of the compounds (g/hL a.a.) depends on the alcoholic content of both fractions (% v/v). In this way, the relative impact of distillation sub-fractions could be evaluated. Therefore, after the CCD was performed, a 3-level-4-factor RSM could estimate a second-degree polynomial model with all the compiled data.

3.3.7.1. Response surface models

In this study, sum-of-squares type III was used to calculate the error terms for statistical significance of linear and quadratic main factor effects and the 2-way interaction factor effects [27]. Non-significant effects ($p > 0.05$) were ignored to obtain more accurate estimation models. The RSM estimated response for each compound was calculated using the following second-degree polynomial function with four factors:

$$\hat{Y} = b_0 + \mathrm{block1} + b_1 \cdot \mathrm{HTV} + b_2 \cdot \mathrm{HDV} + b_3 \cdot \mathrm{pH} + b_4 \cdot \mathrm{CFR} + b_{1,1} \cdot \mathrm{HTV}^2 + b_{2,2} \cdot \mathrm{HDV}^2 \;\; b_{3,3} \cdot \mathrm{pH}^2 + b_{4,4} \cdot \mathrm{CFR}^2 + b_{1,2} \cdot \mathrm{HTV} \cdot \mathrm{HDV} + b_{1,3} \cdot \mathrm{HTV} \cdot \mathrm{pH} + b_{1,4} \cdot \mathrm{HTV} \cdot \mathrm{CFR} + b_{2,3} \cdot \mathrm{HDV} \cdot \mathrm{pH} + b_{2,4} \cdot \mathrm{HDV} \cdot \mathrm{CFR} + b_{3,4} \cdot \mathrm{pH} \cdot \mathrm{CFR} \qquad \text{Eq. 3.1}$$

Where $\hat{Y}$ is the estimated response for each compound (concentration in heart-Cut fraction). *HTV, HDV, pH* and *CRF* are the coded factor values (±1). *block1* is the blocking variable; $b_{i,j}$ are regression coefficients whose subscripts stand for: 0 is the interception of the function, 1 is the HTV, 2 is the HDV, 3 is the pH and 4 is the CFR.

As it has been introduced in **section 3.3.1**, adding a blocking variable to the model allows us to minimize the effect of a known nuisance variable by arranging the experimental runs in similar groups. In our case, we separate the experiments in two groups (blocks) according to their order of execution. In sum, blocking modifies the origin of the coordinates of a group of samples to minimize a nuisance variation, allowing a better fit of the relevant variables of the study. In this study, experiments 1 to 10 were used to determine b_0 + *block1*, while experiments 11 to 20 were used to determine b_0.

Therefore 40 experimental points were extracted from the 20 experimental assays, because for every assay 2 heart fractions were analyzed (heart-1 and heart-2), specifically 30 data points (without counting central point replicates) and 16 regression constants (counting the blocking variable).

For compounds with low levels, concentration values below the detection limit were considered as 0 g/hL a.a., and values between detection and quantification limits were considered as the average of both limits.

3.3.7.2. Desirability function approach

Multi-objective optimization aims to calculate one optimal solution that groups several objectives simultaneously. Derringer & Suich (1980) [26] suggested transforming the estimated responses (RSM) into a range of acceptability values between 0 (undesirable) and 1 (very desirable). For optimizing, desirability functions are based on three response types: Nominal-The-Best (NTB-type) to obtain a target value, Larger-The-Best (LTB-type) to maximize the response, and Smaller-The-Best (STB-type) to minimize the response. A more detailed mathematical explanation can be found in Costa, Lourenço & Pereira (2011) [28].

Starting from the assumption that each compound has a positive or negative aroma effect, LTB- and STB-type functions were used in this study. Curvature between inflection points of the function was not considered. In order to transform the estimated response to a linear desirability function, the following concentration points were used for each compound:

- Lower limit point: the compound odor threshold, assigned to a desirability value of 0 (for the LTB-type) or 1 (for the STB-type). This limit was selected since it makes no sense to do an organoleptic optimization of a compound below the consumer perception.
- Upper limit point: the maximum concentration analyzed in all CCD assays, assigned to a desirability value of 1 (for the LTB-type) or 0 (for the STB-type).

Therefore, the individual desirability of each compound increased (LTB-type) or decreased (STB-type) proportionally to the concentration range above the odor threshold, to maximize or minimize the response according to its positive or negative odor effect on the spirit, respectively. **Figure S3.1** (supplementary information) shows a graphical example of the implementation of the desirability function in this study. Odor description, desirability function-type, odor thresholds and maximum concentration for each compound are shown in **Table 3.2**.

Table 3.2. Compounds groups, names, odor quality, odor desirability, odor threshold and Heart cut maximum levels throughout CCD, and concentrations, standard deviation and significant differences ($p<0.05$) between distillation fractions of the 6 RSM Center Point replicates) [a]*.*

Compound		Odor quality	Odor desirability	Odor threshold	Heart-cut maximum levels of CCD	Fraction concentration		
Group	Name					Head-cut	Heart-1 cut	Heart-2 cut
Alcohol content	Ethanol (% v/v)	Alcoholic	-	-	71.0	87.7±0.4a	70.5±0.5b	55.2±1.4c
Head compounds	Acetaldehyde	Pungent	STB	25.0 [b]	171	839±102a	121±10b	9.68±0.64c
	Acetal	Fruity/Sherry	-	0.250 [b]	38.0	153±25a	25.5±1.7b	2.67±0.29c
	Ethyl acetate	Solvent	STB	12.5 [c]	35.4	227±34a	18.5±1.9b	3.72±0.43b
Fruity esters	Ethyl butyrate	Fruity	LTB	0.005 [b]	0.263	1.95±0.78	d. - n.q.	n.d.
	Ethyl hexanoate	Fruity	LTB	0.0013 [b]	0.956	2.20±0.98a	0.699±0.065b	d. - n.q.
	Ethyl octanoate	Fruity	LTB	0.0005 [b]	1.21	1.13±0.43a	0.980±0.113a	0.090±0.031b
	Ethyl decanoate	Floral/Brandy	LTB	0.105 [d]	0.14	0.520±0.092a	0.117±0.007b	0.087±0.024b
Tail compounds	Ethyl lactate	Lactic	-	25.0 [b]	28.7	0.396±0.112c	10.3±1.2b	35.6±3.6a
	Furfural	Burned	-	5.10 [c]	0.480	n.d.	d. - n.q.	0.600±0.056
	β-phenylethanol	Rose	-	2.50 [b]	2.00	0.149±0.024c	0.763±0.077b	2.62±0.26a
Terpenic compounds	Linalool	Floral/Muscat	LTB	0.250 [e]	2.43	0.197±0.017c	1.72±0.11a	0.576±0.028b
	α-terpineol	Floral	-	75.0 [e]	2.00	0.248±0.022b	1.78±0.18a	1.81±0.14a
Higher alcohols	1-hexanol	Mown grass	-	5.00 [f]	1.29	0.295±0.03b	1.26±0.09a	0.334±0.01b
	1-propanol	Fusel-like	-	208 [b]	51.5	26.3±4.7c	48.8±5.8a	38.1±3.5b
	2-methyl-1-butanol	Fusel-like	-	7.50 [b]	26.4	6.59±1.05b	25.8±2.5a	6.73±0.43b
	3-methyl-1-butanol	Fusel-like	-	7.50 [b]	168	41.9±5.2c	162±16a	55.3±3.8b
Others	Methanol	-	-	167 [b], 200 [g]	92.2	108±18a	75.9±9.2b	99.5±10.6a
	Acetic acid	Vinegar-like	-	50.0 [e]	16.0	247±23a	9.67±0.84b	8.04±0.54b

[a] Except ethanol (% v/v), all compounds are expressed in g/hL a.a. “d. - n.q.” means detected in at least one replicate, but not quantified. “n.d.” means no detected in any replicate. “-“ means there is not enough information. “LTB” means Larger-The-Best. “STB” means Smaller-The-Best.

[b] Referenced in [4]. [c] Referenced in [39]. [d] Referenced in [40]. [e] Referenced in [41]. [f] Referenced in [42]. [g] European legal limit [32].

To group several individual functions in a single multi-objective solution, Derringer & Suich (1980) [26] suggested obtaining an overall desirability by calculating the geometric mean of the individual desirability of the compounds involved in each optimization. Thus, optimization aims to find the values of the factors that maximize an overall desirability.

3.3.8. Sensory analysis

Sensory analysis was performed in the tasting room of the Facultat d'Enologia of Universitat Rovira i Virgili in compliance with standard NF V09-105 [29]. The training period was conducted in 14 sessions of 1 hour each with a selection of 17 assessors. During training sessions, samples of ethyl alcohol of agricultural origin (40 % v/v) (Alcohol Suave, Bodegas y Destilerías Lehman S.A., Tortosa, Spain) were spiked at several levels of ethyl acetate as a Glue-like descriptor (0 to 300 mg/L range), linalool as a Terpenic descriptor (0 to 12 mg/L range) and ethyl hexanoate as a Fruity descriptor (0 to 4 mg/L range). Moreover, assessors were taught to differentiate spirits fractions by identifying the Tail-like descriptor using tail cut fractions from spirit samples of a previous research [10] diluted at several levels.

For the sensory characterization of optimal distillation strategies (3 Muscat and 3 Macabeo), assessors scored samples using 4 aroma attributes (Terpenic, Fruity, Glue-like and Tail-like) and a hedonic test, both using 11-point scales from 0 to 10. Two sessions were held to replicate the analysis for each assessor, using Latin square designs [27]. During the first 10 minutes of both sessions, assessors analyzed the optimizations produced with the Muscat 2016 wine. Then, after 10 minutes of rest, Macabeo 2016 wine optimizations were analyzed during another 10 minutes. For data analysis, the Product Characterization tool of XLSTAT-Sensory statistical package was applied to check if the scores given by the judges were significantly different (ANOVA model: Score = product effect + judge effect + session effect). In both training and analysis, 5 mL of samples were served in black glass cups and assessors had access to drinking water.

3.3.9. Statistical analysis

The CCD, RSM and the Desirability Function Approach were performed with STATISTICA 7.0 statistical package. ANOVA, Tukey's HSD test ($p < 0.05$), Spearman correlation test, Principal Component Analysis (PCA) and Product Characterization (a XLSTAT-Sensory tool) were performed with XLSTAT 2017 statistical add-in for Microsoft Office.

3.4. Results and discussion

3.4.1. Center point analyses

3.4.1.1. Volatile composition of Head and Heart fractions

Center point (six replicates) was applied to analyze the distillation kinetics of volatile compounds and their variance. **Table 3.2** presents the studied compounds (18 quantified compounds out of 20 calibrated, showed in **Table S3.1**) grouped according to their distillation behavior and physical-chemical characteristics [10,24,30]. For all the studied compounds, the table includes their odor descriptors, odor desirability function-type, odor thresholds, maximum levels found in all assays of CCD and their concentration through the distillation of the center point.

In the center point, compositions of all compounds showed significant differences at least between two fractions. Head compounds group (acetaldehyde, acetal and ethyl acetate (C_2)) and fruity esters group (ethyl butyrate (C_4), ethyl hexanoate (C_6), ethyl octanoate (C_8) and ethyl decanoate (C_{10})) were mostly distilled during the head-cut due to their high vapor pressure and/or high solubility in ethanol. In spirits distillations, the head-cut is implemented to reduce the content of negative impact aromas (such as of acetaldehyde and ethyl acetate) and toxic compounds (such as methanol) in the product [4]. The C_4-C_{10} ethyl esters are known for their high positive impact on spirits aroma, having low odor thresholds and providing fruity notes [4]. Even though methanol showed higher concentrations in the head-cut than in heart-1, it is not considered to be a head compound, since it presented high levels in both the first and the last distillation fractions [31] and its acceptance level depends on its legal regulation [32] rather than its aroma impact. Acetic acid also showed high concentrations in the head-cut due to its formation by the hydrolysis of ethyl acetate [4], despite its high boiling point (118 ºC) [33] and high water solubility (K_{OW} = -0.17). Although, in the heart-cut it presented significantly lower values than its threshold.

The higher alcohols group (1-propanol, 1-hexanol, 2-methyl-1-butanol and 3-methyl-1-butanol) and the terpenic compounds group (linalool and α-terpineol) tended to distill during heart-1, due to their higher boiling point and/or solubility in water with respect to head and fruity ester compounds groups. At high levels, higher alcohols are known for their negative impact on the spirit's aroma (fusel-like flavors). In turn, terpenic compounds are known for their high positive impact, providing the typical flowery notes from Muscat wines [4]. In our distillates, some of the compounds of both groups, like 1-propanol and α-terpineol, showed much lower concentrations than their odor thresholds (**Table 3.2**).

Tail compounds (furfural, ethyl lactate and β-phenylethanol) distilled mainly in the last fraction (heart-2), given their high boiling points and/or water solubilities. Tail compounds are considered a defect in young wine spirits, especially furfural compounds that give burned and sweet aroma notes. However, they can add positive characteristics to other type of spirits, e.g. β-phenylethanol may provide a positive rose flavor and furfural may contribute to toasted wood aroma [4].

Methanol, recognized as a toxic compound, presented much lower concentrations than the legal limit of 200 g/hL a.a. for wine spirits in all collected fractions [32]. In addition, ethyl carbamate, a carcinogen compound of Group 2A according to the International Agency for Research on Cancer [34], was not detected in any fraction with an analytical detection threshold of 0.2 mg/L (40 % alc. v/v).

3.4.1.2. System variance

Traditionally, in the spirits industry, distillation columns do not operate at adiabatic conditions and this hinders the production of distillates with consistent composition. In this study, heat insulation significantly improved the experimental reproducibility of the distillation runs. However, it was difficult to completely avoid or control external variables with this distillation system [6]. The relative standard deviations calculated from data of **Table 3.2** for head-cut were high, especially for ethyl lactate (28 %), ethyl octanoate (38 %), ethyl butyrate (40 %) and ethyl hexanoate (44 %). Nevertheless, relative standard deviations in the compositions of the fractions that give rise to the product (heart-1 and heart-2) were below 12 % in all compounds. The effect of room temperature and distillation time on the composition of center point replicates was checked by simple regression with no significant differences (p < 0.05) (data not shown).

3.4.2. Response surface analysis

Linear, quadratic and 2-way interaction regression coefficients, as well as adjusted correlation coefficients of the model for the rescaled factors (Eq. 3.1), are shown in **Table 3.3**. In supplementary information, **Table S3.2** shows other statistical parameters to measure the goodness of fit of the models and **Figure S3.2** presents contour plot examples that can help the interpretation of the models, explained by regression coefficients of **Table 3.3**.

Some compounds presented a statistically significant lack of fit F-test (p < 0.05) due to their low concentrations in heart-2 (C_4-C_6-C_8-C_{10} ethyl esters) and heart-1 (β-phenylethanol) fractions (**Table S3.2**). This can be explained because when the concentrations of the replicates are around quantification limit, a large deviation is produced between analytical values above the limit and those below; when the concentrations of all replicates are below the quantification limit, replicates have identical or very similar concentration values, which imply a pure error that tends towards zero. In both situations, the lack of fit F-test may be significant. Therefore, in these cases, the goodness of fit is analyzed by the analysis of variance of sums of squares (< 0.0001 for all models) and the adjusted correlation coefficient.

Blocking coefficients were significant in almost all models. Most volatile compounds presented positive blocking coefficients (acetal and C_2-C_4-C_6-C_8 ethyl esters) except for acetaldehyde. The remaining compounds had a negative coefficient, except for acetic acid, which was in equilibrium with ethyl acetate. Thus, the system’s rectification could change through the days of experimentation by an unknown external factor that we could not control. In supplementary information, **Figure S3.2** shows counter plot examples that can help the interpretation of the models explained by regression coefficients of **Table 3.3**.

To clear up the following discussion of the effects of the studied factors on distillation time during the head-cut, Tukey’s (HSD) test pairwise comparisons after a multi-way ANOVA ($p < 0.05$) were performed with head-cut distillation time (dependent variable) and pH, CFR and HDV factors (explanatory variables). Significant differences were found between 30 and 100 mL/min CFR levels and between all HDV levels. The pH showed no significant effects. Thus, higher CFR (higher reflux) and higher HDV (larger head-cut volume) increased distillation time (data not shown).

3.4.2.1. Distillation-cuts (HTV and HDV) factors

As can be seen in **Table 3.3**, HTV and HDV linear regression coefficients of each compound presented identical signs (+ or -) as long as both effects were significant, since both factors depend on distillation kinetics previously explained in **section 3.4.1.1.**

Ethanol, head compounds, fruity ethyl esters and linalool tend to distill in higher concentrations at the beginning of the distillation [10]. Hence, larger HDV increased their extraction during the head-cut and consequently reduced their levels in the heart-cut. In addition, since the concentration of these compounds in the boiler is significantly reduced during the last fractions, a larger HTV dilutes them in the heart cut. Consequently, both factors present negative linear coefficients. On the contrary, tail compounds and α-terpineol tend to form and distill in later fractions of the process. Thus, larger HDV increased their concentrations in the heart-cut because there is a displacement of the tail cut, since heart-cut keeps the recovered volume, as well as an increase of the distillation time. Likewise, larger HTV increased tail compounds levels in the heart-cut by adding last distillation fractions. Therefore, both factors presented positive linear coefficients. Higher alcohols and methanol presented no significant effects by HDV. The HTV negatively affects the concentration of higher alcohols by dilution and depletion over time in the boiler [10] while methanol concentration is positively affected since its relative concentration (g/hL a.a.) increases in the last fractions [31].

3.4.2.2. pH factor

The distillation of wine occurs in an acidic hot environment with pH between 2.8 and 4.0 [35], and temperatures ranging between 78 and 100 ºC. This medium favors the formation or reaction of many volatile compounds present in wine. Therefore, the pH of the raw material can be an essential factor for plan distillation strategies, which, in turn, can be easily modified by the producer.

Table 3.3. Compounds Heart-cut concentration models. Significant regression coefficients values with their standard errors of linear (L), quadratic (^2) and 2-way interaction (-) effects by HTV, HDV, pH and CFR rescaled factors (±1), and equation's adjusted correlation coefficient (R-adj) [a].

Compound / Coefficient	Intercept	Block1	HTV (L)	HDV (L)	pH (L)	CFR (L)	HTV(^2)	HDV(^2)	pH(^2)	CFR(^2)	HTV-HDV	HTV-pH	HTV-CFR	HDV-pH	HDV-CFR	pH-CFR	R-adj
Ethanol (% v/v)	65.3 ±0.1**	-0.14 ±0.116	-3.90 ±0.13**	-0.692 ±0.148**	0.190 ±0.148	n.s.	1.30 ±0.22**	n.s.	-0.477 ±0.227*	n.s.	-0.208 ±0.179	n.s.	n.s.	n.s.	n.s.	n.s.	0.986
Acetaldehyde	80.5 ±1.3**	-4.38 ±1.52**	-30.5 ±1.8**	-24.6 ±2.1**	7.86 ±2.08**	-8.78 ±2.08**	11.5 ±3.1**	n.s.	n.s.	n.s.	8.29 ±2.52**	n.s.	2.88 ±2.52	4.35 ±2.33*	n.s.	n.s.	0.972
Acetal	16.8 ±0.5**	0.951 ±0.534*	-7.04 ±0.54**	-5.57 ±0.64**	-1.75 ±0.64**	-1.8 ±0.64**	2.65 ±0.94**	n.s.	3.85 ±1.14**	-1.19 ±1.14	1.93 ±0.77**	n.s.	n.s.	-1.27 ±0.71*	-0.982 ±0.714	n.s.	0.952
Ethyl acetate	13.1 ±0.5**	1.05 ±0.54*	-4.44 ±0.57**	-6.62 ±0.67**	-2.56 ±0.67**	-1.04 ±0.67	1.66 ±0.99	n.s.	2.99 ±1.03**	n.s.	2.54 ±0.81**	n.s.	n.s.	n.s.	-0.938 ±0.748	1.41 ±0.75*	0.933
Ethyl butyrate	0.091 ±0.003**	0.014 ±0.007*	-0.037 ±0.008**	-0.062 ±0.01**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.024 ±0.012*	n.s.	n.s.	-0.017 ±0.011	-0.017 ±0.011	0.017 ±0.011	0.832
Ethyl hexanoate	0.469 ±0.014**	0.062 ±0.016**	-0.155 ±0.017**	-0.159 ±0.02**	n.s.	0.030 ±0.020	0.058 ±0.03*	n.s.	-0.071 ±0.031**	n.s.	0.052 ±0.024**	n.s.	n.s.	n.s.	-0.041 ±0.022*	0.063 ±0.022**	0.930
Ethyl octanoate	0.67 ±0.021**	0.133 ±0.025**	-0.215 ±0.03**	-0.133 ±0.035**	n.s.	n.s.	0.081 ±0.051	n.s.	n.s.	n.s.	n.s.	0.048 ±0.042	n.s.	n.s.	n.s.	0.067 ±0.039	0.871
Ethyl decanoate	0.109 ±0.001**	n.s.	-0.008 ±0.003**	-0.005 ±0.004	n.s.	-0.007 ±0.004*	n.s.	n.s.	n.s.	n.s.	0.005 ±0.004	n.s.	n.s.	-0.005 ±0.004	0.004 ±0.004	0.005 ±0.004	0.546
Ethyl lactate	20.0 ±0.2**	-1.78 ±0.26**	6.66 ±0.31**	1.95 ±0.36**	-1.74 ±0.36**	n.s.	-2.62 ±0.54**	n.s.	n.s.	n.s.	n.s.	-0.958 ±0.441**	n.s.	n.s.	n.s.	n.s.	0.975
Furfural	0.313 ±0.013**	n.s.	0.125 ±0.018**	0.059 ±0.021**	n.s.	n.s.	-0.049 ±0.032	n.s.	n.s.	n.s.	0.033 ±0.026	n.s.	n.s.	n.s.	n.s.	n.s.	0.796
β-phenylethanol	1.49 ±0.01**	-0.133 ±0.017**	0.512 ±0.021**	0.116 ±0.024**	n.s.	n.s.	-0.202 ±0.036**	n.s.	n.s.	n.s.	0.051 ±0.03	-0.032 ±0.03	n.s.	n.s.	n.s.	n.s.	0.979
Linalool	1.3 ±0.02**	-0.055 ±0.019**	-0.321 ±0.021**	-0.071 ±0.024**	0.511 ±0.024**	-0.043 ±0.024*	0.12 ±0.036**	n.s.	-0.077 ±0.037*	n.s.	n.s.	-0.173 ±0.029**	n.s.	n.s.	n.s.	0.029 ±0.027	0.982
α-terpineol	1.82 ±0.01**	-0.145 ±0.017**	n.s.	0.067 ±0.022**	-0.238 ±0.022**	n.s.	n.s.	n.s.	-0.128 ±0.034**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.918
1-hexanol	0.909 ±0.006**	-0.045 ±0.007**	-0.260 ±0.009**	n.s.	0.012 ±0.01	-0.011 ±0.01	0.097 ±0.015**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.985
1-propanol	45.1 ±0.3**	-4.37 ±0.35**	-3.13 ±0.42**	n.s.	0.692 ±0.491	-0.73 ±0.491	1.06 ±0.73	n.s.	n.s.	n.s.	n.s.	-0.706 ±0.595	n.s.	n.s.	n.s.	n.s.	0.938
2-methyl-1-butanol	18.6 ±0.2**	-1.43 ±0.18**	-5.36 ±0.22**	n.s.	0.401 ±0.261	n.s.	2.00 ±0.39**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.978
3-methyl-1-butanol	122 ±1**	-10.0 ±1.1**	-30.1 ±1.4**	n.s.	2.47 ±1.59	-1.91 ±1.59	11.2 ±2.4**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.976
Methanol	85.8 ±0.5**	-8.42 ±0.57**	6.36 ±0.7**	n.s.	n.s.	-1.48 ±0.81*	-2.71 ±1.21**	n.s.	n.s.	n.s.	n.s.	-1.23 ±0.99	n.s.	n.s.	n.s.	n.s.	0.954
Acetic acid	9.49 ±0.18**	0.396 ±0.266	-0.689 ±0.292**	-2.79 ±0.34**	n.s.	-0.786 ±0.341**	n.s.	1.03 ±0.52*	n.s.	n.s.	1.19 ±0.41**	n.s.	n.s.	n.s.	n.s.	n.s.	0.857

[a] Except ethanol, all compounds are expressed in g/hL a.a. HTV means the percentage (%) of Heart-2. HDV means Head-Cut Volume. CFR means Cooling Flow Rate. $p < 0.05$; * $p < 0.001$; ** $p < 0.0001$; n.s. means no significant.

Table 3.3 shows that all head compounds presented linear effects by pH, with a positive regression coefficient for acetaldehyde and negative coefficients for acetal and ethyl acetate. In acidic media, acetaldehyde and ethanol react to form acetal [21]. Furthermore, ethyl acetate is formed by acetic acid esterification, catalyzed in acid media. Therefore, low pH accelerated the formation of ethyl acetate in the boiler (wine) and increased its concentration in the vapor phase (product) since it is much more volatile than acetic acid. Acetal and ethyl acetate also presented positive quadratic effects with pH, showing lower levels in the 2.45 – 3.20 pH range. Hence, low pH favored acetal and ethyl acetate concentration in the heart-cut and decreased acetaldehyde concentration.

In the case of fruity esters, their values were below their quantification limits in the last fractions, due to depletion over time. For this reason, the sum of squares regression did not find linear significant differences with pH, given the small variation of their concentration in heart-2. Nevertheless, variations due to pH can be explained with 2-way interaction effects. Fruity esters showed a positive coefficient of HTV-pH interaction for ethyl octanoate, positive coefficients of pH-CFR interaction for all fruity esters, and negative coefficients of HDV-pH interaction for ethyl butyrate and ethyl decanoate. Low pH could favor the formation of fruity esters from their carboxylic acids, as happened with ethyl acetate – acetic acid equilibrium, thus low pH favors removing fruity esters in the boiler due to their formation at the beginning of distillation. Running out of these esters due to pH interaction effects is more noticeable with high values of HTV and CFR (longer esterification time). On the contrary, fruity esters exhaustion is reduced when the head-cut volume is small, since there is a much shorter time for esterification (negative HDV-pH interaction coefficients). Therefore, low pH accelerates the formation of fruity esters by esterification, behavior that would decrease their levels in the spirit if the head-cut volume is too large.

In relation to tail compounds, ethyl lactate had a negative linear effect with pH, like ethyl acetate. Even though pH should favor the generation of furfural during distillation due to Maillard reactions [18–20,36], this effect was not significant.

Linalool and α-terpineol presented positive and negative linear effects with pH, respectively, since linalool and other terpenic compounds tend to transform to α-terpineol in catalyzed hot acid media [12–16]. Terpenic compounds also presented positive quadratic effects with pH, showing lower levels in the 1.70 – 2.45 pH range. In the case of higher alcohols and ethanol, they presented positive effects with pH, probably due to their reaction by esterification at low pH.

3.4.2.3. Cooling flow rate (CFR) factor

Previous work had shown that high internal refluxes during the heart-cut can significantly alter the distillation behavior throughout the distillation [10]. The present study focused on head-cut reflux strategies; hence, CFR was applied during the head-cut fraction only and in a narrow flow range to avoid masking the effects of other factors.

Head compounds were efficiently removed during the head-cut with high CFR values, due to their high volatilities and high solubilities in ethanol [11], and therefore showed negative linear effects with CFR.

Ethyl hexanoate showed a positive linear effect with CFR, indicating that high rectification held up its distillation during the head-cut, favoring its recovering in the heart-cut [10]. The C_4-C_8 fruity esters also showed pH-CFR and HDV-CFR interaction effects. Positive pH-CFR coefficients indicate that high rectification avoids C_4-C_8 fruity esters distillation during the head-cut and slows down their formation by esterification (at high pH). Negative HDV-CFR coefficients indicate that both factors slowed down the distillation process and, consequently, increased the time for the esterification reaction during the head-cut. Ethyl decanoate (C_{10}) presented the opposite effects with CFR and pH-CFR coefficients, although its correlation coefficient was the lowest observed (Radj = 0.546), therefore analysis of these results should be done with care.

Linalool showed a negative linear effect with CFR, but a positive pH-CFR interaction. High rectification increases the distillation time and therefore the degradation of linalool. However, this effect diminished with high pH since it slows down the reaction [12–16].

Higher alcohols and methanol presented a negative effect with CFR, except for 2-methyl-1-butanol, indicating that high refluxes during the head-cut favored their recovery in the heart-cut. Tail compounds were not affected by CFR, and acetic acid had the same behavior as its ester form (ethyl acetate).

3.4.3. Multi-objective optimizations

Desirability individual functions were constructed according to odor desirability, odor threshold and the maximum levels found during the experimental distillations (**Table 3.2**). To compute specific overall desirability functions, only compounds with a maximum concentration above their aroma threshold were considered. Three optimizations were calculated to obtain specific products with defined organoleptic characteristics: head off-flavors reduction (acetaldehyde and ethyl acetate STB-type), terpenic enhance (linalool LTB-type + acetaldehyde and ethyl acetate STB-type) and fruity ester enhance (C_4-C_8 esters LTB-type + acetaldehyde and ethyl acetate STB-type). Acetaldehyde and ethyl acetate were included in all optimizations to minimize organoleptic defects. Ethyl decanoate (C_{10}) was not included in fruity ester enhance since it presented a considerably low fitting correlation coefficient (Radj. = 0.546). Results of the calculated optimal distillation conditions are shown at the top of **Table 3.4.**

3.4.3.1. Head off-flavors reduction (HOR)

This strategy should reduce the acetaldehyde and ethyl acetate contents in the heart-cut. The largest HTV (240 mL) should dilute these compounds in the heart-cut, the largest HDV (20 mL) should favor their recovery in the head-cut, the lowest pH (1.70) should enhance acetaldehyde degradation, and the highest CFR (100 mL/min) should concentrate these compounds in the head-cut.

3.4.3.2. Terpenic enhance (TEN)

This strategy should significantly increase the content of linalool in the heart-cut. Linalool is the only terpenic compound that showed concentrations above its aroma threshold (**Table 3.2**) and the most important for Muscat spirits [37,38]. Low HTV (16.7 %) should concentrate linalool in the heart-cut, the largest HDV (20 mL) should clean the heart-cut from acetaldehyde and ethyl acetate, the highest pH (3.20) should reduce linalool degradation, and the highest CFR (100 mL/min) should reduce linalool loss in the head-cut.

Table 3.4. Distillation conditions, desirability values and predicted and analytical composition of head off-flavors reduction, terpenic enhance and fruity esters enhance optimums calculated with RSM and desirability function approach [a].

Distillation conditions	Head off-flavor reduction	Terpenic enhance	Fruity Esters enhance	Head off-flavor reduction	Terpenic enhance	Fruity Esters enhance	Head off-flavor reduction	Terpenic enhance	Fruity Esters enhance
HTV (%)	100	16.7	66.7	100	16.7	66.7	100	16.7	66.7
HDV (mL)	20.0	20.0	5.00	20.0	20.0	5.00	20.0	20.0	5.00
pH	1.70	3.20	2.95	1.70	3.20	2.95	1.70	3.20	2.95
CFR (mL/min)	100	100	100	100	100	100	100	100	100
	Predicted values (RSM) for Muscat 2015			*Analytical values for Muscat 2016*			*Analytical values for Macabeo 2016*		
Desirability value	0.993	0.806	0.658						
Ethanol (% v/v)	61.1	67.6	64.8	56.8 ±0.0 a	62.9 ±0.0 c	61.2 ±0.2 b	56.5 ±0.2 a	63.3 ±0.2 c	61.6 ±0.5 b
Compounds (g/hL a.a.)									
Acetaldehyde	**27.1**	**77.4**	**88.0**	15.5 ±0.3 a	24.9 ±3.1 b	37.1 ±2.6 c	21.5 ±0.6 a	50.5 ±3.2 b	63.7 ±3.8 c
Acetal	11.7	12.7	19.1	8.01 ±0.07 a	9.47 ±0.8 a	17.2 ±1.4 b	10.1 ±0.2 ab	7.38 ±0.01 a	14.2 ±2.7 b
Ethyl acetate	**8.44**	**8.38**	**18.1**	41.9 ±1 c	12.3 ±1.8 a	30.2 ±1.1 b	36.4 ±5.5 c	9.10 ±0.30 a	19.4 ±0.8 b
Ethyl butyrate	0.000	0.022	**0.172**	0.235 ±0.003 a	d.-n.q.	0.296 ±0.005 b	0.218 ±0.046	d.-n.q.	0.256 ±0.005
Ethyl hexanoate	0.119	0.386	**0.648**	1.06 ±0.03 b	0.800 ±0.008 a	1.25 ±0.03 c	0.848 ±0.056 b	0.706 ±0.027 a	1.19 ±0.01 c
Ethyl octanoate	0.287	0.751	**0.795**	1.50 ±0.01	1.53 ±0.06	1.50 ±0.14	1.16 ±0.02 a	1.33 ±0.13 a	1.64 ±0.02 b
Ethyl decanoate	0.097	0.104	0.106	0.319 ±0.054	0.450 ±0.077	0.330 ±0.048	0.203 ±0.037 a	0.252 ±0.013 a	0.338 ±0.014 b
Ethyl lactate	28.7	15.2	18.6	52.2 ±1.8 b	13.4 ±0.5 a	16.4 ±0.1 a	3.90 ±0.15 c	1.12 ±0.14 a	1.84 ±0.26 b
Furfural	0.480	0.244	0.279	d.-n.q.	d.-n.q.	d.-n.q.	d.-n.q.	n.d.	d.-n.q.
β-phenylethanol	2.00	1.17	1.50	3.93 ±0.21 b	2.76 ±0.2 a	3.00 ±0.06 a	2.85 ±0.02 b	1.97 ±0.17 a	2.18 ±0.03 a
Linalool	0.543	**2.03**	1.52	0.128 ±0.003 a	2.17 ±0.14 c	1.62 ±0.03 b	n.d.	n.d.	n.d.
Geraniol	n.c.	n.c.	n.c.	n.d.	0.273 ±0.013 b	0.185 ±0.001 a	n.d.	n.d.	n.d.
α-terpineol	2.00	1.52	1.54	1.01 ±0.06	0.977 ±0.027	0.944 ±0.002	n.d.	n.d.	n.d.
1-hexanol	0.723	1.13	0.831	0.840 ±0.032 a	1.14 ±0.05 b	0.886 ±0.004 a	1.37 ±0.05 a	1.94 ±0.12 b	1.56 ±0.07 a
1-propanol	42.4	48.1	43.8	24.8 ±0.3 a	27.9 ±0.8 b	25.3 ±0.3 a	18.6 ±0.1	21.2 ±1.3	19.2 ±0.6
2-methyl-1-butanol	14.9	23.5	17.3	35.0 ±0.8 a	49.2 ±2 b	38.5 ±0.1 a	19.0 ±0.8 a	27.0 ±1.9 b	21.8 ±0.7 a
3-methyl-1-butanol	99.1	148	113	133 ±2 a	179 ±7 b	142 ±0 a	121 ±4 a	166 ±11 b	135 ±5 a
Methanol	89.2	79.7	85.9	47.9 ±0.8 b	44.2 ±0.6 a	46 ±0.9 ab	44.4 ±0.8	41.8 ±2.4	41.5 ±1.1
Acetic acid	7.46	6.62	11.9	9.98 ±1.18	8.08 ±1.08	10.7 ±0.8	9.17 ±0.61	8.77 ±1.46	11.4 ±0.4

[a] Optimal distillation conditions and desirability values were calculated using the responses in bold. "n.c" means no calculated in the RSM. "n.d." means no detected. "d.-n.q." means detected but no quantified.

3.4.3.3. Fruity esters enhance (FEN)

This strategy included operating conditions that, according to the models, would enhance the fruity esters on the heart-cut. Medium HTV (66.7 %) should balance the reduction of head off-flavors and fruity esters due to dilution, lowest HDV (5 mL) should reduce their loss in the head-cut, high pH (2.95) should reduce fruity ester esterification, and highest CFR (100 mL/min) would reduce losses of fruity esters during distillation of the head-cut.

3.4.4. Evaluation of optimal strategies

Two different wines (Muscat and Macabeo of 2016 vintage year) were distilled with the three optimal strategies to assess RSM models and to confirm chemical analyses with a sensory panel. The main difference between both wines is that Macabeo wine is known for having no detectable terpenic compounds.

3.4.4.1. Optimums chemical analyses

Table 3.4 shows the predicted heart-cut composition of Muscat 2015 wine distilled with the calculated optimal conditions (RSM). In addition, this table shows the mean concentration of heart-cut, standard deviation and significant differences of Muscat and Macabeo 2016 wines distilled with the same optimal conditions.

Most of the times, the assessed strategies performed according to what was predicted by the RSM models. Heart-cuts of both wines distilled with the FEN strategy showed in most cases the highest values of C_4-C_{10} ethyl esters. The TEN strategy applied to the Muscat 2016 wine yielded the highest levels of linalool and geraniol in the heart-cut. Geraniol was not taken into account during RSM since Muscat 2015 wine and spirits showed geraniol levels below its quantification limit. Macabeo had no detectable terpenic compounds in both spirits and wine (data not shown). The HOR strategy showed the lowest values of acetaldehyde; however, it also showed the highest values of ethyl acetate and unexpected high values of other ethyl esters. As explained in **section 3.4.2**, we could infer from the RSM models that ethyl esters increase with low pH; however, RSM models predict a decrease of their concentration with high head-cut and heart-cut volumes, which has not observed in 2016 wine distillates. As can be seen throughout **Table 3.4**, the rest of the compounds have a similar ratio of concentrations between optimal strategies. Thus, deviation of ethyl esters in head off-flavor reduction can be due to compositional changes of wines. Chemical analyses of the three initial wines were carried out to understand this behavior (data not shown) without consistent conclusions. Furthermore, ethyl esters obtained low p-value in the lack of fit F-test, initially attributed to their low levels in heart-2; therefore, the model may generate a wrong prediction of the ethyl esters composition for the HOR strategy.

3.4.4.2. Optimums sensory analysis

Samples of the optimal strategies using both wines (Muscat and Macabeo of vintage 2016) were sensory analyzed by 17 trained assessors by rating Terpenic, Fruity, Glue-like and Tail-like aroma descriptors, plus a hedonic rating test.

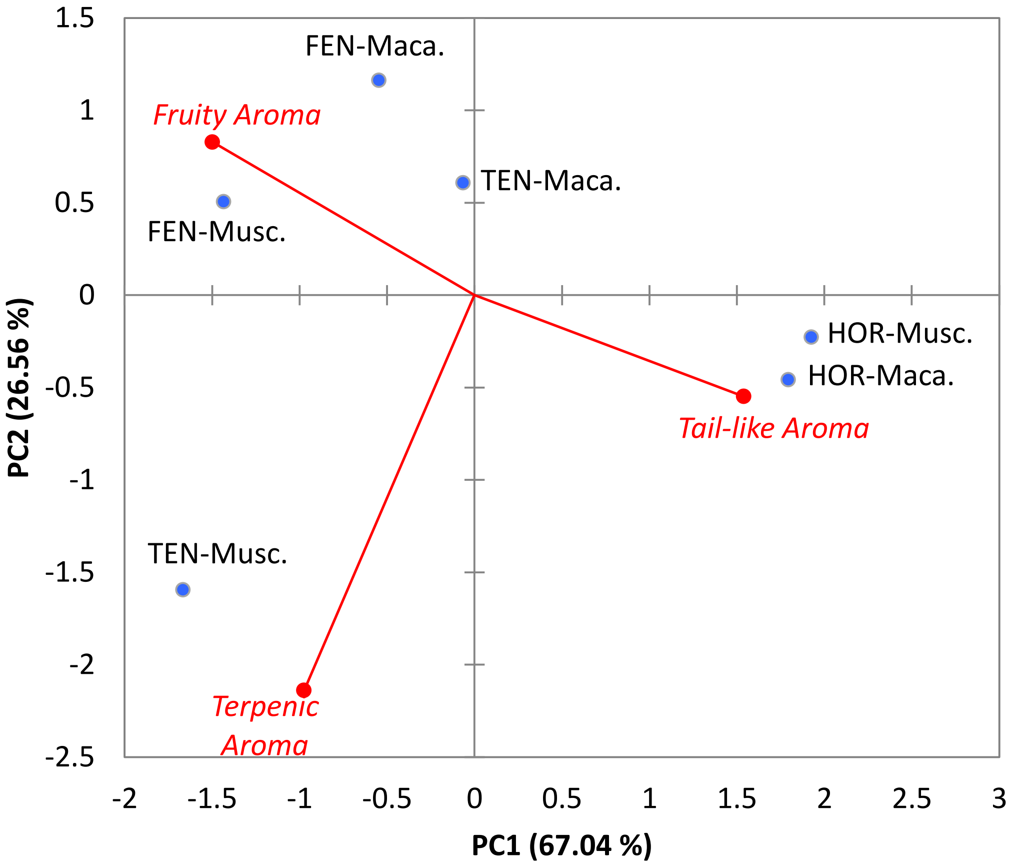

Figure 3.2. PCA biplot of the sensory profile of optimal distillation strategies (HOR, FEN and TEN) using Muscat (Musc.) and Macabeo (Maca.) wines of 2016 vintage and filtering the non-discriminant descriptors ($p < 0.1$).

In order to reflect the aroma differentiation between samples, sensory data was analyzed by PCA (**Figure 3.2**). Two principal components (PC1 and PC2) explained 93.6 % of the variance. Glue-like aroma was not included, as it presented no significant differences ($p < 0.1$). The PC1 axis places Fruity and Terpenic aroma descriptors against Tail-like descriptor, and PC2 places Fruity descriptor against Terpenic and Tail-like descriptors. This arrangement in the biplot appears to be related with the compound groups associated to descriptors, explained in **section 3.4.1.1.** The optimal strategies were well differentiated by PCA. Muscat and Macabeo FEN samples presented high intensity of fruity aroma, and Muscat TEN sample presented high intensity of terpenic aroma. Macabeo TEN sample was located near Macabeo FEN sample, but with less fruity intensity. As expected, Macabeo spirits samples showed no terpenic aroma. Muscat and Macabeo HOR samples presented high intensity of tail-like aroma, since this strategy contained last fractions of the distillation to dilute acetaldehyde and ethyl acetate in the heart-cut.

Finally, the preference test was analyzed with ANOVA and then Tukey's HSD test (to check the acceptance between samples) and with the Spearman correlation test (to check the correlation between aroma attributes and consumers acceptance). Hedonic data analysis can only be taken into account as a suggested trend given the small number of assessors and their previous training. Tukey's pairwise compassions (data not shown) showed significant differences ($p < 0.05$) between the Muscat TEN and FEN samples (highest ratings), and Muscat HOR samples (lowest rating). Macabeo samples showed no significant differences. Hedonic rating correlations ($p < 0.05$) showed that samples with terpenic and fruity aromas were scored positively, and samples with glue-like and tail-like aromas were scored negatively (**Figure S3.3**). This hedonic results illustrate how developing and implementing distillation strategies can favor the production of genuine beverages that retain or enhance flavors coming from the initial fermented beverage, like terpenes (grape origin) and fruity ethyl esters (alcoholic fermentation origin).

3.5. Conclusions

High rectifications during distillation of the head-cut resulted in improved spirits, characterized by low content of head compounds and high content of fruity and terpenic compounds. The effect of distillation volumes and pH should be considered and adjusted when different groups of volatile compounds are needed to optimize the results, depending on which aromas should be enhanced or reduced. A larger heart-cut decreased all studied compound levels, except tail compounds (off-flavors), α-terpineol (without odor impact at spirit levels) and methanol (toxic compound). A larger head-cut decreased the concentration of head compounds (off-flavors), at the cost of reducing C_4-C_{10} ethyl esters and linalool levels (positive odors) and increasing the level of tail-cut compounds (longer distillation time). Low pH favored the decomposition of linalool and acetaldehyde to form α-terpineol and acetal, respectively, and favored the formation of ethyl esters by esterification. Sensory analysis corroborated the optimization of aroma compounds in spirits by chemical modeling using RSM. These results reinforce the versatility of this experimental system and deepen its ability to modify the aroma profile of spirit beverages.

Acknowledgments

P. M.-G. appreciates the Ph.D. Grant “Martí Franquès” (2015PMF-PIPF-29) with the support of the Universitat Rovira i Virgili.

3.6. References

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Chapter 4

RAPID SENSORY ANALYSIS TECHNIQUE BY USING RESPONSE SURFACE METHODOLOGY: APPLICATION TO THE STUDY OF ODOUR INTERACTIVE EFFECTS IN MODEL SPIRITS

A study published in: Journal of the Institute of Brewing 2018, 124 (1), pp. 100-105

4.1. Abstract

One of the major objectives of food industry is predicting the sensory profile of a product by chemical analysis. In the case of spirit drinks, their volatile compounds can exhibit odour interactive effects. This study investigated the odour perception of linalool, ethyl hexanoate, ethyl acetate and acetaldehyde with model solutions (in 40% v/v ethanol) on flowery, fruity and glue-like attributes. Design of experiments for sensory analysis and response surface methodology were used to evaluate the aroma interactions of these compounds by sniffing or orthonasal olfaction. Results showed that ethyl hexanoate masks flowery and glue-like descriptors. Acetaldehyde provides a low-intensity fruity odour, which is confused with ethyl hexanoate odour. High levels of ethyl acetate suppress and enhance the fruity descriptor at high and low levels of ethyl hexanoate, respectively. In summary, the studied sensory technique enables the rapid but consistent assessment of the interaction of aroma compounds in an alcoholic spirit. It is suggested that this approach may be a useful tool in the optimization and development of alcoholic products.

4.2. Introduction

The quality of young Muscat brandy (Pisco) depends on its aromatic composition, where terpenic compounds (e.g. linalool) and low-boiling ethyl esters (e.g. ethyl hexanoate) have a remarkable positive odour effect, related with flowery and fruity descriptors, respectively. In contrast, high volatility compounds as acetaldehyde or ethyl acetate are considered off-flavours, with pungent and glue-like odour, respectively. To achieve the highest organoleptic quality and production yield, Piscos are usually produced by batch distillation, where the first fraction is discarded (heads) and the following is the main product (hearts). This methodology allows producers to concentrate or avoid certain aroma compounds according to their physicochemical characteristics [1].

The typical aroma of Pisco comes from linalool [2], which is described as flowery and citrus [1,3]. This terpene alcohol has a low odour threshold in spirits (1 mg/L of ethanol 40% v/v) with respect to its concentration in commercial Piscos (0.170 to 10.4 mg/L 40% v/v) [4]. Linalool is originated during grape ripening and in wines can be found both free as in their glycoside precursors [5]. Due to its physicochemical characteristics linalool tends to distil in the early stages of distillation heart fraction.

Fruity odour in wine spirits mostly comes from low-boiling ethyl esters (C_4-C_{10}) with boiling points between 125.8 and 247.7 ºC [6]. Their origin is mostly microbiological, produced during alcoholic fermentation by yeasts and other microorganisms. Ethyl esters are highly volatile, thus tend to distil in head fraction and low amounts are found in the product [7]. However, ethyl esters have an important aroma impact in Muscat spirits, as they have very low odour thresholds (0.005 to 0.260 mg/L 40% v/v) [1]. Furthermore, in our study, ethyl hexanoate was chosen as reference ethyl ester, since Peña y Lillo et al. [8] found that ethyl hexanoate was the ethyl ester with highest correlation in the volatile composition of the heart fraction. The aroma of ethyl hexanoate is described as apple, banana and violet [1].

One of the most common defects in spirits of agricultural origin is from ethyl acetate, which contributes a glue-like aroma similar to odour of nail polish remover. Ethyl acetate is produced by yeast and bacterial metabolism or formed through the chemical esterification of ethanol and acetic acid. In spirit drinks, ethanol is presented in very high contents (>30% v/v); therefore ethyl acetate formation is favoured. According to its high volatility, ethyl acetate distils in head fractions, however high levels can be found in heart fraction (4.00 to 800 mg/L 40% v/v) in relation with its odour threshold (7.5 mg/L 40% v/v) [1].

Pungent odour is another spirits off-flavour, which comes from acetaldehyde. However, low levels can suggest positive odour in spirits, as sweetish, cut apple or nuts notes [9]. Acetaldehyde is the most important carbonyl compound formed during the alcoholic fermentation. Since is highly volatile, acetaldehyde distil in the head fraction. Spirits can also present high concentrations in hearts if the head fraction is not well adjusted, being a typical defect. In commercial products, acetaldehyde presents a wide range of contents (<2 and 160 mg/L 40% v/v) [1].

A common way to estimate the odour intensities of volatile compounds is the calculation of odour activity values (OAV; the ratio between chemical concentration and odour threshold), in order to predict the sensory profile of food products by chemical analysis. Several studies had determined olfactory thresholds of many volatile compounds present in spirits conditions, however, these experimental values do not take into account odour interactive effects between compounds, such as enhancement or suppression of odours [10]. There are some methods to evaluate aroma interactions, mostly comparing odour intensities in binary mixtures. A much more comprehensive explanation of odour interactive effects can be found in some reviews [11,12].

Few studies had used factorial experiments to evaluate sensory interactive effects in complex mixtures [13–15]. However, no study has focused on interactive odour effects on spirits, where ethanol is a strong-smelling agent that can produce olfactory fatigue [16].

This work was planned with a dual purpose. The first objective was to perform a rapid sensory methodology to analyse a large number of samples of wine distillates by using response surface methodology (RSM), specially designed to do quickly evaluations of distillation strategies to obtain products with specific aromatic profile. The second objective was to analyse the aroma interactive effects between volatile compounds in wine spirit drinks using model solutions.

4.3. Materials and methods

4.3.1. Samples

Ethyl alcohol of agricultural origin was the base spirit (Alcohol Suave, Bodegas y Destilerías Lehman S.A., Tortosa, Spain), with an alcohol degree of 40 % (v/v). Ethanol content was checked with an electronic density meter (Anton Paar GmbH, Graz, Austria). According to the experimental design (**Table 4.1**), 26 aliquots with model solution were spiked at several levels with linalool, ethyl hexanoate, acetaldehyde and ethyl acetate (Sigma-Aldrich, Saint Louis) 12 hours before the experimentation. Samples were stored in sealed amber glass bottles at room temperature. Concentration levels were chosen according to the order of magnitude of common levels in commercial spirits [1].

Table 4.1. Coded concentrations (±1) and odour thresholds of the compounds (factors) for the face centered central composite design with 2 center points (CP) [a]*.*

Sample Number	Linalool	Ethyl hexanoate	Ethyl acetate	Acetaldehyde
1	-1 (0)	-1 (0)	-1 (0)	-1 (0)
2	-1 (0)	-1 (0)	-1 (0)	+1 (300)
3	-1 (0)	-1 (0)	+1 (300)	-1 (0)
4	-1 (0)	-1 (0)	+1 (300)	+1 (300)
5	-1 (0)	+1 (10)	-1 (0)	-1 (0)
6	-1 (0)	+1 (10)	-1 (0)	+1 (300)
7	-1 (0)	+1 (10)	+1 (300)	-1 (0)
8	-1 (0)	+1 (10)	+1 (300)	+1 (300)
9	+1 (10)	-1 (0)	-1 (0)	-1 (0)
10	+1 (10)	-1 (0)	-1 (0)	+1 (300)
11	+1 (10)	-1 (0)	+1 (300)	-1 (0)
12	+1 (10)	-1 (0)	+1 (300)	+1 (300)
13	+1 (10)	+1 (10)	-1 (0)	-1 (0)
14	+1 (10)	+1 (10)	-1 (0)	+1 (300)
15	+1 (10)	+1 (10)	+1 (300)	-1 (0)
16	+1 (10)	+1 (10)	+1 (300)	+1 (300)
17	-1 (0)	0 (5)	0 (150)	0 (150)
18	+1 (10)	0 (5)	0 (150)	0 (150)
19	0 (5)	-1 (0)	0 (150)	0 (150)
20	0 (5)	+1 (10)	0 (150)	0 (150)
21	0 (5)	0 (5)	-1 (0)	0 (150)
22	0 (5)	0 (5)	+1 (300)	0 (150)
23	0 (5)	0 (5)	0 (150)	-1 (0)
24	0 (5)	0 (5)	0 (150)	+1 (300)
25 (CP)	0 (5)	0 (5)	0 (150)	0 (150)
26 (CP)	0 (5)	0 (5)	0 (150)	0 (150)
Odour threshold (mg/L 40% v/v)	1.00 [b]	0.005 [c]	7.50 [c]	10.0 [c]

[a] Numbers in brackets are the experimental concentrations of factors, expressed in mg/L ethanol 40% v/v. [b] Referenced in [4]. [c] Referenced in [1]

4.3.2. Experimental design

4.3.2.1. Central composite design (CCD)

CCD is an experimental design which allows the estimation of linear and quadratic effects on a response variable with a minimum number of experiments. CCD is built with the 2-level fractional factorial design (FFD) points, a centre point and axial points. 2-level factorial design studies all possible combinations of two or more factors at 2 levels. Centre point is an experimental run whose factor values are the median of the 2 levels of the factors establish in the FFD. Centre Point is normally replicated to know the variance of the system. Axial points have the same factor values as centre point, except for one factor whose value is at an α distance from the centre point. For processing data, independent variables (factors) are coded as follows to construct the design: FFD points = ±1; centre point = 0; axial points = ±α (for one factor) and 0 (for other factors).

For the study, a 3-level-4-factor CCD with face centred axial points ($\alpha = \pm 1$) and 2 centre points was designed (26 runs). Factors were the selected compounds: linalool, ethyl hexanoate, ethyl acetate and acetaldehyde, spiked in a model spirit. **Table 4.1** shows all the experiments in the standard order of runs with coded (-1, 0 and +1) and experimental (spiked concentrations) factors values.

4.3.2.2. Response surface methodology (RSM)

RSM, introduced by Box and Wilson [17], aims to screen, model and optimize an experimental design by studying the relationships between the independent variables (spiked compounds) and each response (odour descriptors). Therefore, after the CCD is performed, RSM estimates a second-degree polynomial model with all the compiled data.

In this study, ANOVA has been used to observe the significance of linear and quadratic main factor effects and two-way interaction factor effects. Sum-of-squares type III was used to calculate the error terms for statistical signification. Non-significant effects ($p > 0.05$) were ignored to obtain more accurate estimation models. A lack-of-fit test ($p > 0.05$) was done to check the adequacy of each model. RSM estimated response for each compound was calculated using Eq. 4.1, a second degree polynomial function with four variables:

$$\hat{Y} = b_0 + b_1 \cdot X_1 + b_2 \cdot X_2 + b_3 \cdot X_3 + b_4 \cdot X_4 + b_{1,1} \cdot X_1^2 + b_{2,2} \cdot X_2^2 + b_{3,3} \cdot X_3^2 + b_{4,4} \cdot X_4^2$$
$$+ b_{1,2} \cdot X_1 \cdot X_2 + b_{1,3} \cdot X_1 \cdot X_3 + b_{1,4} \cdot X_1 \cdot X_4 + b_{2,3} \cdot X_2 \cdot X_3 + b_{2,4} \cdot X_2 \cdot X_3 + b_{3,4} \cdot X_3 \cdot X_4 \qquad \text{Eq. 4.1}$$

Where $\hat{Y}$ is the estimated sensory response (flowery, fruity or glue-like descriptor). b_0 is the regression coefficient for the intercept. b_1, b_2, b_3 and b_4 are the coefficients of the main effects. $b_{1,1}$, $b_{2,2}$, $b_{3,3}$ and $b_{4,4}$ are the coefficients of the quadratic effects. $b_{1,2}$, $b_{1,3}$, $b_{1,4}$, $b_{2,3}$, $b_{2,4}$ and $b_{3,4}$ are the coefficients of two-way interactions. Terms X_1, X_2, X_3 and X_4 refer to the coded concentrations of variables (±1). During this study, subscripts stand for the following terms: *0* means intercept, *1* means linalool, *2* means ethyl hexanoate, *3* means ethyl acetate and *4* means acetaldehyde.

*4.3.3. **Rapid sensory analysis***

Taste panel was composed of 18 third-year students of oenology (with experience in wine tasting) at Universitat Rovira i Virgili during 5 sessions in different days of 30 to 40 minutes each one. All sensory analyses were carried out in the tasting room at the faculty of Enology of Universitat Rovira i Virgili in compliance with standard NF V09-105 [18]. The first session performed was an only one training session to determine assessors' sensory capabilities, and during the other 4 sessions the CCD was completed.

The selected descriptors were named as flowery, fruity and glue-like, which described the aromas of linalool, ethyl hexanoate and ethyl acetate, respectively. Preliminary studies found that acetaldehyde was not well discriminated by semi-trained assessors (data not shown), however it has been reported its ability to change the perception of other compounds [14]. For both reasons, it was introduced in the experimental design as a factor, but not as an odour response. Samples were analysed by orthonasal olfaction, since is more discriminant than retronasal route in Muscat spirits [19].

4.3.3.1. Training session

Assessors were taught to relate the odour of the compounds linalool, ethyl hexanoate or ethyl acetate, with the descriptors flowery, fruity or glue-like, respectively, in a 6-point scale from 0 to 5. Three samples in different levels of each compound were used as training scale (9 samples), prepared with the concentrations of the coded factor levels (-1, 0 and +1) of the CCD (**Table 4.1**). -1, 0 and +1 coded concentrations were related to 0, 2 and 4 values of the 6-point scale, respectively. The value 0 of the 6 point-scale was included to differentiate the aroma of ethanol with respect to the aroma of the studied compounds, and the value 5 of the 6-point scale was not trained but considered in case of odour enhancement between compounds at their high concentrations.

4.3.3.2. Design of experiments for sensory analysis

Spirits contain a large amount of ethanol. Therefore olfactory capabilities of the assessors may decrease. In preliminary studies, it has been observed that it is not suitable to evaluate more than six samples in a continuous session, needing a break for every three samples (data not shown). However, our CCD consisted of 24 samples plus two centre points, and just 18 assessors.

Design of experiments for sensory analysis ensures acceptable statistical results when a small group of judges evaluate a large set of products. With this methodology, products are analysed with an overall frequency as homogeneous as possible during all the experimentation and during each session, and with a random order to avoid bias. The design of experiments for sensory analysis used in this study is showed in **Table 4.2**, where each sample had 8 or 9 replicates.

4.3.3.3. CCD orthonasal sensory procedure

In the four sessions, 3 samples of 5 mL were placed in front of each panellist in transparent glasses covered with empty plastic cell-culture dishes to ensure a homogeneous head-space and to prevent evaporation. Samples were ordered from left to right side according to **Table 4.2**. Panellists scored the 3 descriptors by orthonasal with the 6-point scale.

Table 4.2. Design of experiment for sensory analysis for 18 assessors and 26 samplers.

	Sampler Number (*Table 4.1*)											
	First Session			Second Session			Third Session			Fourth Session		
Tasting order Assessor number	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
1	3	18	1	2	21	4	10	26	24	15	14	11
2	8	24	11	25	13	21	4	5	22	16	12	14
3	5	20	15	22	8	16	20	9	23	3	4	2
4	21	17	22	4	1	2	14	8	3	11	24	15
5	7	26	16	10	1	5	17	25	2	18	24	20
6	12	9	13	2	26	25	6	1	12	15	25	16
7	25	6	19	24	22	23	16	19	13	5	3	21
8	10	23	14	6	4	5	18	21	7	19	11	20
9	14	8	12	3	2	1	7	4	6	10	15	24
10	17	9	24	15	3	6	8	1	5	23	10	19
11	19	13	26	20	11	9	13	12	25	9	22	1
12	23	6	7	4	2	3	21	19	17	11	15	10
13	1	4	3	26	14	17	24	11	10	25	7	8
14	11	25	15	24	7	18	22	16	23	5	17	26
15	22	18	20	23	12	19	3	26	9	21	6	13
16	16	5	10	9	7	8	2	20	18	1	17	4
17	12	10	11	21	19	20	6	18	22	14	13	9
18	13	15	14	18	16	17	12	23	7	26	2	8

4.3.4. Statistical analysis

CCD, ANOVA, lack-of-fit test and regressions models for RSM were performed with STATISTICA 7.0 statistical package. Design of experiments for sensory analysis was performed with XLSTAT 2016 statistical ad-in for Microsoft Office.

4.4. Results and discussion

The purpose of this work is to study the interactions between compounds and their aromas, which are determined by regression models that contain main, quadratic and two-way interaction coefficients. To avoid confusions between interaction terms throughout the article, the term "two-way interaction effect" has been used to describe significant statistical two-way interactions, and the term "odour interactive effect" to describe sensory interactions. Therefore, an odour interactive effect indicates that a compound enhance or reduce the perception of another compound. On the other hand, a two-way interaction effect shows how two compounds produce an odour variation when both are present (which can or cannot produce a sensory interactive effect on the studied odour).

Table 4.3. RSM estimate effects of flowery, fruity and glue-like orthonasal responses, calculated with coded concentration values (±1) of linalool (X_1), ethyl hexanoate (X_2), ethyl acetate (X_3) and acetaldehyde (X_4) [a].

Orthonasal response	Coefficient name	Regression Coefficient	Standard Error	p-value (ANOVA)
Flowery	b_0	1.56	0.097	<0.001
	b_1	0.373	0.116	0.002
	b_2	-0.261	0.116	0.026
Glue-like	b_0	1.8	0.105	<0.001
	b_2	-0.552	0.125	<0.001
	b_3	0.396	0.125	0.002
Fruity	b_0	2.01	0.086	<0.001
	b_2	0.928	0.103	<0.001
	b_4	0.233	0.103	0.025
	$b_{2,3}$	-0.246	0.109	0.025
	$b_{2,4}$	-0.353	0.109	0.001

[a] Coefficients are related to Eq. 4.1 for each response.

4.4.1. Regression model

Table 4.3 shows the regression results of the sensory evaluation of the CCD samples, calculated with the Eq. 4.1. As can be seen, panellists could identify and match the three descriptors with their respective trained compound: flowery with linalool (b_1 coefficient), fruity with ethyl hexanoate (b_2) and glue-like with ethyl acetate (b_3), indicating the reliability of the training session. Moreover, odour interactive effects between compounds were also observed in all descriptors, which will be discussed in the following sections.

Concentrations of the selected compounds in the CCD samples were much higher than their odour thresholds (**Table 4.1**). However, the relation between concentration and odour intensity differs in each compound, and that is why OAV should not be compared between compounds. Regardless, concentration and odour intensity relationships are usually modelled with a sigmoidal function [20]. On the contrary, in this study were found no quadratic effects for any descriptor. Therefore, it has been assumed that the intermediate concentration used in the CCD was located in the logarithmic phase of the theoretical odour intensity function and the maximum concentration on the plateau of the sigmoidal function.

In short, the models of this study should be interpreted as a quick technique to evaluate potential odour interactive effects between volatile compounds, but not as accurate odour intensity estimation models.

4.4.2. Flowery and glue-like descriptors

Flowery and glue-like descriptors are discussed together as both presented similar odour interactive effects with ethyl hexanoate (**Table 4.3**).

Regarding to the flowery descriptor, the regression coefficient of linalool had a positive value (b_1 coefficient), as can be expected since it was the trained compound. However, ethyl hexanoate appeared to negatively affect the flowery perception (b_2). Flowery perception decreased with high concentrations of this ester. This effect is also shown in **Figure 4.1**, where the highest score of flowery descriptor occurred with high concentrations of linalool and low concentrations of the ethyl hexanoate. Peña y Lillo et al. [8] studied the correlations between volatile compounds, distillation fraction, blending process and main sensory attributes of Pisco by principal component analysis. This study showed that ethyl hexanoate and linalool were the highest correlated chemical markers of the blended Pisco heart fraction, where linalool odour was the main sensory attribute.

However, that relationship might be due to the common aroma of hearts of Muscat spirits, since it has been observed that ethyl hexanoate decreased the intensity of linalool odour in all levels of our concentration range.

Glue-like descriptor, as expected, was associated with ethyl acetate with positive regression coefficient (b_3), since it was the trained compound for glue-like perception. However, ethyl hexanoate decreased the intensity of glue-like descriptor at all tested levels (**Figure 4.2**), as flowery descriptor. Their chemical-structure likeness (structure-odour relationship) could lead to confusion of both esters' odour perception [21].

These results allow us to establish that ethyl hexanoate has a masking effect on linalool and ethyl acetate odours and consequently on flowery and glue-like descriptors.

4.4.3. Fruity descriptor

The fruity descriptor was the odour response more affected by the studied volatile compounds. As expected, ethyl hexanoate had a positive regression coefficient (b_2), since it was the trained compound for fruity descriptor. However, there was an important odour interactive effect of acetaldehyde, with a linear positive regression coefficient (b_4). Furthermore, a negative regression coefficient of the two-way interaction between acetaldehyde and ethyl hexanoate also influenced the fruity perception ($b_{2,4}$). As can be seen in **Figure 4.3**, acetaldehyde increased the fruity descriptor at low concentrations of ethyl hexanoate, but without providing high odour intensity values. Several studies have shown that acetaldehyde has a fruity odour [22,23]. Coetzee et al. [14] found that acetaldehyde had odour interactive effect with the fruity descriptor using wine model solutions, showing enhancement at low concentrations and suppression at high concentration. However, an enhancement of the ethyl hexanoate perception by acetaldehyde should be discarded, since the increase of fruity descriptor by acetaldehyde was much higher in samples without ethyl hexanoate (0 mg/L 40% v/v). Thus, in our study assessors could perceive slight fruity notes of acetaldehyde which, in turn, was confused with the trained fruity odour of ethyl hexanoate. In addition, a slight masking effect on fruity odour can be observed in **Figure 4.3** at high levels of ethyl hexanoate.

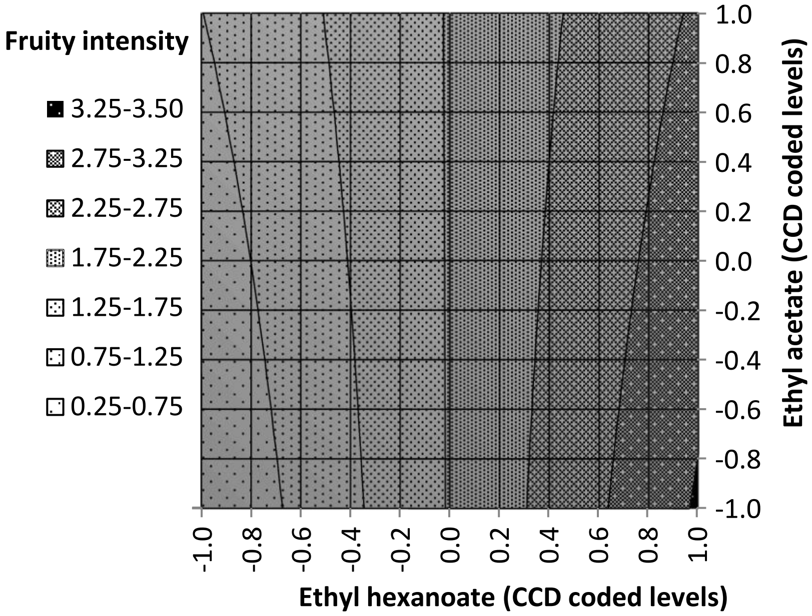

Figure 4.1. Response surface contour plot of flowery sensory response with respect to linalool and ethyl hexanoate coded concentrations (±1.00). Concentrations of ethyl acetate and acetaldehyde were both fixed at -1.00 coded level.

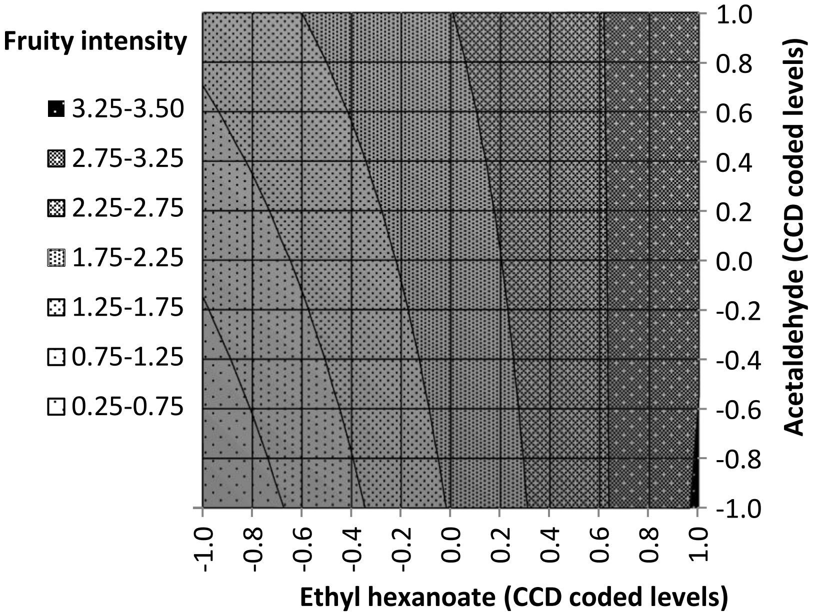

Figure 4.2. Response surface contour plot of glue-like sensory response with respect to ethyl acetate and ethyl hexanoate coded concentrations (±1.00). Concentrations of linalool and acetaldehyde were both fixed at -1.00 coded level.

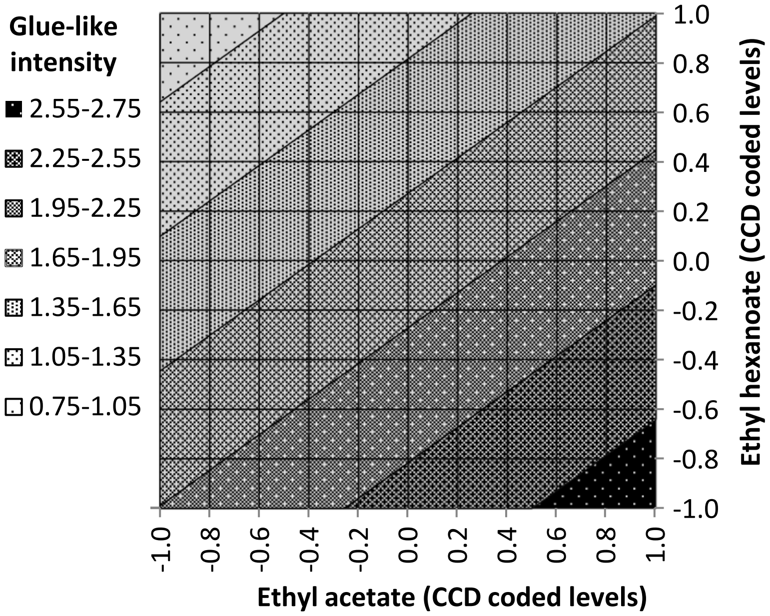

Figure 4.3. Response surface contour plot of fruity sensory response with respect to ethyl hexanoate and acetaldehyde coded concentrations (±1.00). Concentrations of linalool and ethyl acetate were both fixed at -1.00 coded level.

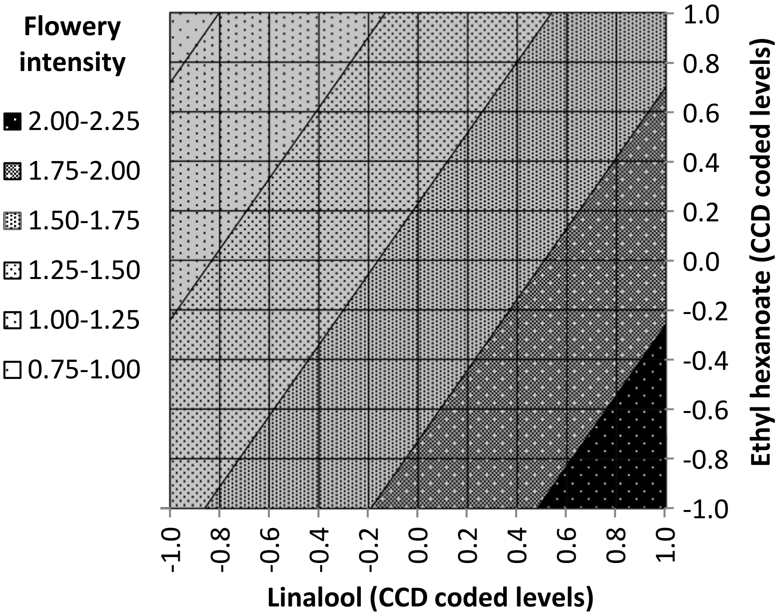

Figure 4.4. Response surface contour plot of fruity sensory response with respect to ethyl hexanoate and ethyl acetate coded concentrations (±1.00). Concentrations of linalool and acetaldehyde were both fixed at -1.00 coded level.

A similar effect can be observed in **Figure 4.4** with a two-way interaction effect between ethyl hexanoate and ethyl acetate ($b_{2,3}$ coefficient). High levels of ethyl acetate enhanced the fruity perception at low levels of ethyl hexanoate, since ethyl acetate could provide a fruity odour at low concentrations [3,23]. Otherwise, high levels of ethyl acetate suppressed the fruity perception at high concentrations of ethyl hexanoate, suggesting a masking odour effect. Thus, as previously mentioned, their structure-odour relationship could lead to confusion of both odour perceptions [21].

Finally, linalool could also provide a fruity odour [22,23], however odour interactive effects were not found in this study.

4.5. Conclusions

In this work, RSM has shown the odour interactive effects between linalool, ethyl hexanoate, ethyl acetate and acetaldehyde on flowery, fruity and glue-like descriptors. Ethyl hexanoate showed a sensory masking effect on linalool and ethyl acetate, in relation to flowery and glue-like odour perception. Acetaldehyde and ethyl acetate increased fruity perception when samples had low ethyl hexanoate levels. High levels of ethyl acetate showed a masking effect on fruity descriptor at high levels of ethyl hexanoate. Finally, highlight that RSM allowed to easily evaluate odour interactive effects between volatile compounds in a medium that produces high olfactory fatigue after setting an only training session,; showing an innovative and easy tool for future sensory multi-interaction studies.

Acknowledgments

P. M.-G. appreciates the Ph.D. Grant “Martí Franquès” (2015PMF-PIPF-29) with the support of the Universitat Rovira i Virgili.

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Chapter 5

FRUIT SPIRIT MATURATION UNDER DIFFERENT STORAGE CONDITIONS ANALYZED BY MULTI-WAY ANOVA AND ARTIFICIAL NEURAL NETWORKS

A study under review at 2018/05/03.

5.1. Abstract

For producers, distributors and restaurateurs, it is essential to understand the variation of the volatile composition of bottled spirits under different storage conditions. Given the scarce information found in this regard, the present study investigates the effect of pH, temperature, light exposure and time of storage on 18 major volatile compounds of a fruit spirit. To carry out this longitudinal study, a central composite design was applied repeatedly over a year. Multi-way ANOVA and artificial neural networks were used to analyze and model the process. The results show that high temperatures (45 ºC) sharply reduce most spirit compounds, especially acetaldehyde, ethyl esters and linalool. In addition, under standard conditions, most compounds undergo a concentration decrease during the first 20 days of storage and then their composition becomes stable. Most of the other studied conditions showed noticeable effects, although without significant compositional differences.

5.2. Introduction

To ensure food-safety and sensory properties of a product, controlling shipping time and shelf life is key for the food and beverage industry. Shipping time is the time taken by a product to get into the market, and in the case of spirits, it should be short enough to guarantee the stability of their aroma profile. Shelf life is the period after which the beverage loses its organoleptic qualities and cannot be commercialized. Since a solution with an alcohol concentration higher than 37.5 % v/v is microbiologically stable, aroma losses in spirits are due to physicochemical transformations, and therefore its labeling is not legally required.

Usually alcoholic beverages need a maturation time after their distillation to stabilize their organoleptic characteristics before bottling. For instance, according to Peruvian regulations [1], Peruvian pisco (Peru's national wine spirit) must rest a minimum of 3 months in inert recipients prior to packaging and marketing to ensure its quality. In addition, unaged cachaça and rum remain for some time in stainless steel tanks for their aroma stabilization [2].

Most of the scientific literature focuses on wood aging, which causes an aroma contribution from wood maceration and oxidative reactions [3–6]. These reactions only occur if noticeable amounts of air or oxygen are added (in barrels or tanks); hence, oxidative reactions do not occur with the amounts of oxygen that remain in a bottle of a distilled spirit [7]. In the case of whiskey, brandy, rum and other aged beverages, aging under suitable oxidative conditions could favor a more pleasant aroma.

Few studies consider the maturation of spirits in inert vessels. Flouros *et al.*, (2003) [8] studied tsipouro (Greek pomace brandy) in PET and PVC bottles at room temperature without light exposure, observing that only furfural levels increased significantly. Rodríguez Madrera *et al.*, (2011) [9] followed the volatile composition of young cider spirits aged in inert containers made of glass and stainless steel, apparently at room temperature. Researchers noticed a decrease in ethyl acetate, ethyl lactate, diethyl succinate and acetal levels, and an increase in fatty acid ethyl esters levels during 24 months of maturation. Furthermore, they carried out a sensory preference test, where spirits aged 12 months had a higher acceptability than spirits aged 0 or 24 months. Qiao and Sun (2015) [10] studied fenjiu (Chinese grain unflavored spirit) maturation in ceramic and glass containers, and they reported an increase of carboxylic acids and β-phenylethanol levels, and a decrease of ethyl esters levels after 3 years.

In addition to the time of storage, environmental conditions can also affect the sensory shelf life of bottled spirits [11]. For example, distributors may transport and store bottled spirits without refrigeration in places that easily reach 45 ºC, like closed trucks or industrial units under the sun. In addition, consumers and restaurateurs usually store bottled spirits over a long period of time, during which they can undergo compositional changes by light-exposure or prolonged periods in refrigerators (≈5 ºC). Temperature has also been widely used to perform accelerated shelf life tests with other types of alcohol beverages, such as cream liqueurs [11–13].

To prevent turbidity after bottling, producers cool the distillates between +10 to -7 ºC to precipitate long-chain ethyl esters since they show low water solubility [7]. Moreover, Kralj Cigić and Zupančič-Kralj (1999) [14] studied UV light irradiation (sunlight-exposure) on Bartlett pear brandy, observing that the level of ethyl trans-2-cis-4-decadienoate (pear ester) diminished. Refsgaard *et al.,* (1995) [15] also reported that light-exposure changed the sensory characteristics of aquavit (Scandinavian flavored spirit) with the formation of a geranium-leaf odor and taste. Many of aquavit's volatile characteristic compounds decreased and some photoproducts were formed. A non-identified compound with low geranium-leaf odor threshold was detected.

Commercial spirits are produced from a wide range of raw materials, since it is possible to produce ethanol from almost all sugars of agricultural origin, preserving their primary and distinctive aroma composition [16]. pH variation can also affect the levels of the spirits' volatile composition since terpenic [17–21], ester [22,23], furfural [24–26], aldehyde [27] and other compounds undergo transformations. Spirits and their fermented raw materials usually have a similar acidity, with a pH ranging between 2.8 and 5.

The aim of this study was to determine the effect of three storage conditions on a bottled fruit spirit: pH, temperature and light-exposure. Two analyses were carried out: first, volatile compounds levels were monitored during 7 months under the selected storage conditions; secondly, volatile compounds levels were analyzed after one year to check the overall effects of the storage conditions. A central composite design (CCD) was used to minimize the number of experiments while obtaining good statistical reliability. In addition, the repeated measurements of the CCD design were analyzed using multi-way ANOVA (for analysis of variance) and artificial neural networks (for modeling the compounds' evolution).

5.3. Material and methods

5.3.1. Design of experiments (DOE)

Face centered Box-Wilson central composite design comprises a full factorial design with center points and with a group of axial points. With a reduced number of experiments, this design allows the study of the variance response using 3 or 5 levels of different factors. Coded variables are often used when constructing this design (±1 unit range). In this study, a 3-level-2-factor face centered central composite design (CCD) was designed, with 3 replicates of the center point (11 runs). Selected factors were spirit pH (2.8, 3.8 and 4.8) and storage temperature (5, 25, 45 ºC). In addition, three additional replicates of the central point were tested for light-exposure conditions. Thus, DOE consisted of 14 runs shown in **Table 5.1**.

Table 5.1. Design of experiments (DOE) for the present study [a]

Experiment	Nº of assay	Light-exposure	pH	Temperature (ºC)
Central Composite Design	1	No	2.8	5.0
	2	No	2.8	45
	3	No	4.8	5.0
	4	No	4.8	45
	5	No	2.8	25
	6	No	4.8	25
	7	No	3.8	5.0
	8	No	3.8	45
	9 (CP)	No	3.8	25
	10 (CP)	No	3.8	25
	11 (CP)	No	3.8	25
Light-exposure experiment	12	Yes	3.8	25
	13	Yes	3.8	25
	14	Yes	3.8	25

[a] "CP" means Center Point

5.3.2. Fruit spirit

Three spirits of different agricultural origin (40 % v/v spirit from fermented pear juice *Pyrus communis* Conference [28], 40 % v/v spirit from fermented kiwi *Actinidia chinensis* Hayward [29] and 40 % v/v spirit from wine *Vitis vinifera* Muscat [30]) were mixed in equal parts (500 mL each) to proceed with a re-distillation.

A 2 L batch glass distillation system was used, with a copper packed column and an internal partial condenser, as previously described in Matias-Guiu *et al.* (2018) [31]. A partial condenser was controlled with a peristaltic pump (313S, Watson-Marlow Ltd., Falmouth, England) at a constant inlet temperature of 20 ºC. The boiler was heated by a heating mantle (Fibroman-C 1000 mL, JP Selecta S.A., Abrera, Spain) at 205 W.

For the distillation, 1.5 L of the mix of spirits was placed in the boiler with 3 g of pumice stone. The cooling water of the partial condenser ran at 300 mL/min while collecting the head-cut (first 40 mL of distillate), which was discarded. During the heart-cut collection (the next 570 mL of distillate, alc. 79.6 % v/v) the cooling water was stopped. The heart cut was diluted at alc. 40 % (v/v) with distilled water and used as base spirit throughout the experimentation. Diluted base spirit had a pH of 3.8, which is within the usual range of unaged commercial distilled spirits.

According to the established DOE, 14 glass bottles of 100 mL (11 amber tinted and 3 transparent for darkness and light-exposure conditions, respectively) were filled with 50 mL of base spirit. Three samples were adjusted to pH 2.8 with sulfuric acid 98% and another three samples were adjusted to pH 4.8 with pellet sodium hydroxide >98% (Sigma-Aldrich, Saint Louis, USA). Light-exposure samples were placed next to a window without direct sunlight. Samples at 5 and 45 ºC were placed at isothermal conditions without light-exposure. Samples at 25 ºC without light-exposure were placed in an opaque box at room temperature.

Samples were analyzed chemically for 7 months (days 10, 20, 30, 60, 90, 120, 150 and 210). After 7 months, samples were kept at room temperature until one year of maturation (day 365) when they were chemically analyzed again.

5.3.3. Chemical analysis

pH was determined by a pH-meter (Crison Basic 20, L’Hospitalet del Llobregat, Spain) and ethanol content by an electronic density meter (DSA 5000M, Anton Paar, Graz, Austria).

Prior to each analysis, samples at 5 and 45 °C were acclimated at room temperature for 20 minutes. For the analysis, 50 µL of the internal standard solution (400 mg/L of by 2-octanol, Sigma-Aldrich, Saint Louis) was added to 1 mL of sample extracted from each bottle.

The sample’s composition was determined by chromatographic analyses performed using a gas chromatograph fitted with a flame ionization detector (GC-FID) (Agilent 6890, Agilent Technologies, Waldbronn, Germany), an autosampler (Agilent 7683, Agilent Technologies, Waldbronn, Germany) and a capillary polar column (MetaWAX, 60 of length, 0.25 mm ID and 0.5 µm of phase thickness) from Teknokroma (Barcelona, Spain). Injection (2 µL) was done in split mode (1:5). Injector and detector temperatures were at 250 ºC and 260 ºC, respectively. Oven temperature program was: 40 ºC (5 min), 7 ºC/min up to 100 ºC (15 min), 3 ºC/min up to 140 ºC and 2 ºC/min up to

200 ºC (5 min). Column-head flow ramp was 0.5 mL/min (28 min) and 5 mL/min^2 up to 1.1 mL/min (67 min) of helium as carrier gas.

Quantifications were carried out by interpolation into calibrations built with synthetic solution doped with all compounds at different levels. Reagents' CAS, supplier companies and Kovats retention indices are shown in **Table S5.1** in Supporting Information. Detection and quantification limits were determined by Signal-to-Noise ratio (S/N) of 3 and 10, respectively. Throughout the study, concentration values below detection limit were considered as 0 mg/L, and values between detection and quantification limits were considered as the average of both limits.

5.3.4. Modeling and statistical analysis

The most common method for analyzing CCD is the Response Surface Methodology (RSM) [32]. However, in the present longitudinal study, CCD assays were analyzed repeatedly over time, which generates more data points than those usually provided to RSM. Therefore, the following predictive and statistical methods have been used.

5.3.4.1. Multi-way ANOVA

Fruit spirit maturation during first 7 months was analyzed by Tukey's HSD test pairwise comparisons after multi-way ANOVA ($p < 0.05$) between DOE storage conditions and storage period (explanatory variables) and volatile compounds concentrations (dependent variables). The aim of using multi-way ANOVA was to achieve an in-depth analysis of variance of all factor levels.

Fruit spirit analysis after 1 year was also performed by Tukey's HSD test pairwise comparisons after ANOVA ($p < 0.05$). Two tests were carried out. In the first test (TEST A), the variance of the composition of central point replicates was analyzed between first and one year analysis at both light-exposure levels. In the second test (TEST B), the variance of the composition of CCD at one year was analyzed.

ANOVA and Tukey's HSD tests were performed with XLSTAT 2017 statistical add-in for Microsoft Office.

5.3.4.2. Artificial neural network (ANN)

To visualize the results better, ANN was used to develop fitting models of compounds' concentrations for the first 7 months of maturation under factor effects. ANN is a powerful nonlinear regression technique widely used to build mathematical models of any kind of process. The main advantage of ANN from other methodologies is that ANN does not require a priori specification of a suitable fitting function. A more extensive explanation can be found in Kuhn and Johnson (2013) [33]. CCD are usually analyzed with RSM's quadratic equation systems; however in this case, that system wouldn't be able to fit the evolution of the compound's concentration over time which follows a sigmoidal curve. Furthermore, some studies have shown that ANN models have consistently better fittings of CCD responses than RSM [34–36].

In the present study, ANN functions were a two-layer feed-forward network, with linear transfer function between inputs and neurons of the hidden layer (Eq. 5.1), sigmoid transfer function in neurons of the hidden layer (Eq. 5.2) and a linear transfer function between the hidden layer and the output (Eq. 5.3).

$$I_j = \sum_{i=1}^{n} \left(W_{ij} \cdot X_i \right) + \theta_j \qquad \text{Eq. 5.1}$$

where *W* are the connection weights between inputs (*i* subscript) and neurons (*j* subscript), *X* are the input values and *Θ* the bias.

$$f(I_j) = \frac{1}{1 + exp\left(-I_j\right)} \qquad \text{Eq. 5.2}$$

$$Output_o = \sum_{j=1}^{n} (W_{jo} \cdot f(I_j)) + \theta_o \qquad \text{Eq. 5.3}$$

where *W* are the connection weights between neurons (*j* subscript) and outputs (*o* subscript) and *Θ* the bias.

The global equation would be as follows:

$$Output_o = \sum_{j=1}^{n} \left(W_{jo} \cdot \frac{1}{1 + exp\left(-\left(\sum_{i=1}^{n} (W_{ij} \cdot X_i) + \theta_j\right)\right)} \right) + \theta_o \qquad \text{Eq. 5.4}$$

The number of neurons was set during the analysis, depending on the fitting performance and the overfitting of the responses. Inputs were CCD factors and time factor (both explanatory variables) and outputs were each compound concentration (response). Therefore, a single neuron implies 6 fitting parameters and 2 neurons imply 11 fitting parameters, since each model consists of 3 inputs and 1 output. Inputs and outputs were normalized within a uniform range of 0.1 – 0.9. Models were trained with the Levenberg-Marquardt algorithm. Data (99 points for each compound) was randomly divided into training, validation and test sets with a ratio of 80%, 15% and 5%, respectively. The performance of the network was evaluated with the Mean Squared Error (MSE). Since data points are limited in this study, ANN was retrained 20 times (each training session started with different initial weights and biases, and different training, validation and test sets) to ensure a good generalization. After retraining, the network with the lowest MSE was selected for the analysis. ANN models were obtained using the Neural Network Toolbox of MATLAB R2015b software package.

Table 5.2. ANOVA model probabilities corresponding to F of Fisher's F test (p-values) of model, main and two-way interaction effects [a].

Compounds (mg/L)	Model	Time	pH	Temp.	Time/pH interaction	Time/temp. interaction	pH/temp. interaction
Acetaldehyde	**< 0.0001**	**< 0.0001**	**0.0123**	**< 0.0001**	0.9685	**< 0.0001**	0.8200
Acetal	**< 0.0001**	**< 0.0001**	**0.0021**	**< 0.0001**	0.7013	**< 0.0001**	**< 0.0001**
Ethyl acetate	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.6903	**< 0.0001**	**0.0134**
Ethyl butyrate	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.3699	**< 0.0001**	0.4375
Ethyl hexanoate	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.1716	**< 0.0001**	0.0095
Ethyl octanoate	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.4877	**< 0.0001**	0.0954
Ethyl decanoate	**< 0.0001**	**< 0.0001**	0.1137	**< 0.0001**	0.3336	**< 0.0001**	0.1528
Ethyl lactate	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.0027**	0.1947	**< 0.0001**
Furfural	**< 0.0001**	**< 0.0001**	0.3477	0.3477	0.9379	0.9379	0.1991
β-phenylethanol	**< 0.0001**	**< 0.0001**	**0.0008**	0.0560	0.2947	0.0590	0.1682
1-hexanol	**< 0.0001**	**< 0.0001**	**0.0008**	**0.0325**	**0.0393**	**< 0.0001**	0.2889
1-propanol	**< 0.0001**	**< 0.0001**	**0.0027**	**0.0004**	0.1403	**0.0094**	0.2103
2-methyl-1-butanol	**< 0.0001**	**< 0.0001**	**0.0009**	**0.0006**	0.1164	**0.0095**	0.2356
3-methyl-1-butanol	**< 0.0001**	**< 0.0001**	**0.0014**	**0.009**	0.0973	**0.0015**	0.2218
Linalool	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.0170**	**0.0001**	**< 0.0001**
α-terpineol	**0.0165**	0.2878	0.1637	**0.0039**	0.1839	0.7547	**0.0002**
Methanol	**< 0.0001**	**< 0.0001**	*0.0297*	**< 0.0001**	0.3710	**0.0015**	0.3403
Acetic acid	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.0025**	**< 0.0001**

[a] Values in bold are significant by ANOVA and Tukey test ($p < 0.05$). Values in italic are significant just by ANOVA ($p < 0.05$). Temp. means temperature.

5.4. Results

5.4.1. Multi-way ANOVA of spirit evolution over 7 month

Table 5.2 shows ANOVA results (*p*-values) for each factor and their two-way interactions. The factors that show more significant differences on the composition of the distillate are time and temperature, followed by pH. Time/temperature interaction also presents significant differences in more than three-quarters of the studied compounds. On the other hand, few compounds present significant differences by time/pH interaction and no compound showed significant differences for light-exposure (data not shown).

To represent ANOVA effects, **Table 5.3** shows means, standard deviations and Tukey's HSD test results for pH and temperature factors, and **Table 5.4** shows the same parameters for the time factor. To explain two-way interaction results, line graphs are used. Ethyl hexanoate interaction plots are shown in **Figure 5.1**, where Y-axis is a continuous variable (mean concentration with ANOVA's confidence interval of 95 % for each discussed compound) and X-axis is a discrete variable (factor levels) where the distance between axis-points may change (i.e. time can have 10, 30 or 60 days between points). Two-way interaction line graphs of the remaining compounds are available in Supporting Information (**Figure S5.1**), given the large number of figures generated during the present study.

It should be noted that significant effects of pH and temperature indicate concentration differences between pH and temperature factor levels without considering the time factor. Significant time/pH and time/temperature interaction effects indicate a stepped or segmented variation throughout the study. For example, ethyl hexanoate has *p*-values < 0.0001 for main effects of pH and temperature (**Table 5.2**), which indicate significant concentration differences between the different levels of temperatures and pH during the 7 months (see **Figure 5.1a, b**). On the other hand, ethyl hexanoate shows no significant effects for time/pH (*p*-value = 0.1716) and significant effects for time/temperature (*p*-value < 0.0001). Therefore, the concentration differences produced by different pH levels remain practically constant throughout the 7 months (**Figure 5.1a**), while the differences produced by different temperature levels vary as function of time (**Figure 5.1b**).

Table 5.3. Compounds means, standard deviations (±) and significant groups (a, b, c) between levels of pH and temperature factors using pairwise multiple comparisons (Tukey HSD Test) after a multi-way ANOVA ($p < 0.05$).

Compounds	pH			Temperature (ºC)		
(mg/L)	2.8	3.8	4.8	5	25	45
Acetaldehyde	58.5 ±17.7 b	63.8 ±16.3 a	61.6 ±17.2 a	67.3 ±3.2 a	67.3 ±4.1 a	47.1 ±27 b
Acetal	28.9 ±9.4 ab	30.4 ±8.8 a	27.5 ±10.5 b	34.4 ±3.6 a	30.6 ±4.4 b	21.7 ±14.1 c
Ethyl acetate	35 ±14.2 b	45.2 ±12.6 a	43 ±13.6 a	49.9 ±4.9 a	45.1 ±8.7 b	28.3 ±17.2 c
Ethyl butyrate	0.389 ±0.214 b	0.484 ±0.167 a	0.457 ±0.193 a	0.541 ±0.102 a	0.498 ±0.116 b	0.282 ±0.246 c
Ethyl hexanoate	3.1 ±1.28 b	3.65 ±1.02 a	3.54 ±1.18 a	4.13 ±0.47 a	3.74 ±0.69 b	2.37 ±1.47 c
Ethyl octanoate	8.85 ±4.28 b	9.72 ±3.8 a	9.68 ±4.03 a	11.1 ±2.7 a	10.2 ±3 b	6.7 ±5 c
Ethyl decanoate	4.91 ±3.3	4.84 ±3.2	4.87 ±3.25	5.68 ±3.1 a	5.07 ±2.99 b	3.72 ±3.45 c
Ethyl lactate	8 ±4.16 c	10.1 ±2.8 b	11.2 ±2 a	11.1 ±2.3 a	10 ±2.9 b	8.23 ±3.97 c
Furfural	0.591 ±0.137	0.577 ±0.129	0.569 ±0.12	0.591 ±0.137	0.577 ±0.129	0.569 ±0.12
β-phenylethanol	0.385 ±0.11 a	0.33 ±0.122 b	0.327 ±0.117 b	0.331 ±0.126	0.337 ±0.115	0.37 ±0.12
1-hexanol	24.1 ±1.4 a	23.3 ±1.2 b	23.5 ±1.3 b	23.9 ±1.4 a	23.4 ±1.1 b	23.5 ±1.4 ab
1-propanol	4034 ±214 a	3929 ±180 b	3954 ±175 ab	4044 ±207 a	3961 ±159 b	3891 ±202 b
2-methyl-1-butanol	188 ±11 a	182 ±9 b	184 ±9 b	188 ±11 a	184 ±8 b	181 ±10 b
3-methyl-1-butanol	827 ±44 a	803 ±36 b	809 ±38 b	825 ±44 a	807 ±33 b	804 ±43 b
Linalool	0.47 ±0.26 c	0.582 ±0.127 b	0.624 ±0.078 a	0.66 ±0.073 a	0.599 ±0.097 b	0.406 ±0.236 c
α-terpineol	1.71 ±0.33	1.77 ±0.11	1.79 ±0.13	1.83 ±0.14 a	1.78 ±0.11 a	1.67 ±0.32 b
Methanol	773 ±47	760 ±46	765 ±36	783 ±39 a	771 ±33 a	737 ±51 b
Acetic acid	17.2 ±6.6 a	9.91 ±5.12 b	8.02 ±3.21 c	8.71 ±4.56 c	10.3 ±5.5 b	15.8 ±6.9 a

Table 5.4. Compounds means, standard deviations (±) and significant groups (letters) between levels of time factor using pairwise multiple comparisons (Tukey HSD Test) after a multi-way ANOVA ($p < 0.05$).

Compounds (mg/L)	Storage period (days)								
	0	10	20	30	60	90	120	150	210
Acetaldehyde	69.5 ±0.5 a	68.1 ±3.1 ab	66.6 ±2.2 ab	67.2 ±2.8 ab	66.2 ±3.7 ab	63.2 ±5.9 b	52.4 ±27.3 c	53.2 ±28.2 c	49.7 ±25.8 c
Acetal	41 ±0.6 a	29.7 ±4.3 b	30.5 ±3.3 b	31.2 ±4.5 b	31.1 ±3.7 b	29.2 ±5.1 b	23.7 ±12.9 c	24 ±13.2 c	22.4 ±12.7 c
Ethyl acetate	55.9 ±0.4 a	48.7 ±6 b	45.4 ±7.7 b	44.9 ±8.6 bc	42.5 ±9.6 bc	38.9 ±10.6 cd	33.7 ±18.2 de	35.2 ±18.6 de	30.9 ±16.5 e
Ethyl butyrate	0.738 ±0.01 a	0.524 ±0.087 b	0.475 ±0.074 bc	0.466 ±0.111 bc	0.467 ±0.121 bc	0.409 ±0.103 cd	0.343 ±0.226 de	0.33 ±0.224 de	0.307 ±0.226 e
Ethyl hexanoate	4.98 ±0.02 a	4.4 ±0.44 b	3.67 ±0.53 c	3.54 ±0.56 c	3.43 ±0.77 c	3.02 ±0.85 d	2.73 ±1.29 de	2.82 ±1.39 de	2.65 ±1.33 e
Ethyl octanoate	16.7 ±0 a	13.7 ±1.2 b	9.69 ±1.26 c	9.12 ±1.41 cd	8.77 ±1.95 d	7.28 ±2.07 e	6.76 ±2.91 ef	6.93 ±3.43 ef	6.25 ±3.36 f
Ethyl decanoate	11.6 ±0.1 a	9.02 ±1.27 b	4.66 ±1 c	4.04 ±1.02 d	3.95 ±0.87 d	2.77 ±0.65 e	2.47 ±0.88 e	2.6 ±1.13 e	2.71 ±1.22 e
Ethyl lactate	14.8 ±0.1 a	14.1 ±1.7 a	9.28 ±1.83 b	9.01 ±2.09 bc	8.88 ±2.45 bc	7.86 ±2.22 c	8.19 ±2.45 bc	7.9 ±2.4 c	8.3 ±2.66 bc
Furfural	0.903 ±0.015 a	0.588 ±0.136 b	0.554 ±0.087 b	0.527 ±0 b	0.527 ±0 b	0.527 ±0 b	0.527 ±0 b	0.527 ±0 b	0.527 ±0 b
β-phenylethanol	0.554 ±0.007 a	0.468 ±0.068 b	0.282 ±0.079 cd	0.284 ±0.082 cd	0.287 ±0.082 cd	0.244 ±0.071 d	0.303 ±0.082 cd	0.319 ±0.059 cd	0.357 ±0.092 c
1-hexanol	25 ±0.2 a	24.7 ±1 ab	23.3 ±1.2 c	23.4 ±1.8 c	23.3 ±0.5 c	21.9 ±0.6 d	23.8 ±1.4 bc	23.8 ±0.7 bc	23.1 ±1.1 c
1-propanol	4114 ±43 a	4089 ±152 a	3941 ±181 abc	3979 ±289 ab	3954 ±86 ab	3763 ±98 c	3984 ±189 ab	3987 ±209 ab	3867 ±157 bc
2-methyl-1-butanol	192 ±1 a	192 ±8 a	183 ±9 b	185 ±14 ab	183 ±4 b	173 ±5 c	184 ±8 ab	185 ±9 ab	179 ±7 bc
3-methyl-1-butanol	845 ±8 a	837 ±32 ab	805 ±38 bc	811 ±61 abc	805 ±18 bc	765 ±20 d	819 ±40 abc	819 ±30 abc	795 ±35 cd
Linalool	0.775 ±0.015 a	0.621 ±0.124 b	0.579 ±0.119 b	0.582 ±0.142 b	0.572 ±0.125 b	0.494 ±0.195 c	0.482 ±0.185 c	0.479 ±0.187 c	0.482 ±0.209 c
α-terpineol	1.84 ±0.01	1.76 ±0.07	1.76 ±0.09	1.8 ±0.15	1.75 ±0.11	1.64 ±0.14	1.78 ±0.18	1.78 ±0.3	1.76 ±0.43
Methanol	796 ±12 a	792 ±28 a	765 ±30 ab	773 ±52 ab	774 ±17 a	734 ±20 b	757 ±49 ab	758 ±68 ab	733 ±44 b
Acetic acid	14.5 ±0.3 a	8.6 ±3.17 cd	8.37 ±4.9 d	9.55 ±6.36 bcd	11.7 ±7.8 abcd	10.6 ±6.4 bcd	14.8 ±9.5 a	12 ±6.3 abc	12.3 ±5.7 ab

5.4.2. ANN models of spirit evolution over 7 month

Table 5.5 shows the number of hidden neurons used for fitting ANN models and fitting performance parameters.

Models of compounds were set with 2 neurons, except for acetaldehyde and ethyl acetate that were modeled using 1 neuron. The increase of the number of neurons produced an overfitting (data not shown), graphically noticeable by comparing the surface plots of ANN results with the ANOVA analysis. This behavior was expected since the factors of CCD were set with a few data points (3 levels) and neural networks have the tendency to predict illogical curves between level points to improve the fit when a high number of hidden neurons are used. Apart from Mean Square Error (MSE), other parameters have been calculated to evaluate the goodness of fit of ANN models. RMSE is the square root of the MSE, another customary performance measurement of ANN models. Mean Absolute Percentage Error (MAPE) measures the mean of the sum of relative errors, considering the experimental value as the reference value. Symmetric Mean Absolute Percentage Error (SMAPE) also measures mean of the sum of relative errors, but considering the mean of the experimental and predicted value as the reference value. SMAPE values are divided by two so that their range is normalized in a percentage. Finally, the correlation coefficient (R^2) has been also included to enrich the discussion, taking into account that R^2 is not the most suitable parameter to analyze nonlinear regressions. MAPE and SMAPE are the most quickly understandable parameters to evaluate regression, since they are expressed in percentage (values close to 0 indicate a lower deviation of the model with respect experimental values). For R^2, values close to 1 show better model-fitting.

Alcohols are the compounds that show the worst fit according to all statistical parameters; however, the interpretation of the goodness of fit of the rest of the compounds varies depending on the parameter analyzed. Since non-linear systems are complex, visual analyses of the fitting results help researchers better assess the suitability of a given model. ANN model of ethyl hexanoate is represented in **Figure 5.2.** ANN representations for the remaining compounds are available in Supporting Information (**Figure S5.2**) given the large amount of figures generated for the present study.

Table 5.5. Modeling and statistical parameters of ANN models for each studied compound concentration [a].

Compound	Neurons	MSE	RMSE	MAPE (%)	SMAPE (%)	R^2
Acetaldehyde	1	0.0019	0.044	6.10	3.10	0.950
Acetal	2	0.0039	0.062	7.77	4.01	0.886
Ethyl acetate	1	0.0089	0.094	12.2	6.43	0.790
Ethyl butyrate	2	0.0049	0.070	11.9	6.06	0.876
Ethyl hexanoate	2	0.0034	0.059	8.62	4.45	0.926
Ethyl octanoate	2	0.0013	0.036	6.60	3.31	0.967
Ethyl decanoate	2	0.0017	0.041	9.17	4.48	0.967
Ethyl lactate	2	0.0025	0.05	8.56	4.39	0.934
Furfural	2	0.0087	0.093	16.6	5.96	0.858
β-phenylethanol	2	0.0176	0.133	39.5	21.9	0.730
1-hexanol	2	0.0163	0.128	24.4	12.9	0.386
1-propanol	2	0.0132	0.115	20.5	10.5	0.310
2-methyl-1-butanol	2	0.0144	0.120	22.7	11.9	0.355
3-methyl-1-butanol	2	0.0167	0.129	26.4	14.0	0.333
Linalool	2	0.0025	0.050	7.83	3.93	0.916
α-terpineol	2	0.0029	0.054	6.55	3.25	0.641
Methanol	2	0.0093	0.097	13.7	6.97	0.462
Acetic acid	2	0.0074	0.086	17.4	7.97	0.758

[a] "Neurons" means the number of hidden neurons used in ANN models. "MSE" means Mean Squared Error. "RMSE" means Root-Mean-Square Error. "MAPE (%)" means Mean Absolute Percentage Error. "SMAPE (%)" means Symmetric Mean Absolute Percentage Error. "R^2" means R-squared coefficient or correlation coefficient.

Figure 5.2 and the other ANN representations (**Figure S5.2**) are composed of 4 graphs. The top left graph shows the regression plot that assesses the goodness of fit (target versus predicted values). The three remaining graphics in the figure correspond to the two-way interactions surface plots between factors (axes x and y) and model response (axis z). For better interpretation of surface plots, the initial analytical concentration is indicated with a red line at initial time (day 0).

5.4.3. Spirit ANOVA analysis after 1 year

Table 5.6 shows the means, standard deviations and Tukey's HSD test comparison between studied factors. Two separate analyses have been carried out, since not all factors interact with all factor levels. The first analysis (TEST A) tests the composition of initial samples (day 0), one year central point samples (day 365) and one year light exposure experiment samples (day 365). The second analysis (TEST B) tests compounds' composition between levels of pH and temperature factors after one year (CCD samples at 365 days).

Time and temperature are the factors that show more significant differences at one year for most compounds (**Table 5.6**), as it has been already seen during the analysis of the aging process (**Table 5.2**). Except for acetaldehyde, furfural, alcohols and acetic acid that remained stable over time, compounds tended to decrease their concentration after one year. β-phenylethanol shows a small concentration reduction with light exposure. The pH effect after one year presented less significant differences than those observed during the aging process, since only ethyl lactate, linalool and acetic acid show significant differences. On the other hand, temperature does maintain many significant differences, since its effect is much more pronounced especially between 25 and 45 ºC, where a sharp decrease of most of the compounds is shown.

5.5. Discussion

5.5.1. Spirit evolution along 7 month

5.5.1.1. pH effect along 7 months

The ANOVA test (**Table 5.2**) presents significant pH main effects and non-significant time/pH effects for acetaldehyde and acetal. Therefore, the variation produced by the pH effect occurred at the beginning of the process and remained constant through time. The pH main effect (**Table 5.3**) shows a small but significant decrease of acetaldehyde and acetal levels with low and high pH, respectively. ANN models of both compounds do not reflect the effect of pH (**Figures S2a** and **S2b**), probably because the variation produced is small. These compositional variations occur since acetaldehyde react in acid media to form acetal [27]. In turn, this reaction could transform the pungent off-flavor of acetaldehyde into the fruity and sherry aroma of acetal [16].

With low pH, ethyl ester levels drop significantly (except for ethyl decanoate) and acetic acid concentration increases (**Table 5.3**). Ethyl lactate shows significant differences for time/pH interaction effects (**Table 5.2**); a lower pH decreases ethyl lactate concentration faster (**Figure S5.1g**). ANN models of most ethyl esters correctly reflect the significant effects described by ANOVA, as can be seen in the case of ethyl hexanoate (**Figure 5.1** and **2)**; the exception however is for ethyl acetate where the ANN model (**Figure S5.2c**) shows a noticeable time/pH effect that is non-significant according to the ANOVA results.

In hydro-alcoholic solutions like fermented beverages and spirits, ethyl esters are in equilibrium with their respective carboxylic acids. However, in a freshly distilled spirit, ester concentrations are above equilibrium since they are more volatile than their respective acid form. In addition, low pH favors even lower ester concentrations [22,23] since the dissociation equilibrium of the carboxylic acid is displaced towards its molecular form, causing a reduction of the anionic form. Hence, the lower pH decreased ethyl acetate and increased acetic acid (**Table 5.3**). The same behavior can be assumed for the other ethyl esters, and it could explain a loss of fruity character.

β-phenylethanol, 1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol show higher levels at lower pH (2.8) than at higher pH (3.8 or 4.8), as can be seen in **Table 5.3**. Although, their concentration variation by pH effect is very small, and ANN models correctly reproduce these trends (**Figures S2i, S2k, S2l** and **S2m**). pH effect may come from the esterification equilibrium of alcohols with certain carboxylic acids. 1-hexanol also showed effects in response to pH (**Table 5.3**) and time/pH interaction, however these variations are very small and time/pH interaction only has an impact the first month. These trends are not well reproduced by ANN models and barely noticeable in ANOVA representations (**Figures S1j** and **S2j**).

Table 5.3 shows that linalool presents significant level differences between the different pH levels, since terpenic compounds react in acid media [17–21]. Like ethyl lactate, linalool concentration decreases over time more rapidly at low pH (**Figure S5.1n**). However, unlike ethyl lactate, the concentration of linalool appears to decrease at pH 3.8 and 4.8 during the first 10 days. These effects are correctly reproduced by ANN representations (**Figure S5.2n**). This could explain the loss of the flowery character of the distillate, especially in white wine and pomace spirits (i.e. Pisco, Orujo and Grappa).

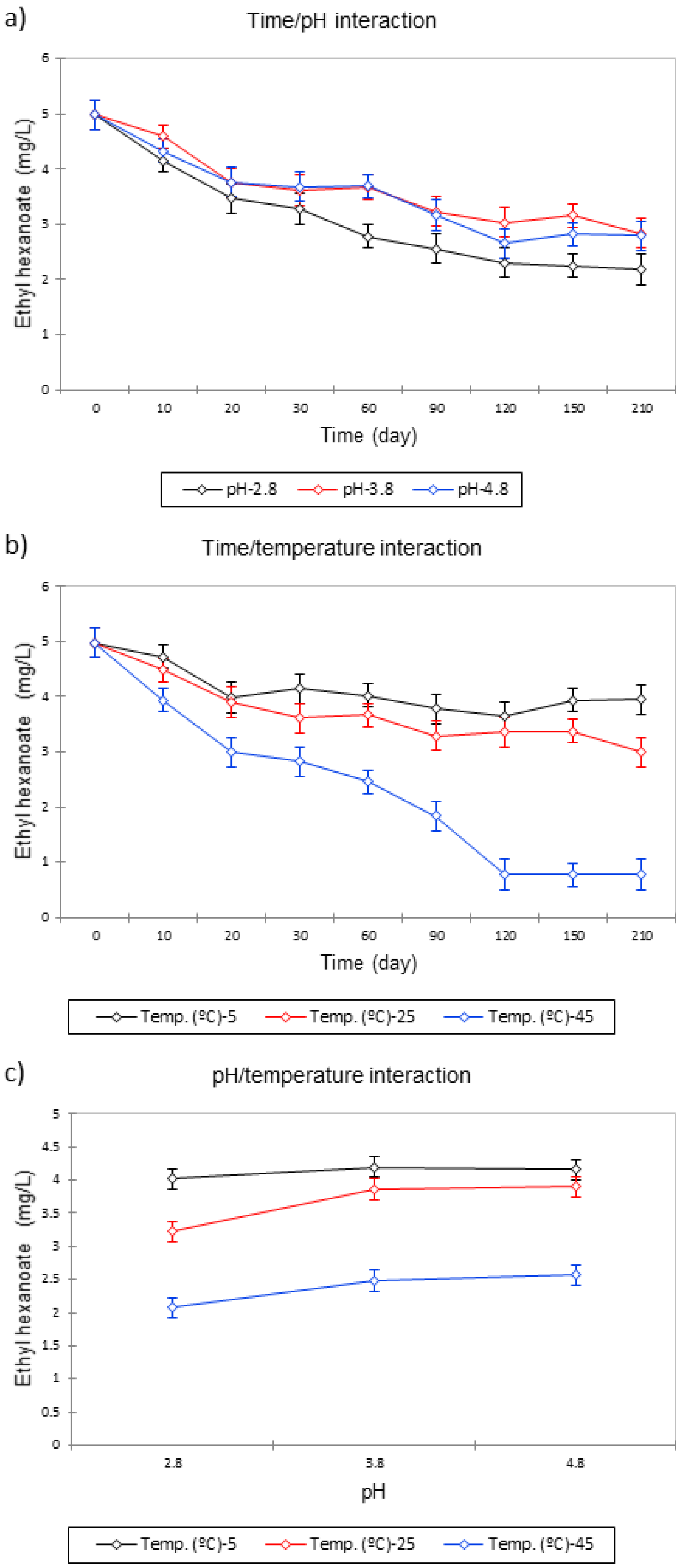

Figure 5.1. Line graphs of ethyl hexanoate mean concentration with confidence intervals of 95 %: two-way interaction effects between studied factors.

5.5.1.2. Temperature effect along 7 months

The temperature factor shows the greatest significant differences for most compounds, except for furfural and β-phenylethanol (**Table 5.2**). In particular, temperature variation between 25 and 45 ºC presents the largest differences, showing a decrease of most compound levels at 45 ºC, except for acetic acid that increases (**Table 5.3**). Again, acetic acid levels exhibit an inverse trend to ethyl acetate, given their equilibrium. The decrease of concentrations due to a high temperature is much more marked with the most volatile compounds (acetaldehyde, acetal and C_2-C_{10} ethyl esters). Regarding terpenic compounds, linalool presents significant differences at the three temperature levels, and α-terpineol between 25 and 45 ºC, since both react in hot acid media [17–21]. Moreover, most compounds present smaller but also significant differences between 5 and 25 ºC conditions, especially ethyl esters, alcohols, linalool and acid acetic. These sharp drops of compound concentrations should clearly affect the complexity of the spirit.

The effect of temperature is closely related to the time factor, since most compounds show significant time/temperature effects (**Table 5.2**). Acetaldehyde and acetal time/temperature interaction effects are well represented in ANOVA line graph plots (**Figures S1a** and **S1b**), although both maturation processes may be better appreciated with surface plots of ANN models (**Figures S2a** and **S2b**). The time/temperature effect indicates no major differences between samples at different temperatures from day 0 to 90. Samples between 90 to 120 days presented a considerable concentration drop. From 120 to 210 days, the concentrations remained stable.

Time/temperature interaction effects for ethyl esters show that a higher temperature reduces their concentration faster (**Figure 5.1** for ethyl hexanoate). These effects are well represented with ANN models (**Figure 5.2** for ethyl hexanoate). Again, acetic acid showed the opposite time/temperature behavior versus ethyl esters (**Figures S1q** and **S2q**). With respect to the comparison between C_2-C_{10} esters, low molecular weight esters seem to be more susceptible to the effect of temperature, since the concentration differences between samples at different temperatures are greater as the ester molecule is smaller. Linalool presents a similar time/temperature effect since it reacts to hot acid media [19], i.e., linalool level decreases when temperature increases

(**Figures S1n** and **S2n**).

Alcohols also showed time/temperature effects, however the variation is small and only during the first month. Methanol, in contrast, presents a small but constant variation in response to the time/temperature effect. Although alcohol effects are barely noticeable on both ANOVA and ANN representations, ANN models may allow a more comprehensible visualization (**Figures S2j, S2k, S2l, S2m** and **S2p**).

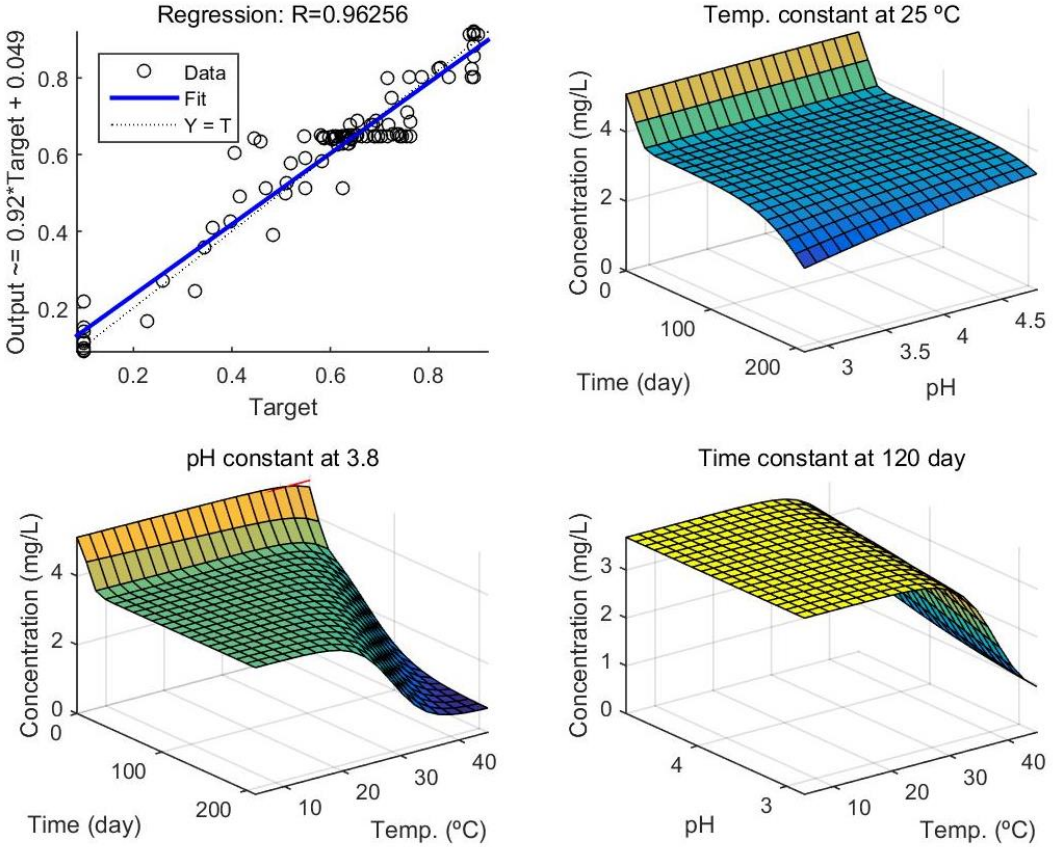

Figure 5.2. ANN model of ethyl hexanoate concentration: regression plot and surface diagrams of the predicted response.

5.5.1.3. Time effect along 7 month

Table 5.4 shows means, standard deviations and Tukey's HSD test of compounds with respect to the effect of storage time. Given the high influence of pH and temperature effects, it is important to emphasize that values and significant differences of **Table 5.4** account for all samples at all pH and temperature levels. For this reason, compositional changes due to the CCD factors may be easier to analyze with ANN surface plots (**Figure 5.2** and **S2**).

Table 5.6. Compounds means, standard deviations (±) and significant groups (letters) between initial composition and light exposure levels after 1 year of bottling (TEST A) and between levels of pH and temperature factors after 1 year of bottling (TEST B), by using pairwise multiple comparisons (Tukey HSD Test, $p < 0.05$) after a multi-way ANOVA ($p < 0.05$).

Compounds (mg/L)	TEST A			TEST B					
	Day 0	Day 365, without light exposure	Day 365, with light exposure	pH			Temperature (ºC)		
				2.8	3.8	4.8	5	25	45
Acetaldehyde	69.5 ±1.2	63.7 ±13.2	68.3 ±4.2	46.7 ±29.7	52.9 ±28	49.6 ±30.9	64.5 ±3.9 a	65.5 ±9.7 a	10.7 ±4.4 b
Acetal	41 ±1.4 a	28.2 ±7.6 b	32 ±1.1 ab	21.8 ±14.2	23.4 ±13	21.3 ±13.7	28.8 ±1.1 a	29.3 ±5.6 a	4.4 ±1.88 b
Ethyl acetate	55.9 ±0.9 a	33.3 ±10.9 b	37 ±2.2 b	20.3 ±6.6	31.1 ±15.7	35.7 ±24.2	41.1 ±13.6 a	34.1 ±12.1 a	9.88 ±2.69 b
Ethyl butyrate	0.738 ±0.023 a	0.382 ±0.17 b	0.39 ±0.042 b	0.186 ±0.186	0.315 ±0.214	0.329 ±0.287	0.359 ±0.15 a	0.409 ±0.137 a	0 ±0 b
Ethyl hexanoate	4.98 ±0.04 a	2.64 ±0.61 b	3 ±0.15 b	1.86 ±0.99	2.51 ±1.18	2.87 ±1.84	3.6 ±0.78 a	2.72 ±0.69 a	0.775 ±0 b
Ethyl octanoate	16.7 ±0.1 a	5.78 ±1.29 b	6.26 ±0.51 b	4.66 ±3.2	5.62 ±3.61	6.44 ±5.21	9.68 ±1.84 a	5.96 ±1.23 b	0.849 ±0.405 c
Ethyl decanoate	11.6 ±0.2 a	3.04 ±0.31 b	3.12 ±0.07 b	2.64 ±1.8	2.82 ±1.56	2.79 ±2.27	4.52 ±0.38 a	3.1 ±0.24 b	0.438 ±0.159 c
Ethyl lactate	14.8 ±0.2 a	11.8 ±0.8 b	11.8 ±0.5 b	7.13 ±0.81 c	11.4 ±2.6 b	16.6 ±1.8 a	13.3 ±4.8 a	11.9 ±4 ab	9.48 ±4.37 b
Furfural	0.903 ±0.034	0.683 ±0.27	0.527 ±0	0.631 ±0.179	0.687 ±0.224	0.656 ±0.224	0.527 ±0	0.76 ±0.22	0.638 ±0.191
β-phenylethanol	0.554 ±0.016 a	0.343 ±0.007 c	0.392 ±0.027 b	0.48 ±0.137	0.389 ±0.09	0.408 ±0.072	0.421 ±0.124 ab	0.355 ±0.039 b	0.525 ±0.047 a
1-hexanol	25 ±0.4	24.9 ±1.2	25 ±0.9	24.5 ±0.4	24.5 ±1	25.4 ±1.1	25.1 ±0.9	25 ±1	24 ±0.2
1-propanol	4114 ±95	4187 ±232	4201 ±147	4046 ±141	4070 ±262	4206 ±269	4183 ±141 ab	4212 ±191 a	3832 ±101 b
2-methyl-1-butanol	192 ±3	192 ±9	193 ±7	188 ±6	187 ±11	195 ±13	193 ±8 ab	194 ±8 a	178 ±5 b
3-methyl-1-butanol	845 ±19	852 ±43	852 ±30	830 ±13	834 ±41	861 ±42	850 ±29	856 ±37	806 ±14
Linalool	0.775 ±0.034 a	0.541 ±0.028 b	0.551 ±0.047 b	0.155 ±0.268 b	0.463 ±0.268 ab	0.609 ±0.096 a	0.594 ±0.117 a	0.464 ±0.269 ab	0.169 ±0.293 b
α-terpineol	1.84 ±0.03 a	1.71 ±0.05 ab	1.68 ±0.08 b	1.3 ±0.65	1.74 ±0.06	1.83 ±0.03	1.8 ±0.05	1.71 ±0.1	1.36 ±0.7
Methanol	796 ±27	826 ±58	832 ±28	778 ±61	790 ±80	815 ±84	823 ±28 a	833 ±46 a	698 ±26 b
Acetic acid	14.5 ±0.6	12.2 ±0.7	13.9 ±2.4	31.4 ±3.3 a	11.2 ±1.6 b	10.2 ±1.8 b	16.2 ±10.1	15.8 ±9.4	17.7 ±14.2

In most compounds, two marked concentration drops over time were produced, the first between 0 and 90 days and the other between 90 and 210 days. During the first 10 days of the first drop, we observed a sharp reduction in the levels of acetal, ethyl acetate, ethyl butyrate, furfural, linalool and acetic acid. In turn, the remaining ethyl esters, β-phenylethanol and 1-hexanol show a larger reduction between days 10 and 20. In the second drop, most compounds (except furfural and alcohols) presented lower mean values and higher standard deviations in the last samples due to the variation produced by time/pH and time/temperature interactions (especially at the level of 45 ºC).

5.5.1.4. pH/temperature interaction effect along 7 months

The pH/temperature effect for acetal concentration observed by ANOVA (**Table 5.2**) is statistically significant (< 0.0001) but small. High levels of both factors favor the reduction of acetal concentration (**Figure S5.1a**). The ethyl esters ethyl acetate, ethyl hexanoate (**Figure 5.1** and **2**) and ethyl lactate showed pH/temperature interactions (**Table 5.2**). Low pH and high temperatures favor the reduction of their concentration. However, their ANN representations only show a clear pH/temperature effect with the ethyl lactate model (**Figure S5.2g**), which is the one that shows the most significant differences in the ANOVA test (p <0.0001). Terpenic compounds behaved similarly; linalool showing a strong effect and α-terpineol showing a weak effect (**Figure S5.1n** and **S1o**). Finally, low pH with elevated temperatures increases acetic acid concentration, a behavior related to ethyl acetate results.

5.5.1.5. ANN models evaluation

At first sight, surface analysis after ANN modeling allows a faster interpretation of the results with respect to ANOVA. In this study, ANN representations have been compared with ANOVA results to check ANN fitting ability.

According to their fitting quality parameter values (MSE, RMSE, MAPE, SMAPE and R^2), acetaldehyde, acetal, ethyl ester and terpenic compounds evolutions are well represented by their respective ANN model, while alcohol evolutions are not (**Table 5.5**). ANN models with bad fitting quality parameters show variations in the studied factors that are smaller than the experimental errors. In contrast, ANN models with good fitting quality parameters show variations in the studied factors that are much larger than the experimental errors. However, according to the ANOVA tests, the evolution of almost all compounds are well represented by their respective ANN surface plot, except for the ethyl acetate model (**Figures S2c**) which fits the data well (**Table 5.5**) even though its pH effect differs from ANOVA results. Consequently, ANN models were useful to represent the observed evolution of the studied compounds independently of the values of their fitting quality parameters.

Both methodologies, ANOVA and ANN modeling, are complementary. ANOVA is a more sensitive test and allows an exhaustive analysis of the results, although it requires a considerable interpretation effort. In turn, the representation of the ANN models with surface diagrams greatly simplify the visualization and analysis of the results, providing a useful estimation of the responses within the studied range of Design of Experiment (DOE) factors. ANN models though do not pick up some significant effects that showed very small concentration variations according to ANOVA. Therefore, both methodologies are mutually reinforced to easily achieve a more comprehensive and accurate analysis of longitudinal studies.

5.5.2. Storage conditions effects after 1 year

The TEST A column of **Table 5.6** shows means, standard deviations and significant differences (ANOVA) of the evolution of the composition of the central point (standard conditions with and without light exposure) after one year. Ethyl esters have been the compounds that showed the greatest variation during this period. C_2, C_4 and C_6 esters have been reduced by 45 %; C_8 and C_{10} were reduced by 70% approx. Furthermore, significant effects of the light exposure factor are small, as was already observed during the first 7 months.

In most cases, our results agree with the few studies about aging without wood found in the literature, even though many of these studies used spirits from different raw materials. Studies about light-exposure are scarce and without comparable conclusions [14,15]. Qiao *et al.* (2015) [10] (fenjiu spirit) and Mangas et al. (1996) [3] (cider spirit) observed an increase of organic acids; however, the present study observed a decrease of acetic acid, like the study of Dieguez *et al.*, (2002) [37] that used a raw material (grape pomace spirit) similar to that used in our study. Like in our study, Madrera *et al.*, (2011) [9] observed a strong decrease of acetal concentration. Regarding alcohols, Qiao *et al.* (2015) [10]

reported no significant differences with 1-propanol and methanol, a behavior that we confirm with these and the other alcohols.

The TEST B column of **Table 5.6** shows means, standard deviations and significant differences between factor levels (multi-way ANOVA) of the 1 year composition. pH effects on the spirit are small. Esters and linalool are the compounds that have shown a greater variation of their concentration (decreasing at low pH), widely explained throughout the discussion. No studies about the effect of pH on the volatile composition of spirit drinks have been found in the literature. Our results suggest that since the first days of storage, low pH reduces relevant compounds associated with fruity and terpenic flavors (ethyl esters and linalool) and reduces pungent aroma (acetaldehyde), but after one year of stabilization no significant concentration variations were observed.

After one year of maturation, high temperatures reduced negative “head-cut” aromas (acetaldehyde, acetal and ethyl acetate) and pleasant aromas (particularly linalool and fruity ethyl esters) (**Table 5.6**). Dieguez *et al.*, (2002) [37] studied the variation of volatile organic acids in fresh spirits at low (5 ºC) and room temperature during 7 months, and found no significant differences with any carboxylic acids: However, in the present study, at 5 ºC ethyl esters showed higher levels and acid acetic showed lower levels. No studies about storing spirit beverages at high temperatures (45 ºC) have been found. Similar studies performed with bottled wine at different temperatures were compared to the present work, although the composition of fermented beverages differs from their distillates. Recamales *et al.*, (2011) [38] found that temperature fluctuation had the greatest impact on the volatile compounds in white wine compared with the other factors studied (light-exposure, storage period and bottle position), which also happened in the present study with samples at 45 ºC. In wines, ethyl esters appear to be more sensitive and susceptible to changing temperature conditions than other volatile components [39]. Robinson *et al.*, (2010) [40] noticed that high temperatures decrease levels of ethyl octanoate and ethyl decanoate in wine, among other compounds. Benitez *et al.*, (2006) [41] studied the effect of high temperature on fortified wines, reporting a decrease of almost all volatile compounds, in particular ethyl esters, acids and alcohols. Regarding head-cut compounds, Recamales et al., (2011) [38] reported a decrease of acetaldehyde in white wine due to temperature variations. Nevertheless, other studies also have documented an increase of furfural and ethyl esters with an increasing temperature in bottled wines [39], contrary effects to those found in the present study. Young wine contains residual sugars (which react and form furfurals by Maillard reactions [24]) and abundant organic acids (which react to form esters); whereas in young spirits (recently distilled) there are no residual sugars and the ester form predominates instead of the acid one.

Maintaining bottled fruit spirits at 45 ºC reduces most compound levels during the first 7 months, remaining stable for the following 5 months. Consequently, distributors and producers may be warned of storing spirit beverages in hot environments for long, since the flavor of the product will be damaged. On the other hand, maintaining spirits at reasonably high temperatures could be useful for producing "clean" distillates, although at the cost of losing positive aromas.

Acknowledgments

P. M.-G. appreciates the Ph.D. Grant “Martí Franquès” (2015PMF-PIPF-29) with the support of the Universitat Rovira i Virgili.

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Chapter 6

CONCLUSIONS

The main conclusion of this thesis is that batch distillation columns with an internal partial condenser have a great ability to produce very different spirit drinks from the same raw material with marked chemical and organoleptic characteristics by setting the optimal operational strategies.

Furthermore we can conclude that:

- With regard to the operational conditions of the distillation, a drastic reduction of the internal rectification after high rectification during head fractions increase terpenic compounds levels in the subsequent fractions, producing a spirit enriched in floral notes. The drastic cooling flow reduction also increases higher alcohols and esters and decreases head compounds levels in heart fractions.
- Initial wine pH and distillation fractions volumes may be adjusted to perform an optimal strategy, depending on which aromas are meant to be enhanced or reduced.
    - Wine pH: acid media favors the decomposition of linalool and acetaldehyde to form α-terpineol and acetal, respectively, and favors the formation of ethyl esters by esterification.
    - Fractions volumes: larger head-cut decrease head compounds (off-flavors), at the cost of reducing C_4 – C_{10} ethyl esters and linalool levels (positive odors) and increasing the level of tail-cut compounds (longer distillation time).
- With regard to the aroma interaction effects, ethyl hexanoate (fruity odor) shows a sensory masking effect on linalool (flowery odor) and ethyl acetate (glue-like odor). Acetaldehyde and ethyl acetate increase fruity perception at low levels of ethyl hexanoate. High levels of ethyl acetate show a masking effect on the fruity descriptor at high levels of ethyl hexanoate.
- With regard to storage conditions after distillation, low pH reduces relevant compounds associated to fruity and terpenic flavors (ethyl esters and linalool) and pungent aroma (acetaldehyde) during the first seven month, however this effect is barely significant after one year.

- Maintaining bottled fruit spirits at 45 ºC reduces considerably most compounds levels. Distributors and producers may be warned of storing spirit beverages in hot environments which could clearly damage the product flavor. However, maintaining spirits at high temperatures could be useful for producing non-aromatic spirits.

Appendices

SUPPLEMENTARY INFORMATION

For the bound format, supplementary data is provided in digital format to avoid generating unnecessary paper expenses, according to the sustainability criteria of Universitat Rovira i Virgili. For this purpose, a CD-ROM that contains a file called “S_data_thesis_matiasguiu.pdf” is attached to the back cover. For the digital depository TDX (Tesis Doctorals en Xarxa), supplementary data is added below with the aim of unifying into a single document.

The following tables and figures are appended.

For Chapter 3:

Table S3.1. Parameters of calibrated compounds for chemical analysis, ordered according to their chromatographic retention time.

Table S3.2. Goodness of fit statistics parameters for models of Table 3.3.

Figure S3.1. Graphical example of the curve of the desirability function to maximize (LTB-type) or minimize (STB-type) the sensory impact of a compound.

Figure S3.2. Graphical example for the interpretation of the regression models of Table 3.3 using contour plots.

Figure S3.3. Spearman correlations bar chart between aroma descriptors and hedonic rating ($p < 0.05$), carried out with 17 trained assessors.

For Chapter 5:

Table S5.1. Reagents’ CAS, supplier companies and Kovats retention indices.

Figure S5.1. Line graphs of two-way interactions of compounds concentrations, where each compound is associated with a letter of the alphabet (i.e. acetaldehyde figure is **Figure S5.1a**).

Figure S5.2. Representations of ANN models of compounds concentrations, where each compound is associated with a letter of the alphabet (i.e. acetaldehyde figure is **Figure S5.2a**).

Index of the supporting data

Table S3.1. Parameters of calibrated compounds for chemical analysis, ordered according to their chromatographic retention time.

Compounds	CAS	Supplier company	Minimum assay (%)	Calculated Kovats retention indices
Acetaldehyde	75-07-0	Sigma-Aldrich, Saint Louis, USA	99.5	730
Acetal	105-57-7	Sigma-Aldrich, Saint Louis, USA	98.0	872
Ethyl acetate	141-78-6	Sigma-Aldrich, Saint Louis, USA	99.0	878
Methanol	67-56-1	PanReac Química, S.A.U. Castellar del Valles, Spain	99.9	917
Ethyl butyrate	105-54-4	Sigma-Aldrich, Saint Louis, USA	99.7	996
1-propanol	71-23-8	Sigma-Aldrich, Saint Louis, USA	99.5	1033
Ethyl hexanoate	123-66-0	Sigma-Aldrich, Saint Louis, USA	99.0	1167
2-methyl-1-butanol	137-32-6	Sigma-Aldrich, Saint Louis, USA	98.0	1206
3-methyl-1-butanol	123-51-3	Sigma-Aldrich, Saint Louis, USA	98.0	1213
1-hexanol	111-27-3	Sigma-Aldrich, Saint Louis, USA	99.0	1352
Ethyl lactate	687-47-8	Sigma-Aldrich, Saint Louis, USA	98.0	1361
Ethyl octanoate	106-32-1	Sigma-Aldrich, Saint Louis, USA	99.0	1383
2-octanol	123-96-6	Sigma-Aldrich, Saint Louis, USA	97.0	1408
Furfural	98-01-1	Sigma-Aldrich, Saint Louis, USA	99.0	1490
Acetic acid	64-19-7	J.T.Baker Chemicals, Deventer, Holland	99.0	1501
Linalool	78-70-6	Sigma-Aldrich, Saint Louis, USA	97.0	1540
Ethyl decanoate	110-38-3	Sigma-Aldrich, Saint Louis, USA	99.0	1593
α-terpineol	98-55-5	Sigma-Aldrich, Saint Louis, USA	96.0	1723
Ethyl carbamate	51-79-6	Sigma-Aldrich, Saint Louis, USA	99.0	1748
Geraniol	106-24-1	Sigma-Aldrich, Saint Louis, USA	97.0	1885
β-phenylethanol	60-12-8	Sigma-Aldrich, Saint Louis, USA	99.0	1995

Table S3.2. Goodness of fit statistics parameters for models of Table 3.3 [a].

Response	Full R^2	R^2	Adj. R^2	MSE	RMSE	MAPE	DW	Cp	AIC	SBC	PC	Press	Q^2	MS PE	MS LoF	LoF *p*-value	AV *p*-value
Ethanol	0.988	0.987	0.986	0.161	0.401	0.409	2.32	8	-102	-85.4	0.017	10.6	0.984	0.401	0.108	0.999	< 0.0001
Acetaldehyde	0.979	0.976	0.972	31.8	5.64	4.97	2.14	10	217	238	0.033	2339	0.965	28.4	32.858	0.413	< 0.0001
Acetal	0.966	0.961	0.952	2.95	1.72	6.66	1.86	12	75.6	101	0.059	235	0.935	1.86	3.319	0.141	< 0.0001
Ethyl acetate	0.952	0.945	0.933	3.23	1.80	8.12	2.42	11	80.1	103	0.080	241	0.916	1.56	3.765	0.052	< 0.0001
Ethyl butyrate	0.887	0.852	0.832	0.001	0.026	15.7	2.21	8	-428	-412	0.193	0.051	0.792	0.000	0.001	0.000	< 0.0001
Ethyl hexanoate	0.945	0.940	0.929	0.003	0.055	10.7	1.31	10	-339	-318	0.084	0.238	0.906	0.001	0.004	0.018	< 0.0001
Ethyl octanoate	0.904	0.885	0.871	0.009	0.094	10.9	1.44	7	-277	-263	0.146	0.613	0.848	0.002	0.011	0.002	< 0.0001
Ethyl decanoate	0.649	0.592	0.537	0.000	0.010	6.68	1.19	8	-550	-533	0.533	0.006	0.494	0.000	0.000	0.038	< 0.0001
Ethyl lactate	0.984	0.977	0.975	0.971	0.985	4.75	1.79	7	4.77	19.4	0.029	67.5	0.970	0.550	1.094	0.099	< 0.0001
Furfural	0.853	0.807	0.793	0.003	0.058	14.1	2.10	5	-337	-327	0.228	0.224	0.766	0.003	0.003	0.535	< 0.0001
β-phenylethanol	0.984	0.982	0.979	0.004	0.066	4.48	1.44	7	-319	-305	0.023	0.305	0.976	0.002	0.005	0.020	< 0.0001
Linalool	0.987	0.984	0.982	0.004	0.065	3.88	1.88	10	-319	-298	0.022	0.310	0.977	0.004	0.004	0.431	< 0.0001
α-terpineol	0.943	0.924	0.918	0.004	0.060	2.45	1.52	5	-334	-323	0.090	0.237	0.908	0.004	0.003	0.718	< 0.0001
1-hexanol	0.989	0.986	0.985	0.001	0.028	2.01	1.94	6	-425	-413	0.017	0.050	0.983	0.001	0.001	0.977	< 0.0001
1-propanol	0.953	0.944	0.938	1.77	1.33	2.04	2.04	7	40.8	55.4	0.070	121	0.928	3.01	1.406	0.965	< 0.0001
2-methyl-1-butanol	0.984	0.980	0.978	0.499	0.706	2.67	1.93	5	-36.9	-26.4	0.024	32.6	0.976	27.9	15.971	0.922	< 0.0001
3-methyl-1-butanol	0.982	0.978	0.976	18.6	4.31	2.39	1.92	6	181	194	0.027	1232	0.973	0.766	0.425	0.909	< 0.0001
Methanol	0.966	0.958	0.954	4.86	2.20	1.86	1.95	6	101	113	0.052	325	0.948	9.142	3.635	0.986	< 0.0001
Acetic acid	0.888	0.871	0.857	0.850	0.922	7.07	2.03	7	-3.18	11.5	0.163	59.1	0.831	0.535	0.942	0.146	< 0.0001

[a] "Full R^2" means determination coefficient for the model without filtering factors. "R^2" means determination coefficient for the model. "Adj. R^2" means adjusted determination coefficient for the model. "MSE" means mean squared error. "RMSE" means root mean square of the errors. "MAPE" means mean absolute percentage error. "DW" means Durbin-Watson statistic. "Cp" means Mallows Cp coefficient. "AIC" means Akaike's information criterion. "SBC" means Schwarz's bayesian criterion. "PC" means Amemiya's prediction criterion. "Press" means predicted residual error sum of squares. "Q2" means cross-validated R^2. "MS pure error" means mean squared error of pure error. "MS LoF" means mean squared error of lack of fit. "LoF *p*-value" means the p-value of the lack of fit F-test. "AV p-value" means the p-value of the analysis of variance of the model.
krhfp

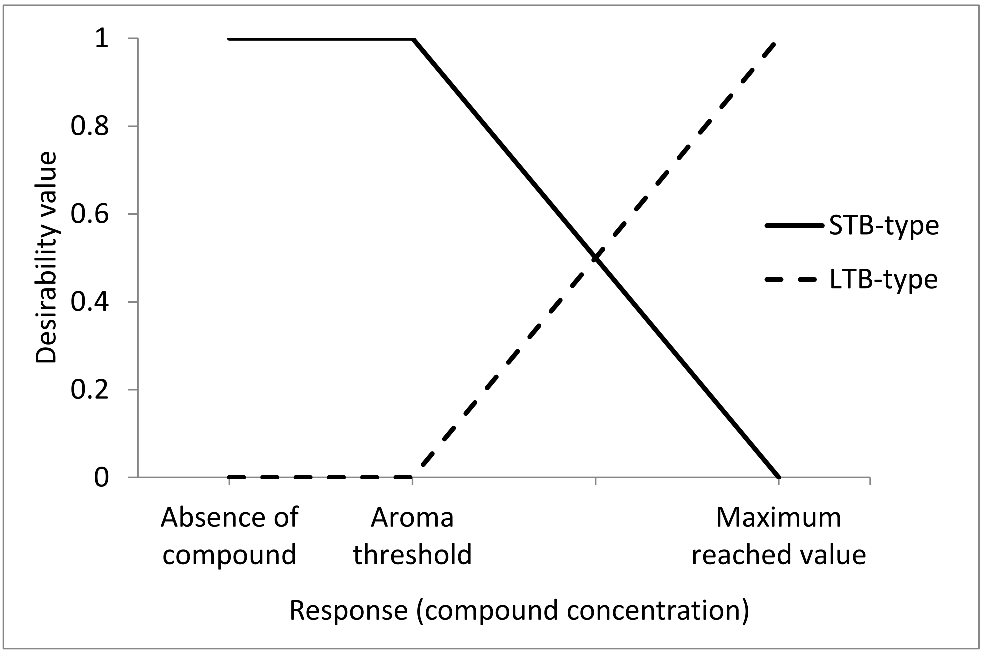

Figure S3.1. Graphical example of the curve of the desirability function to maximize (LTB-type) or minimize (STB-type) the sensory impact of a compound.

Given the large number of compounds and factors, this Figure has been set up to interpret Table 3. For this purpose, an easy model with two factors (F1 and F2) is presented and plotted using contour plots. The differences between plots are the sign of the regression coefficients (+1, -1 or 0). In this way, the reader can quickly imagine the trends of the compounds, by the relationship between the model of this figure and regression coefficients of each model of Table 3.

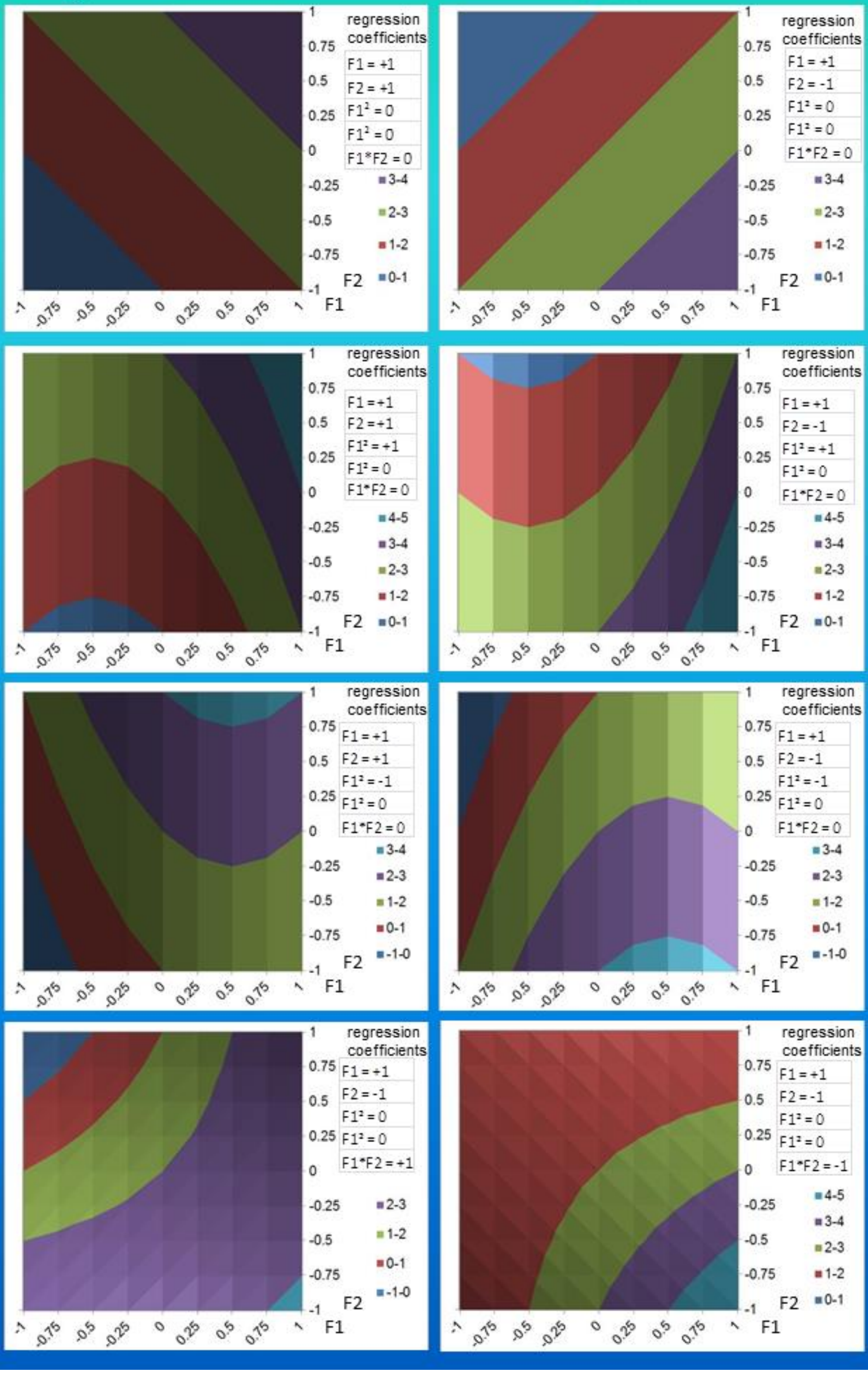

Figure S3.2. Graphical example for the interpretation of the regression models of Table 3.3 using contour plots.

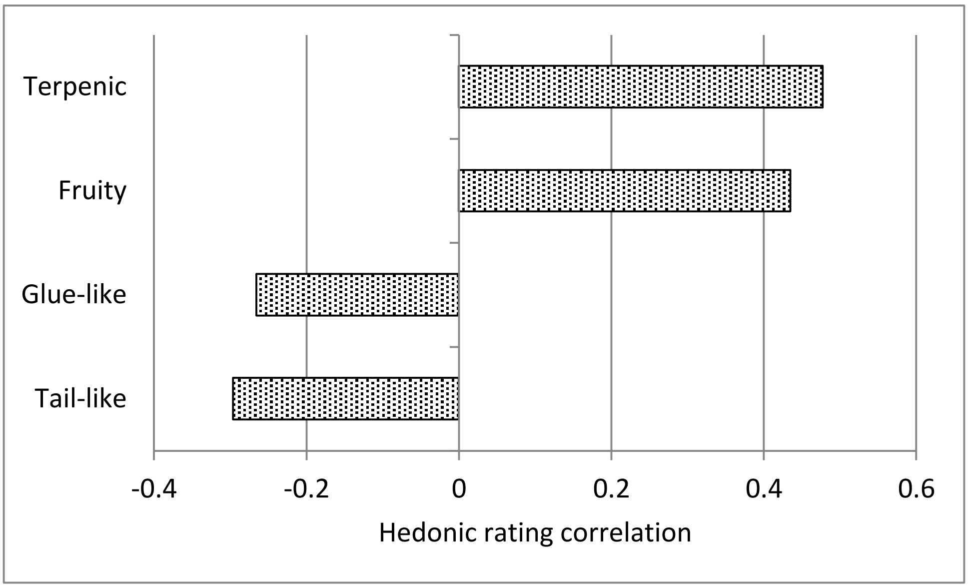

Figure S3.3. Spearman correlations bar chart between aroma descriptors and hedonic rating (p < 0.05), carried out with 17 trained assessors.

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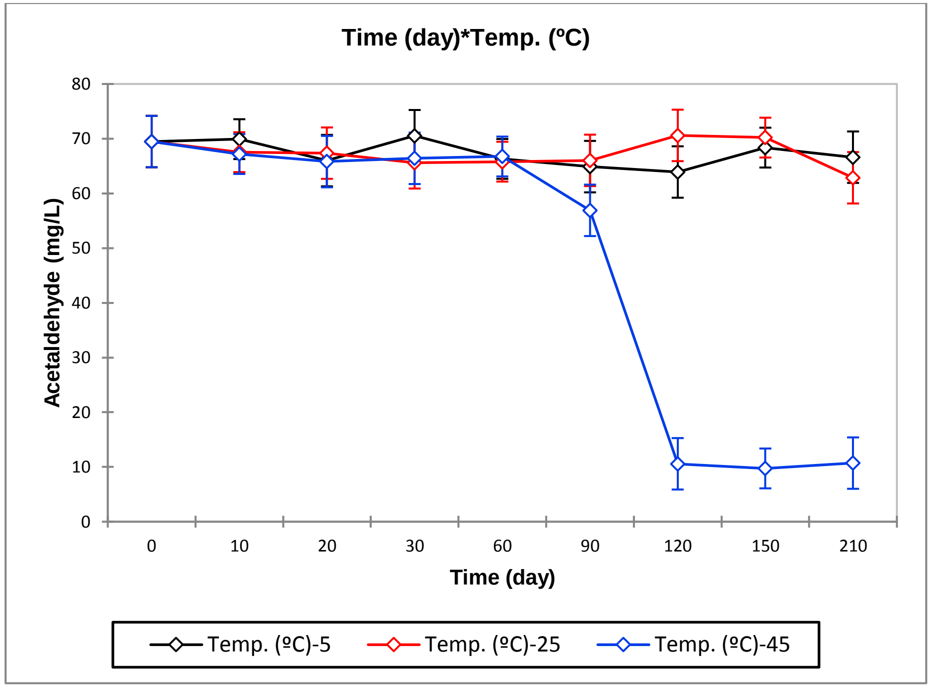
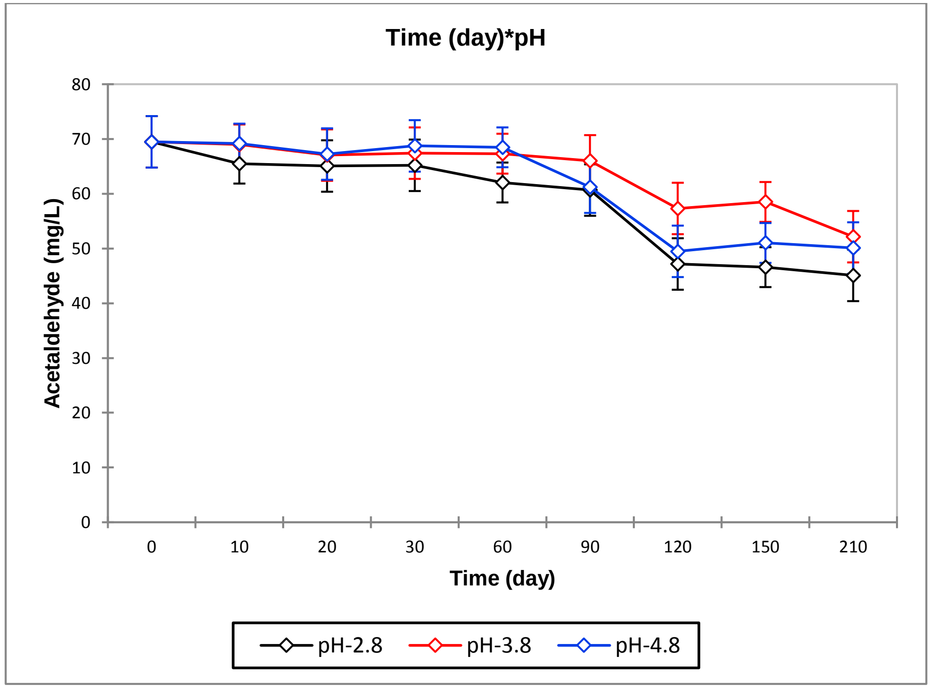
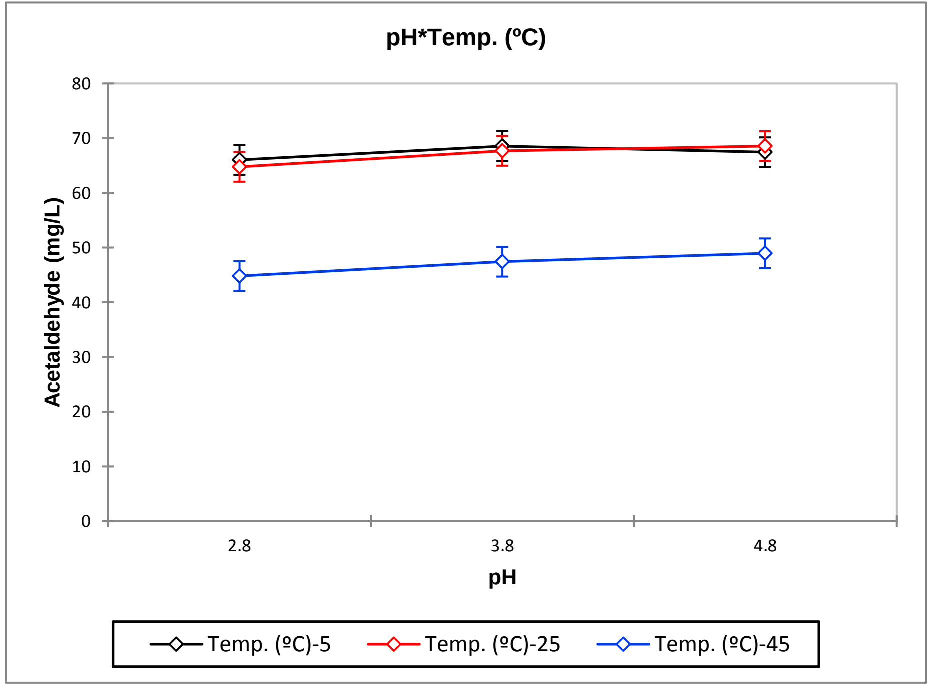
Ethyl decanoate	110-38-3	Sigma-Aldrich, Saint Louis, USA	99.0	1593
α-terpineol	98-55-5	Sigma-Aldrich, Saint Louis, USA	96.0	1723
β-phenylethanol	60-12-8	Sigma-Aldrich, Saint Louis, USA	99.0	1945

Figure S5.1. Line graphs of all compounds mean concentration with confidence intervals of 95 % (except ethyl hexanoate plots which are shown in the manuscript): two-way interaction effects between studied factors.

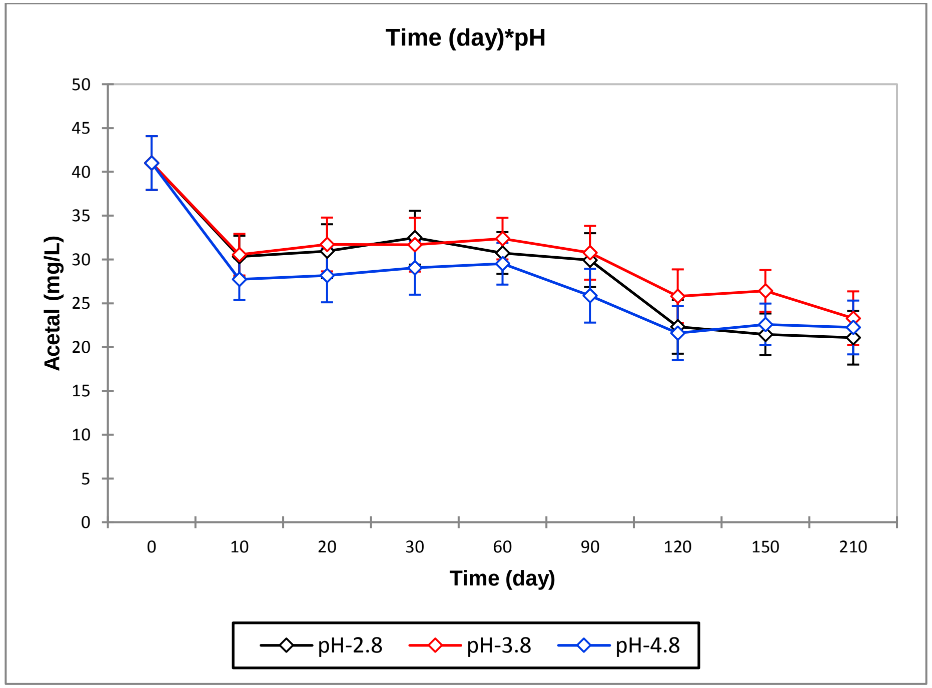
a) Acetaldehyde

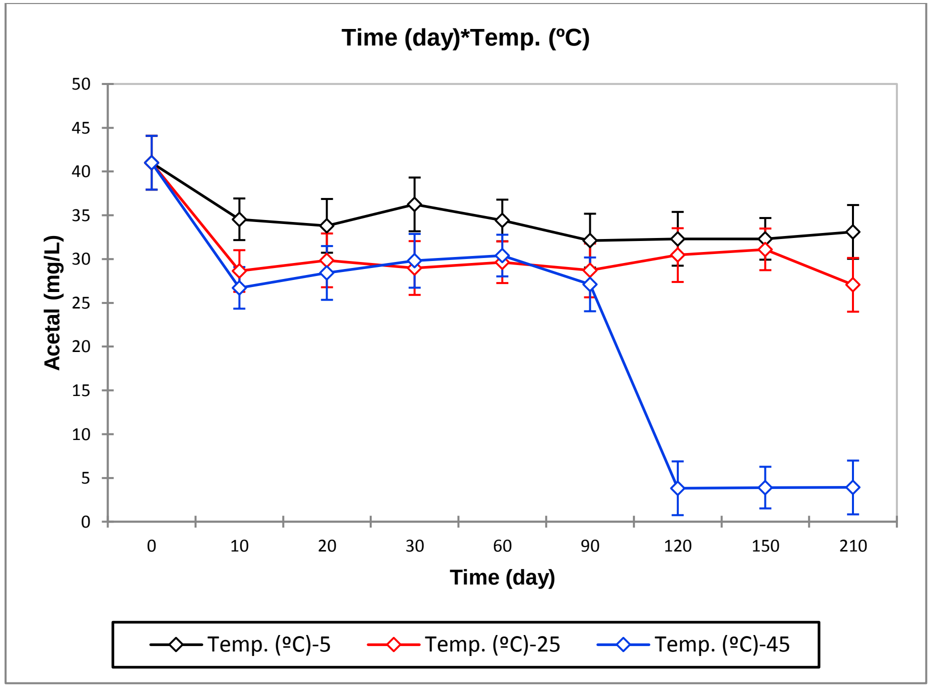


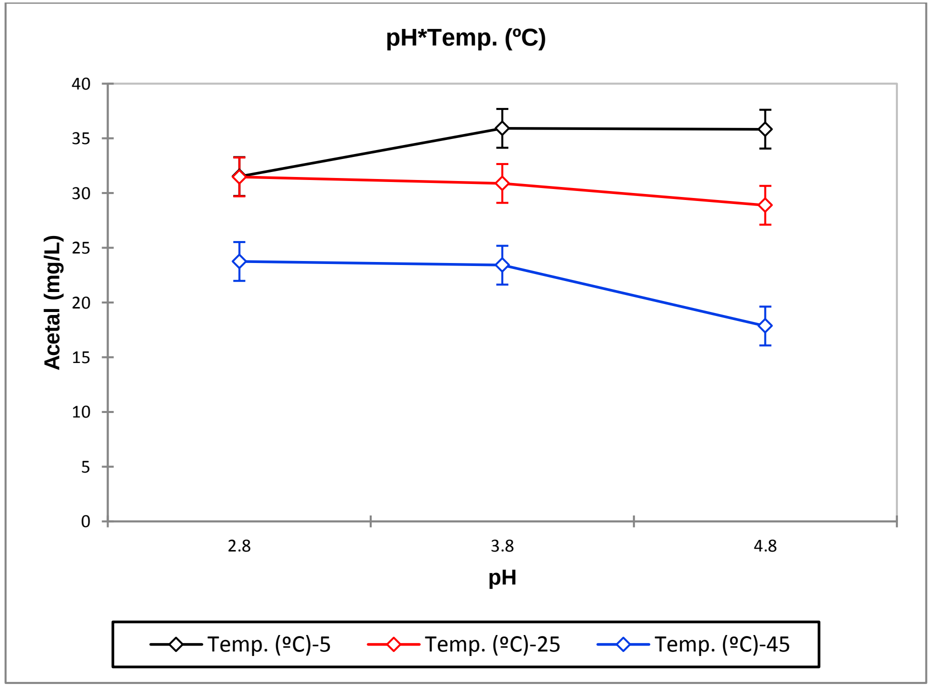




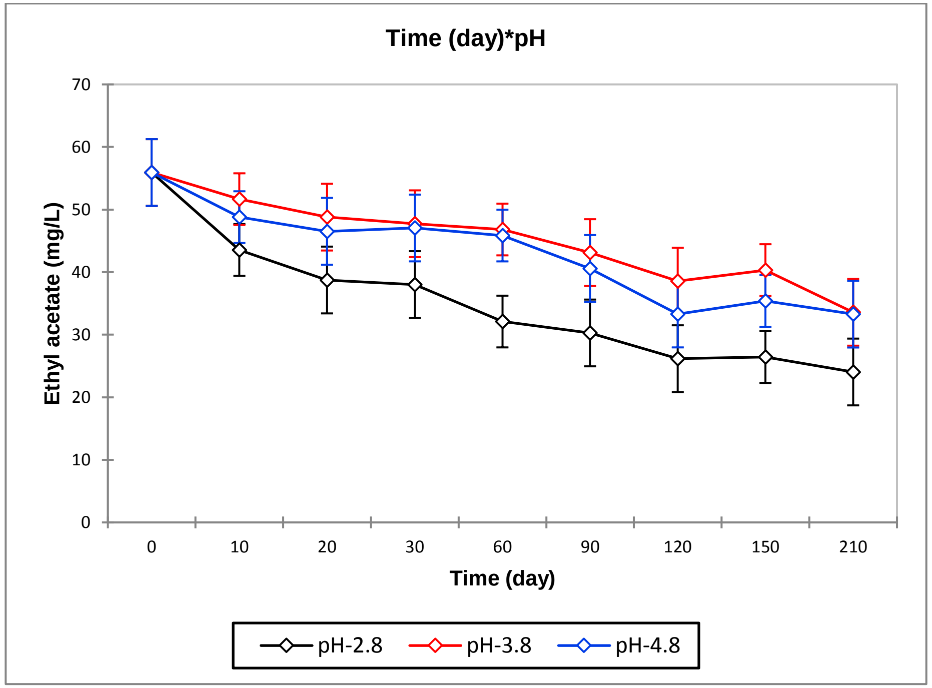
b) Acetal

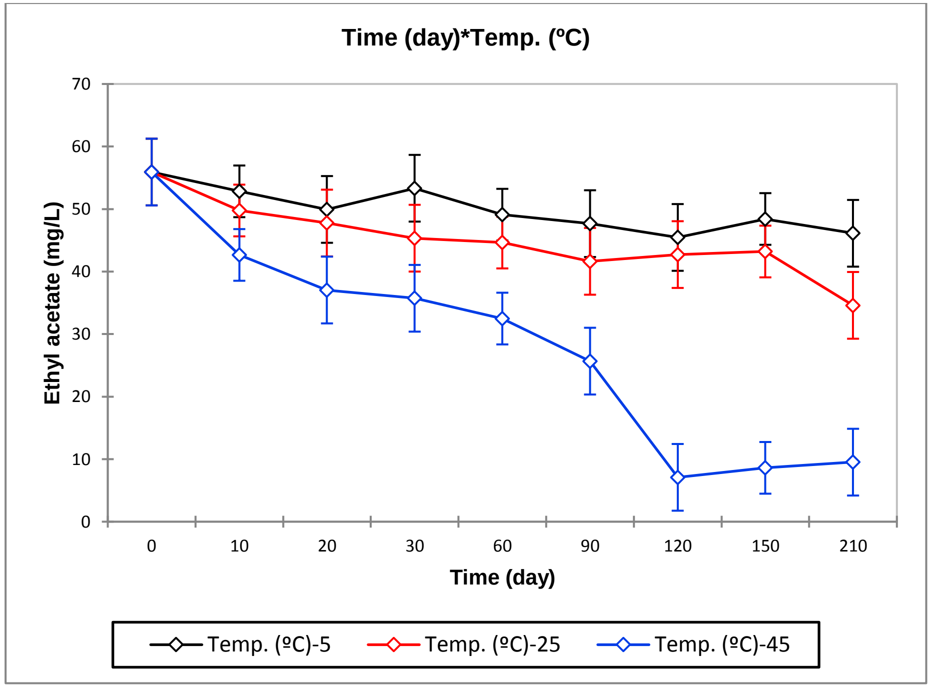

Time (day)*pH
Acetal (mg/L)
50
45
40
35
30
25
20
15
10
5
0
0
10
20
30
60
90
120
150
210
Time (day)
pH-2.8
pH-3.8
pH-4.8

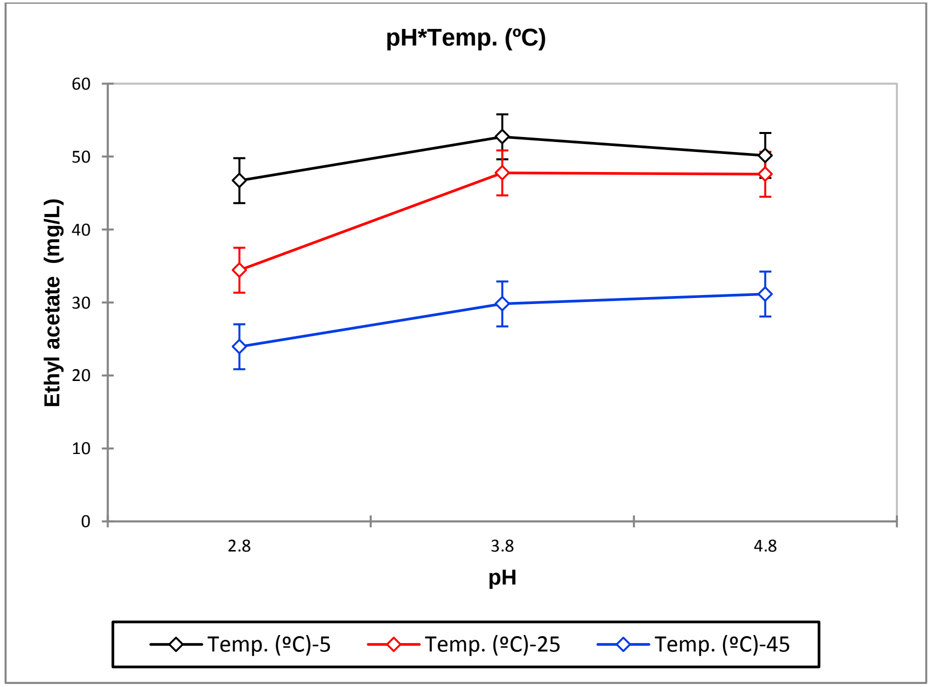

Time (day)*Temp. (ºC)
Acetal (mg/L)
50
45
40
35
30
25
20
15
10
5
0
0
10
20
30
60
90
120
150
210
Time (day)
Temp. (ºC)-5
Temp. (ºC)-25
Temp. (ºC)-45


pH*Temp. (ºC)
Acetal (mg/L)
40
35
30
25
20
15
10
5
0
2.8
3.8
4.8
pH
Temp. (ºC)-5
Temp. (ºC)-25
Temp. (ºC)-45

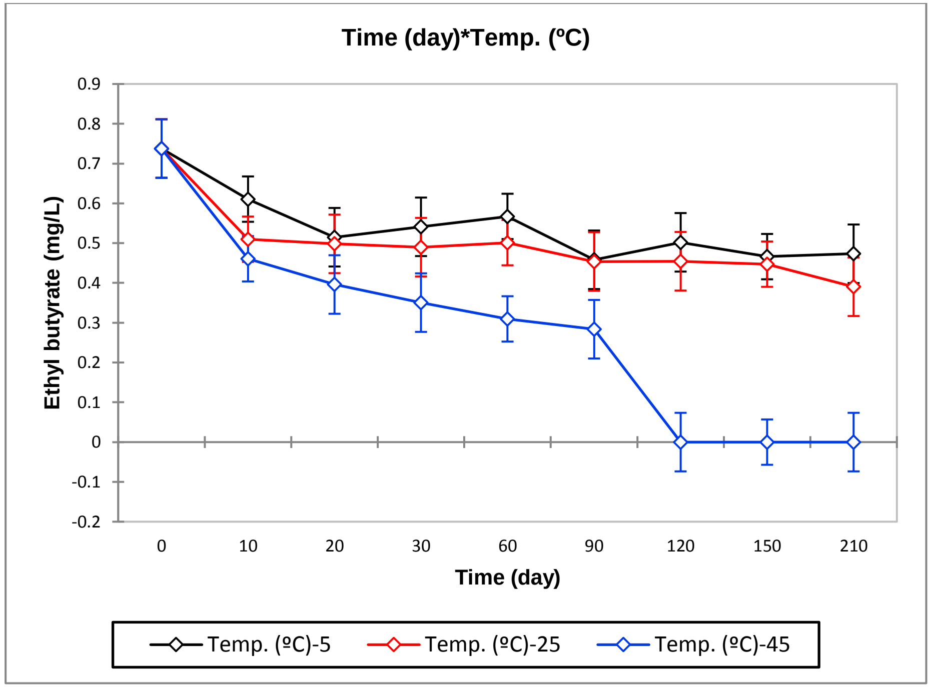
c) Ethyl acetate

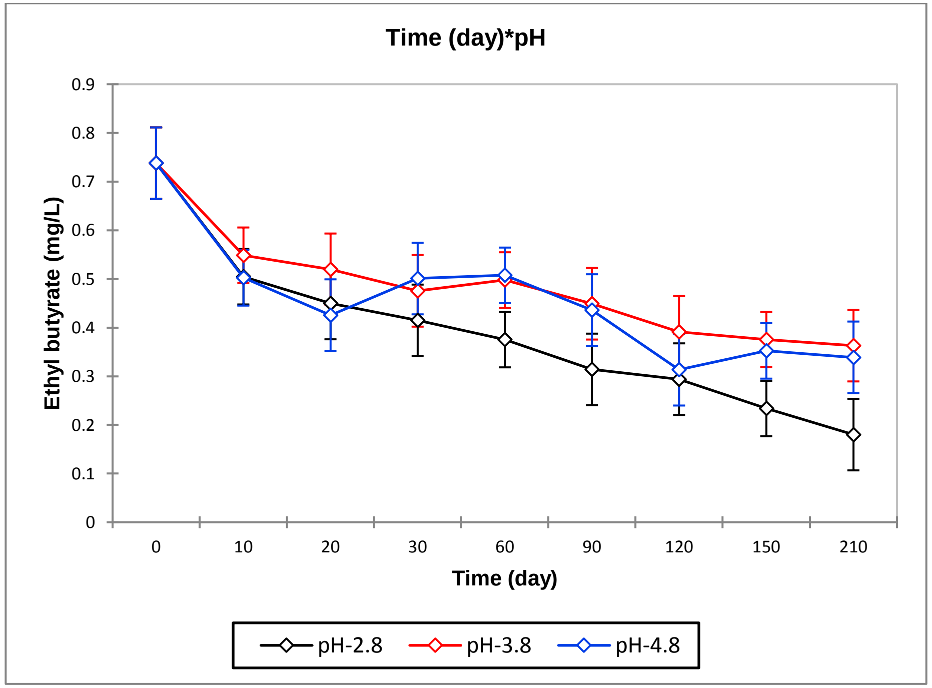


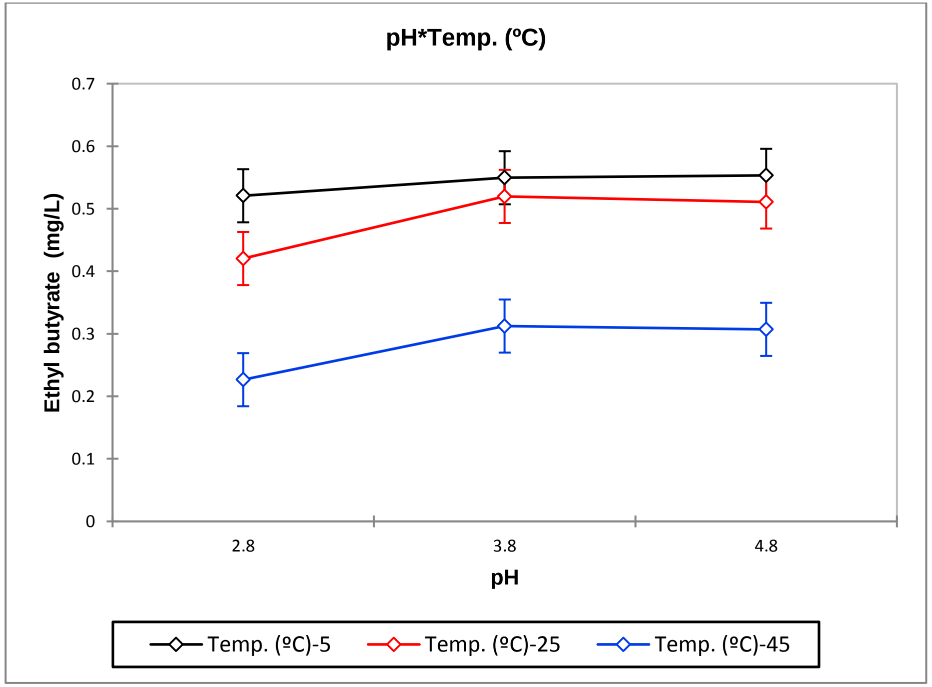




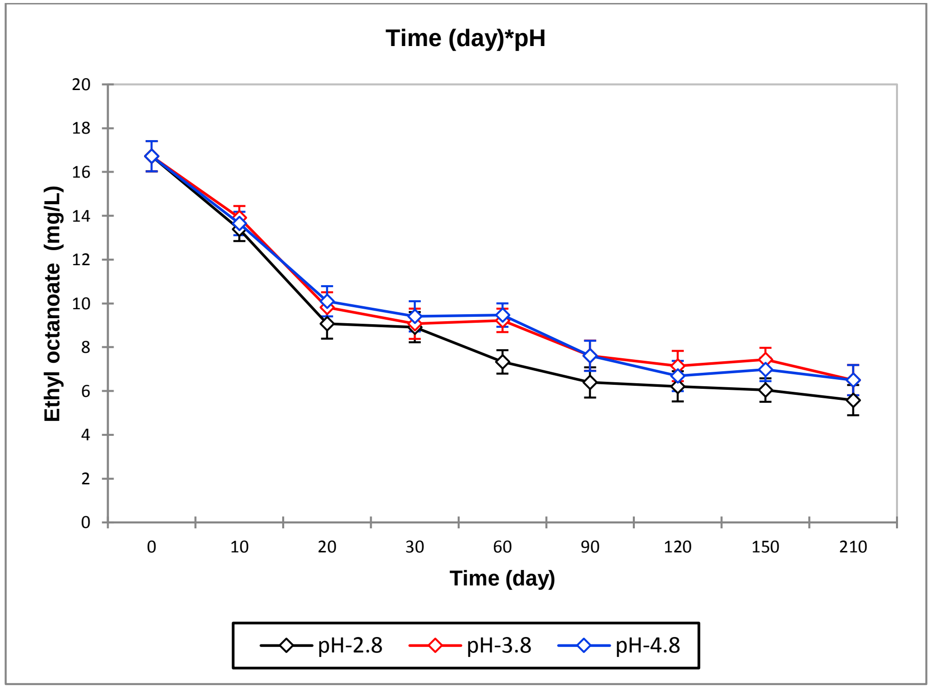
d) Ethyl butyrate

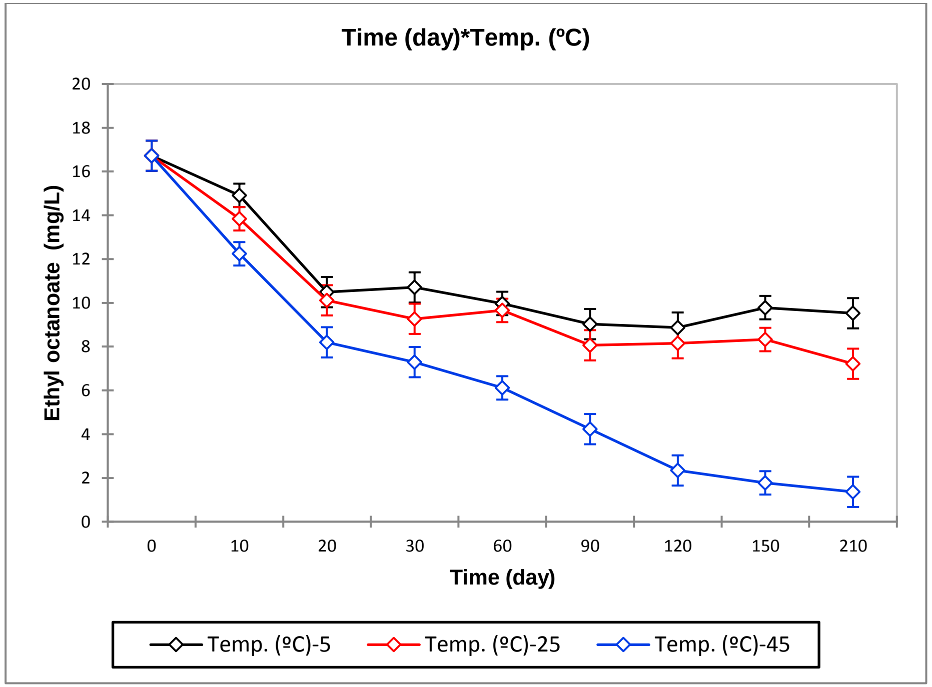


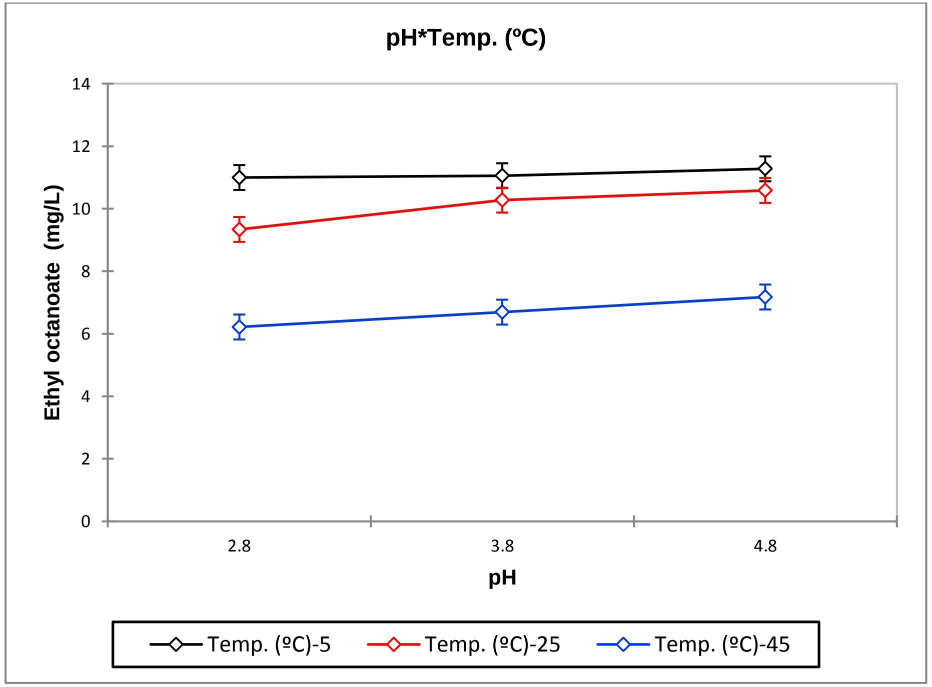




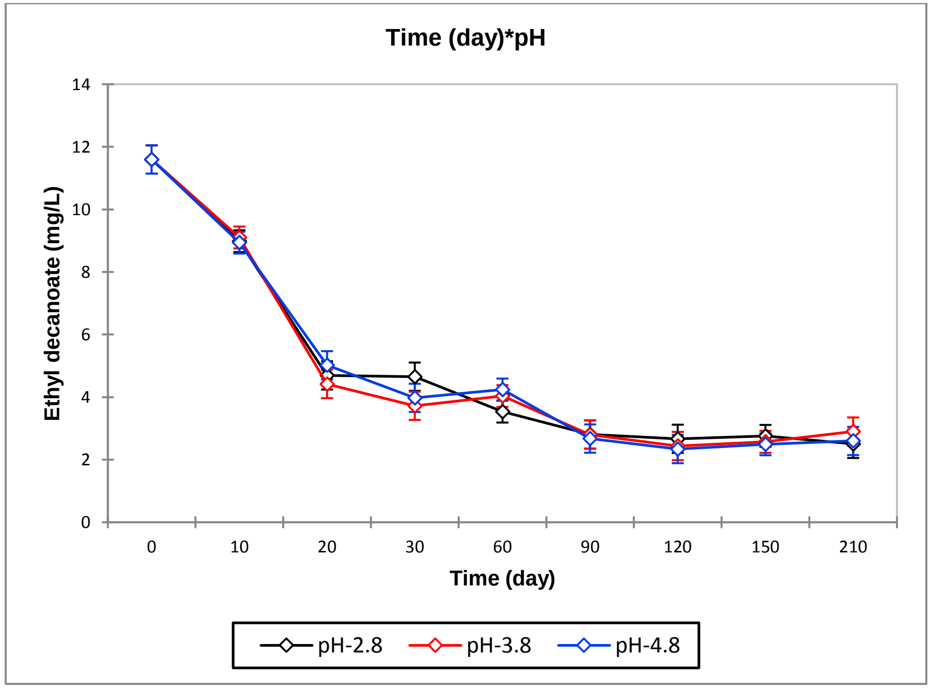
e) Ethyl octanoate

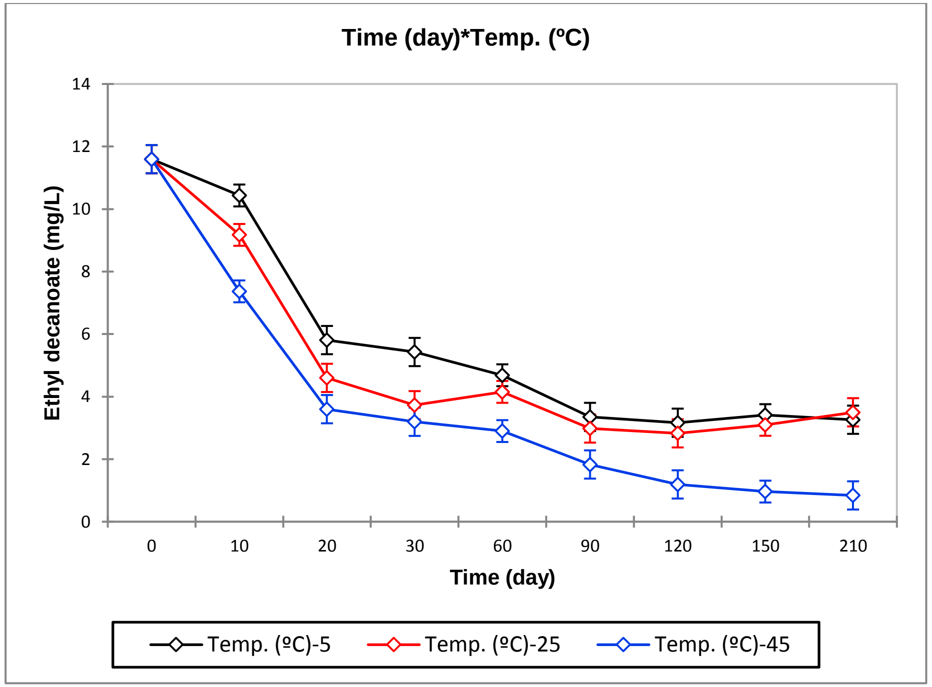


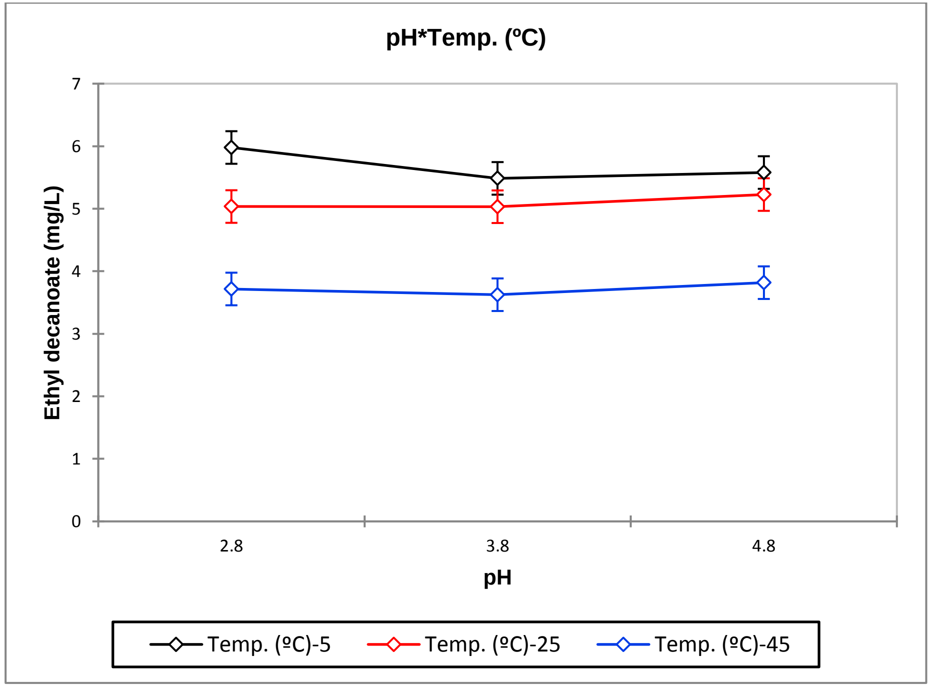




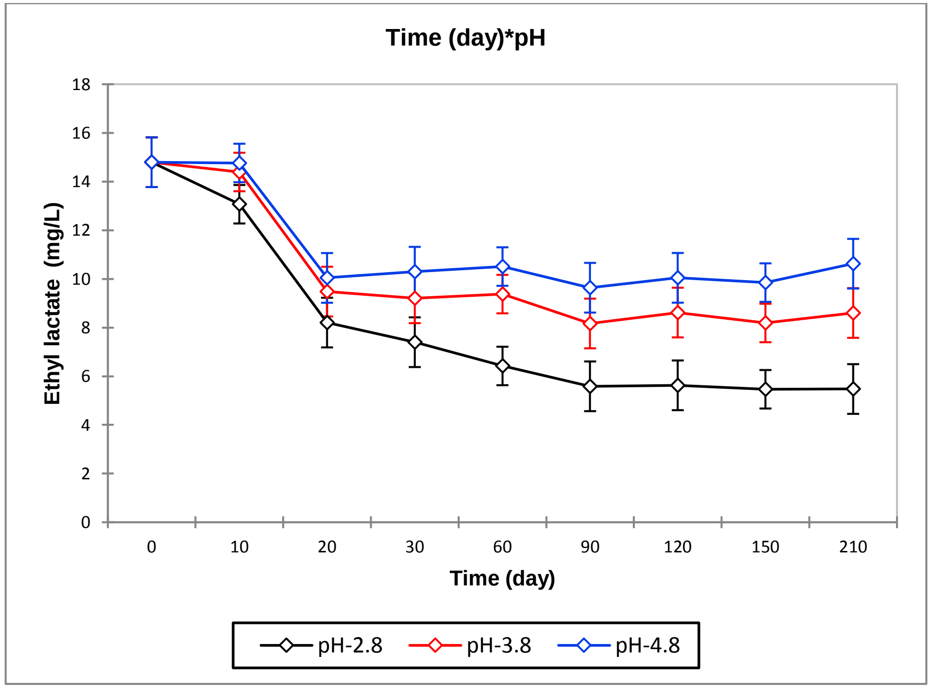
f) Ethyl decanoate

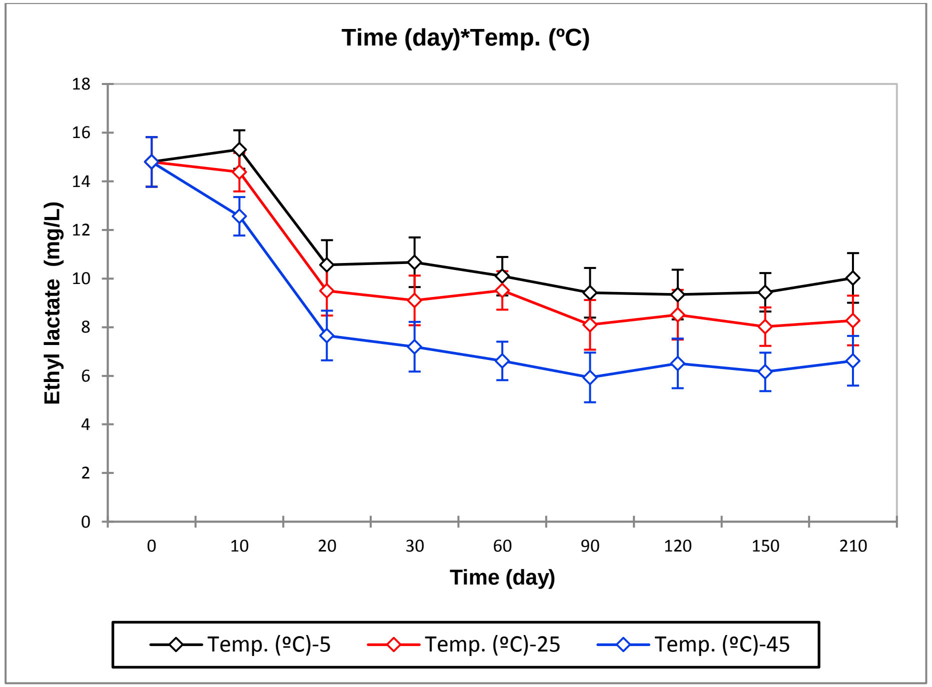


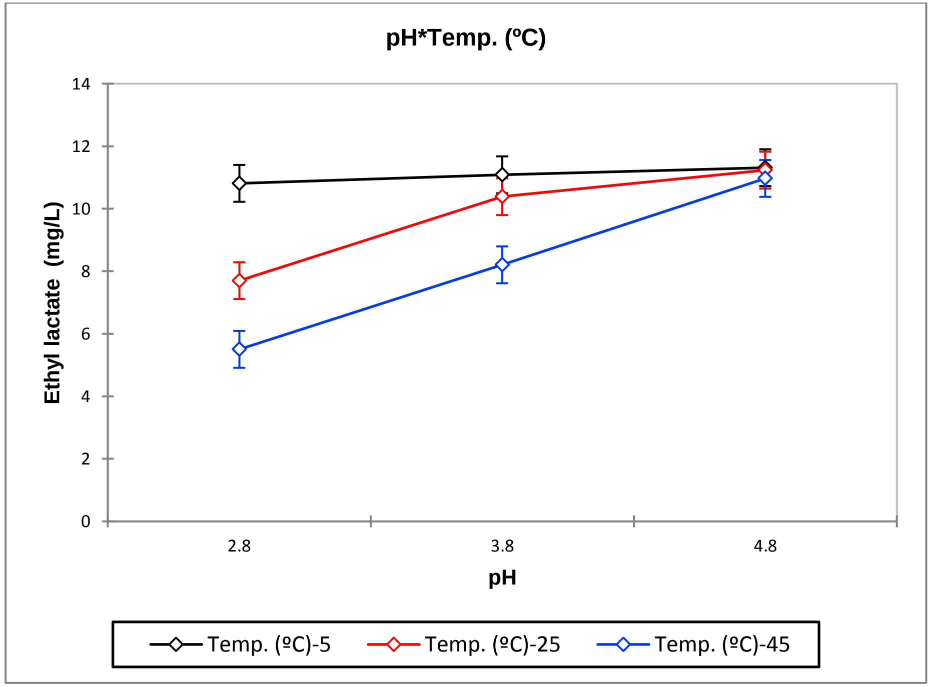




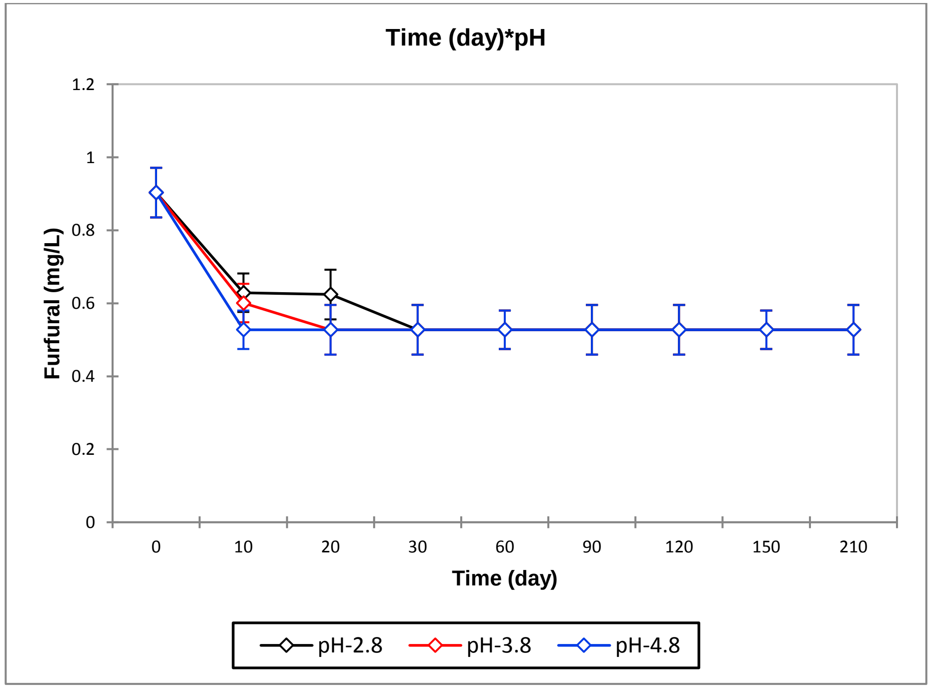
g) Ethyl lactate

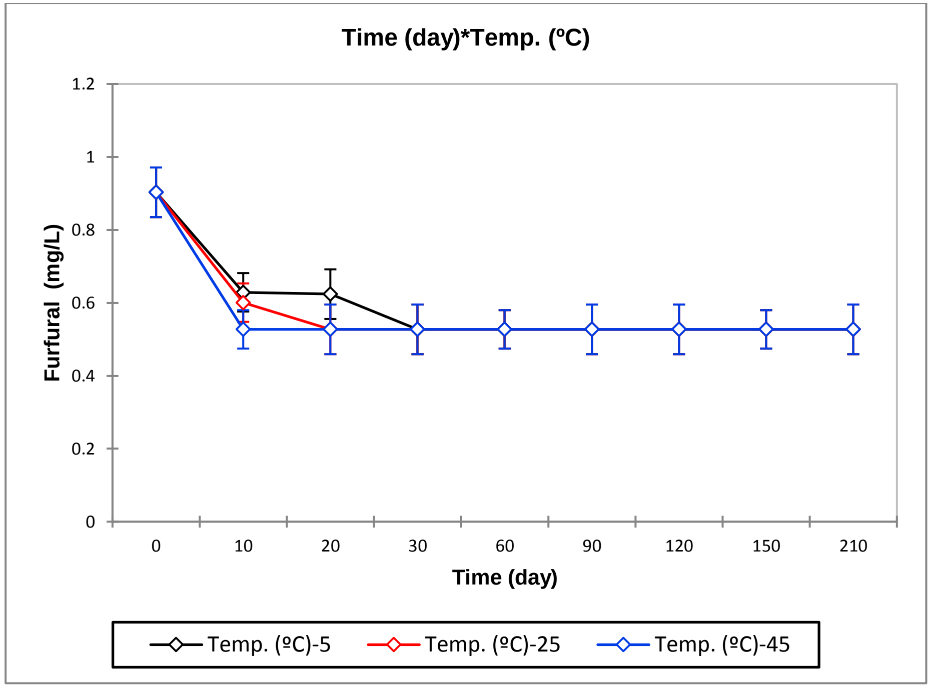


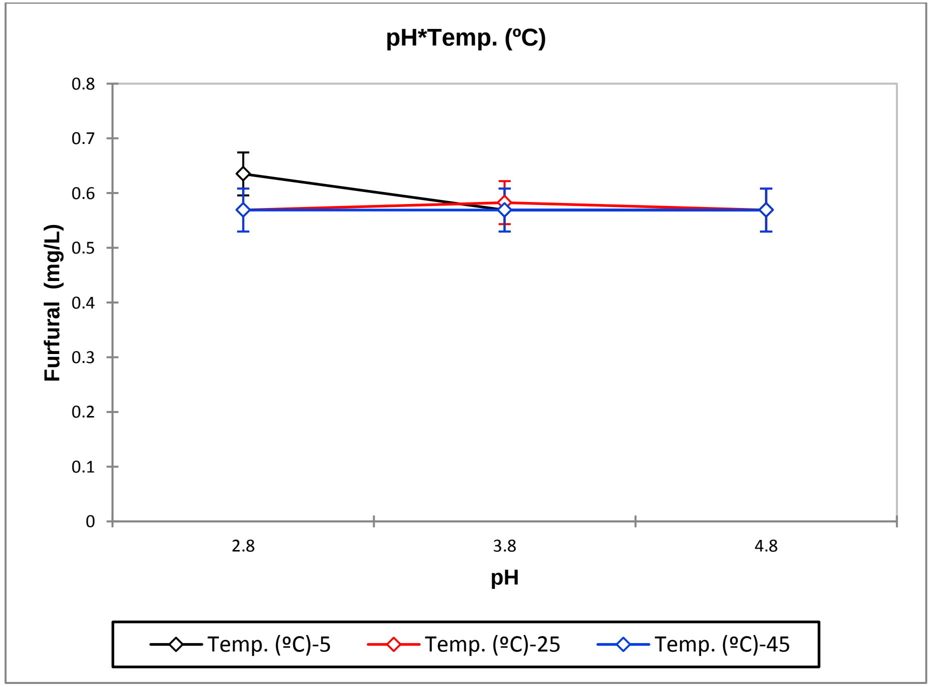




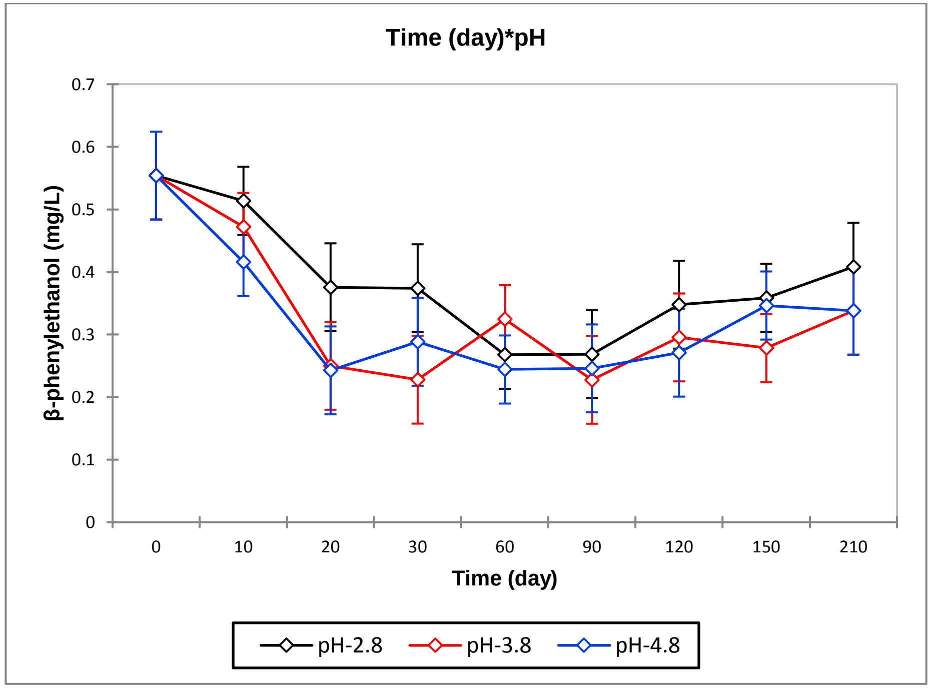
h) Furfural

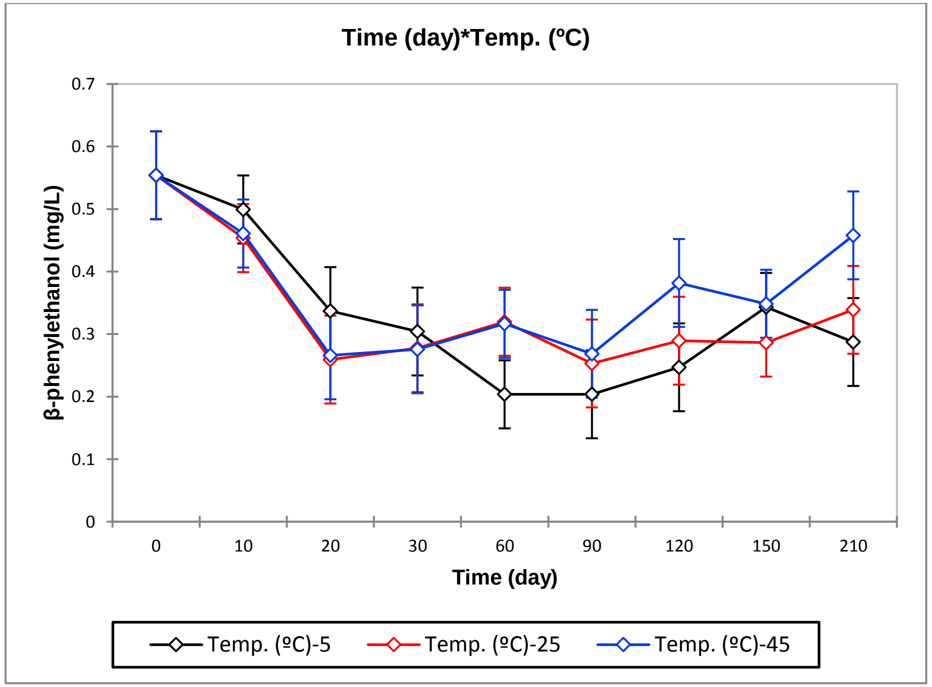

Time (day)*pH
Furfural (mg/L)
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
60
90
120
150
210
Time (day)
pH-2.8
pH-3.8
pH-4.8

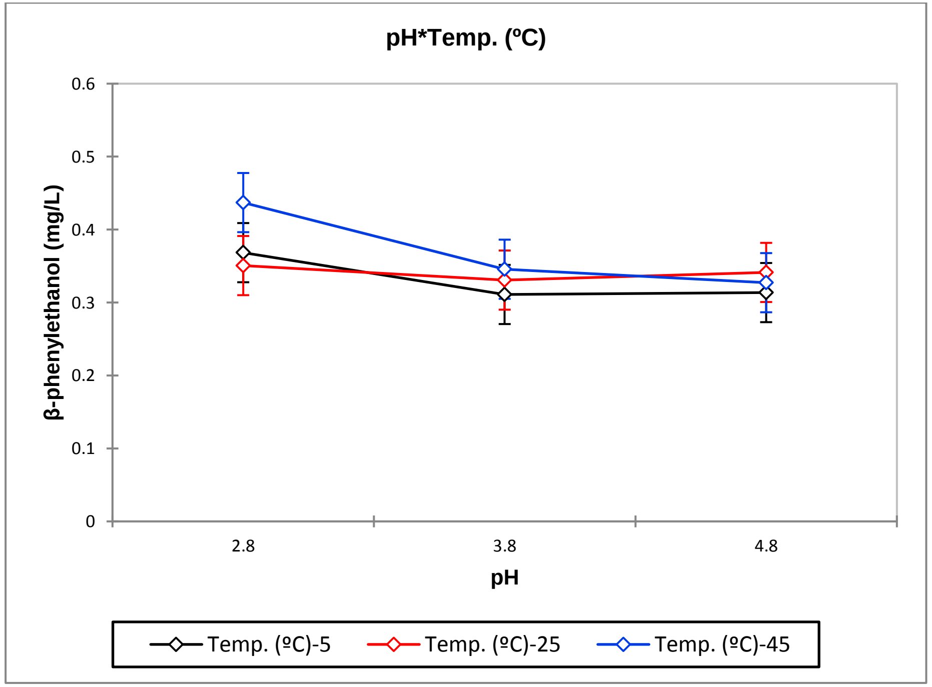

Time (day)*Temp. (ºC)
Furfural (mg/L)
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
60
90
120
150
210
Time (day)
Temp. (ºC)-5
Temp. (ºC)-25
Temp. (ºC)-45


pH*Temp. (ºC)
Furfural (mg/L)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2.8
3.8
4.8
pH
Temp. (ºC)-5
Temp. (ºC)-25
Temp. (ºC)-45

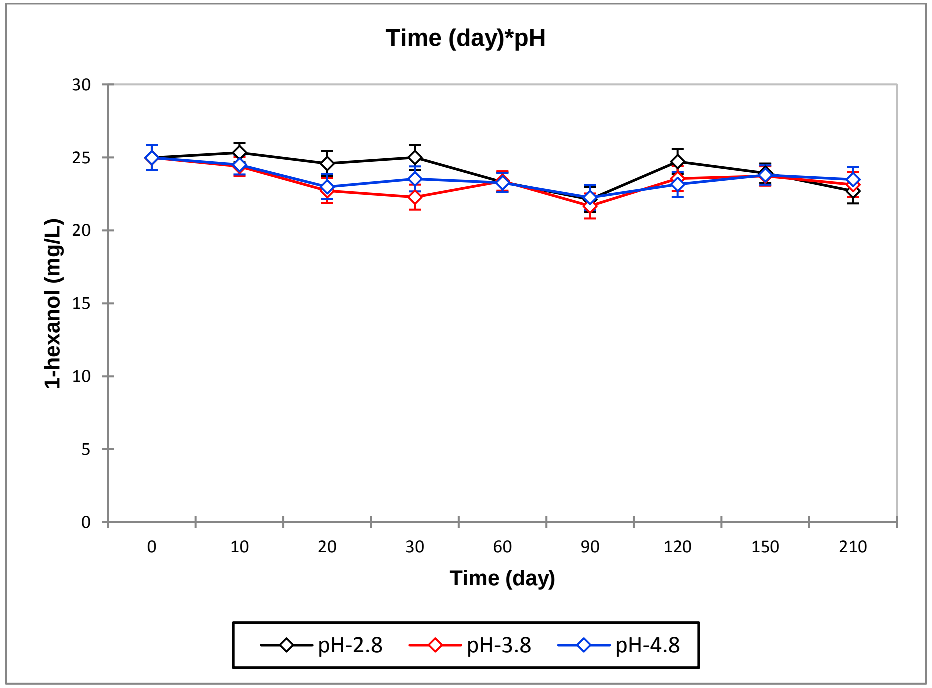
i) β-phenylethanol

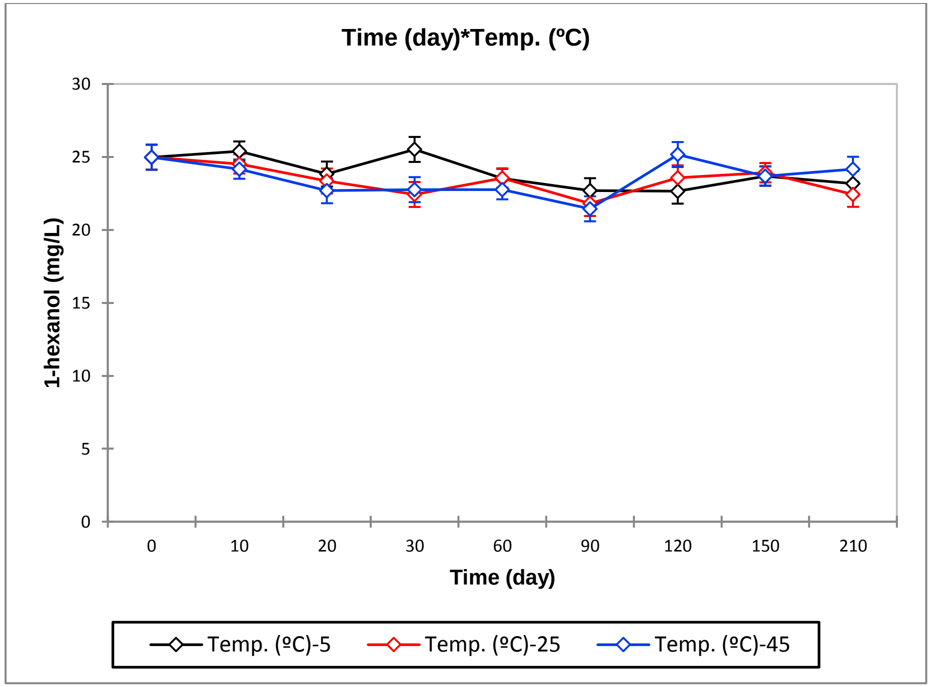


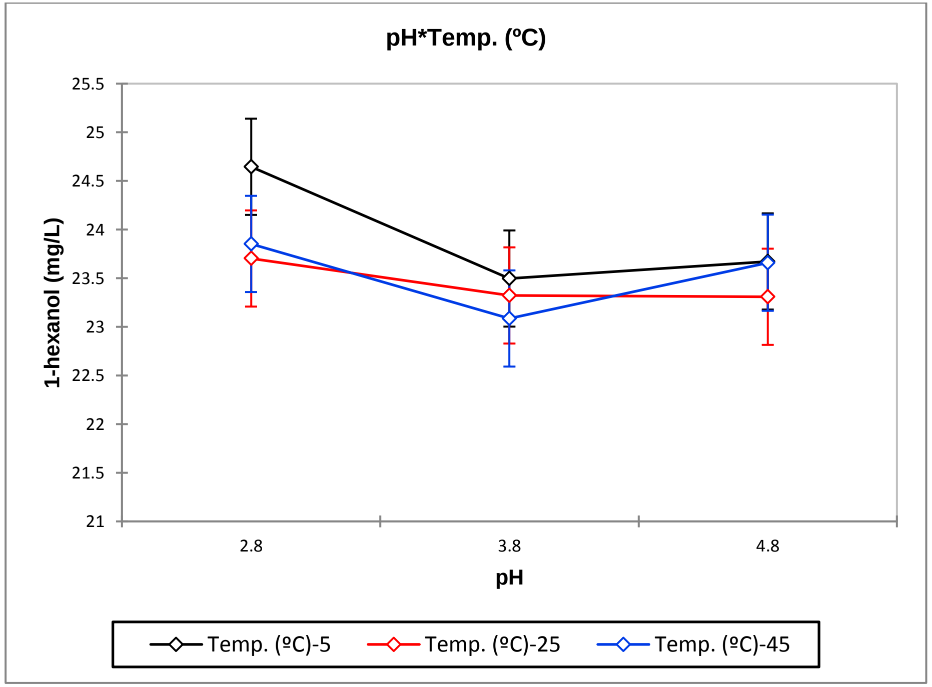




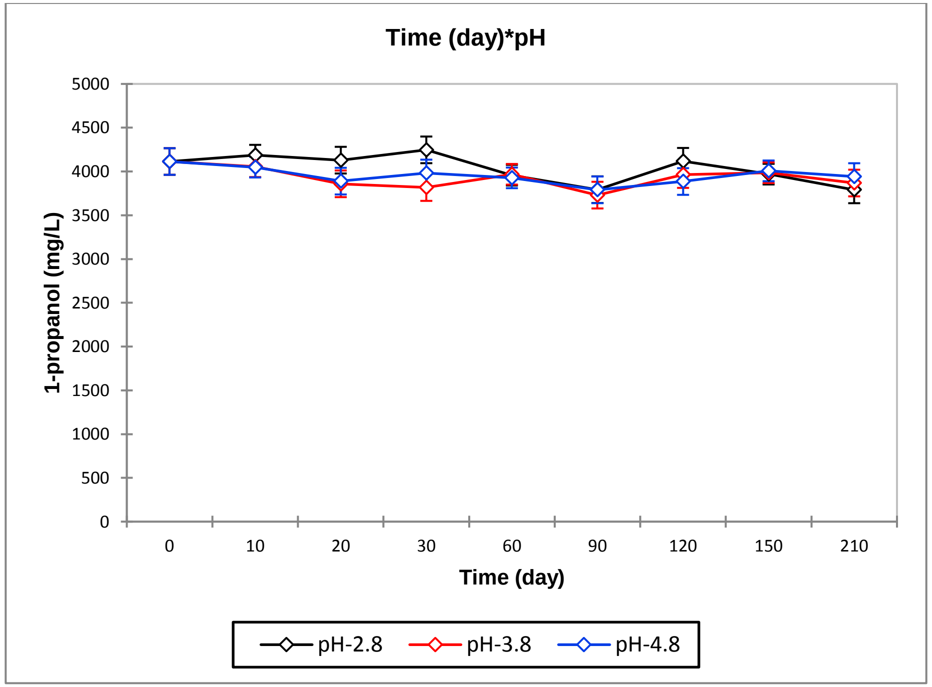
j) 1-hexanol

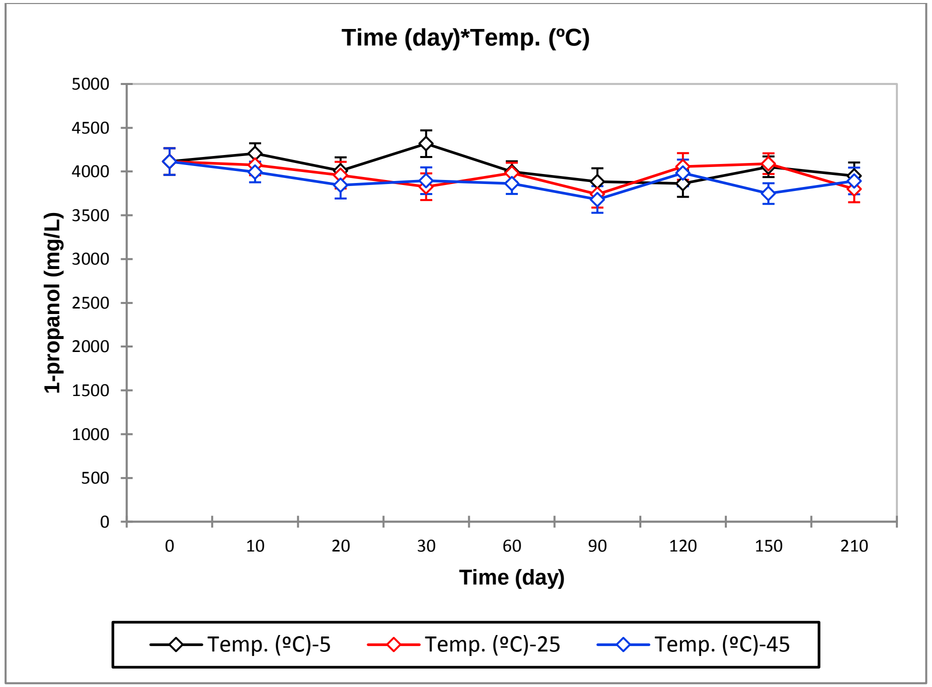

Time (day)*pH
1-hexanol (mg/L)
30
25
20
15
10
5
0
0
10
20
30
60
90
120
150
210
Time (day)
pH-2.8
pH-3.8
pH-4.8

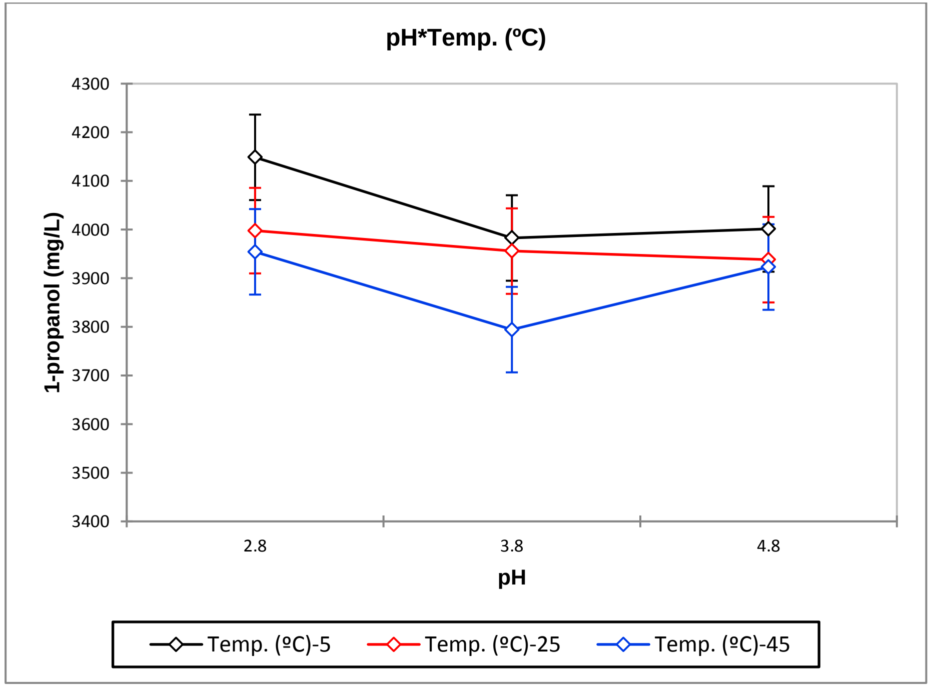

Time (day)*Temp. (ºC)
1-hexanol (mg/L)
30
25
20
15
10
5
0
0
10
20
30
60
90
120
150
210
Time (day)
Temp. (ºC)-5
Temp. (ºC)-25
Temp. (ºC)-45


pH*Temp. (ºC)
1-hexanol (mg/L)
25.5
25
24.5
24
23.5
23
22.5
22
21.5
21
2.8
3.8
4.8
pH
Temp. (ºC)-5
Temp. (ºC)-25
Temp. (ºC)-45

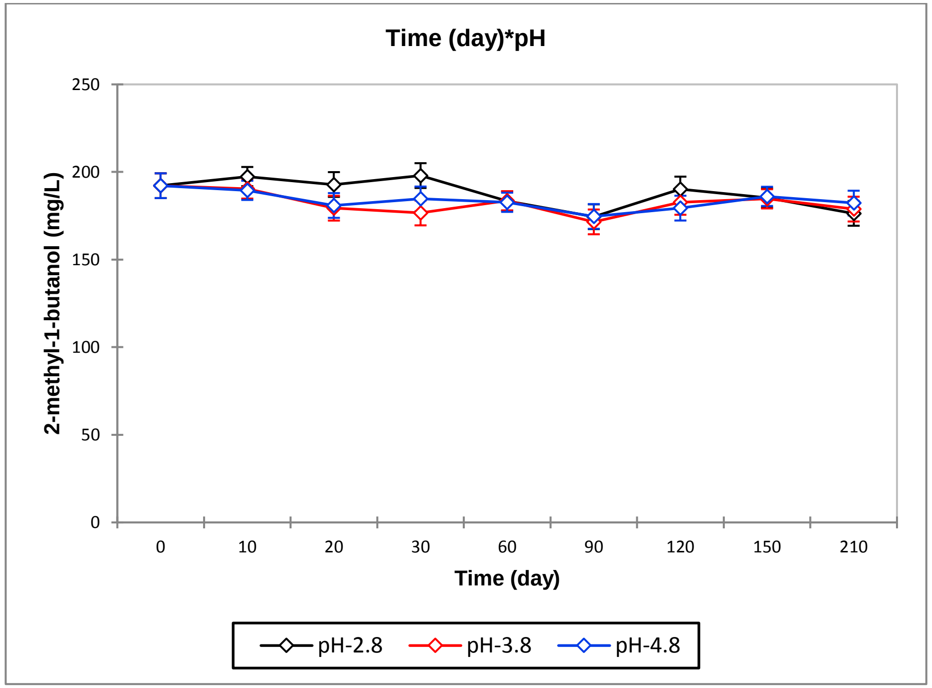
k) 1-propanol

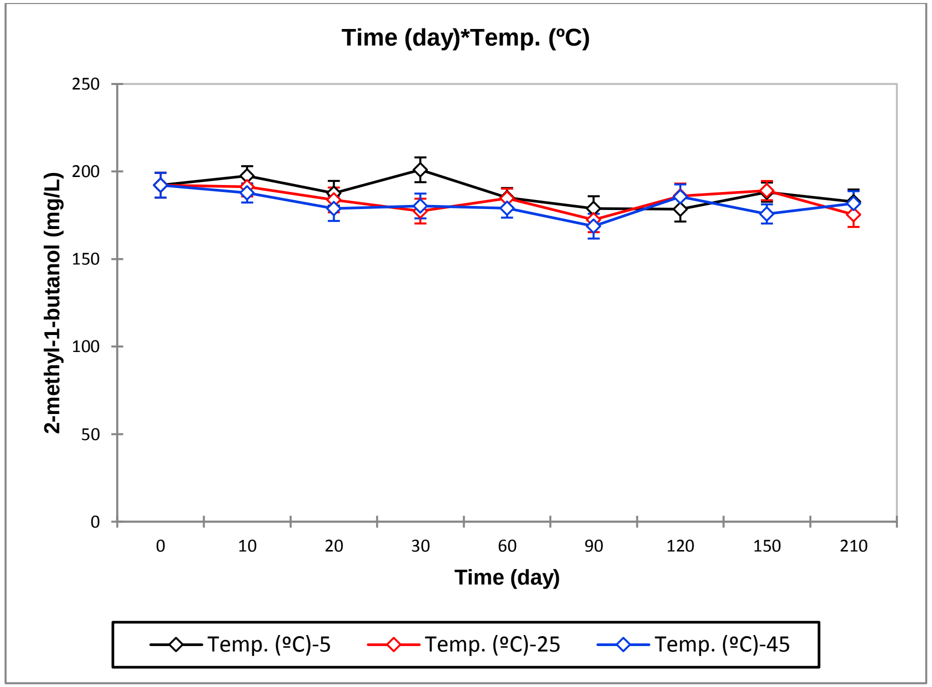

Time (day)*pH
1-propanol (mg/L)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
10
20
30
60
90
120
150
210
Time (day)
pH-2.8
pH-3.8
pH-4.8

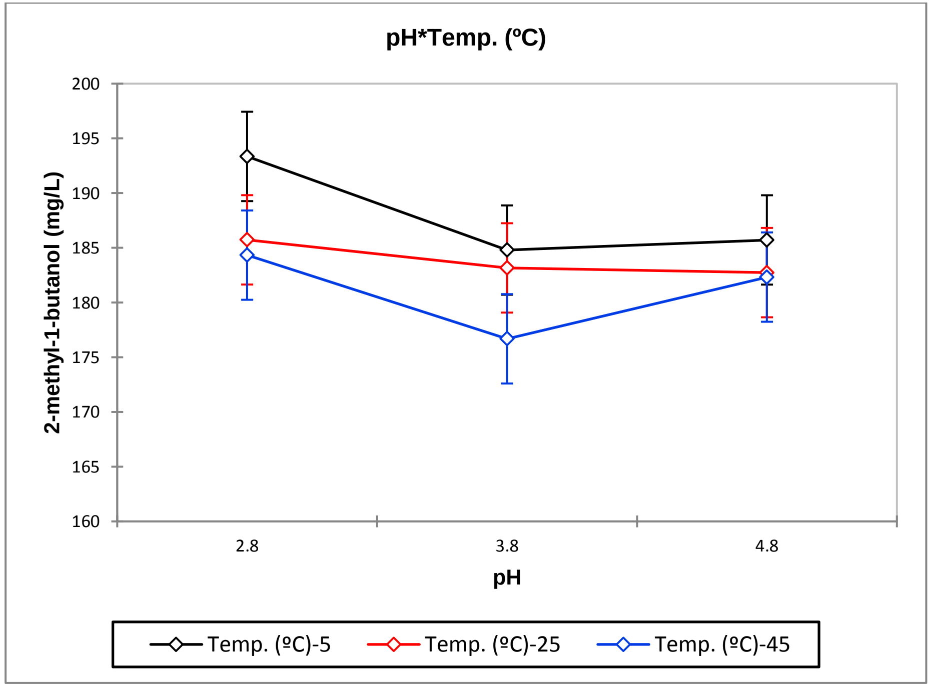

Time (day)*Temp. (ºC)
1-propanol (mg/L)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
10
20
30
60
90
120
150
210
Time (day)
Temp. (ºC)-5
Temp. (ºC)-25
Temp. (ºC)-45


pH*Temp. (ºC)
1-propanol (mg/L)
4300
4200
4100
4000
3900
3800
3700
3600
3500
3400
2.8
3.8
4.8
pH
Temp. (ºC)-5
Temp. (ºC)-25
Temp. (ºC)-45

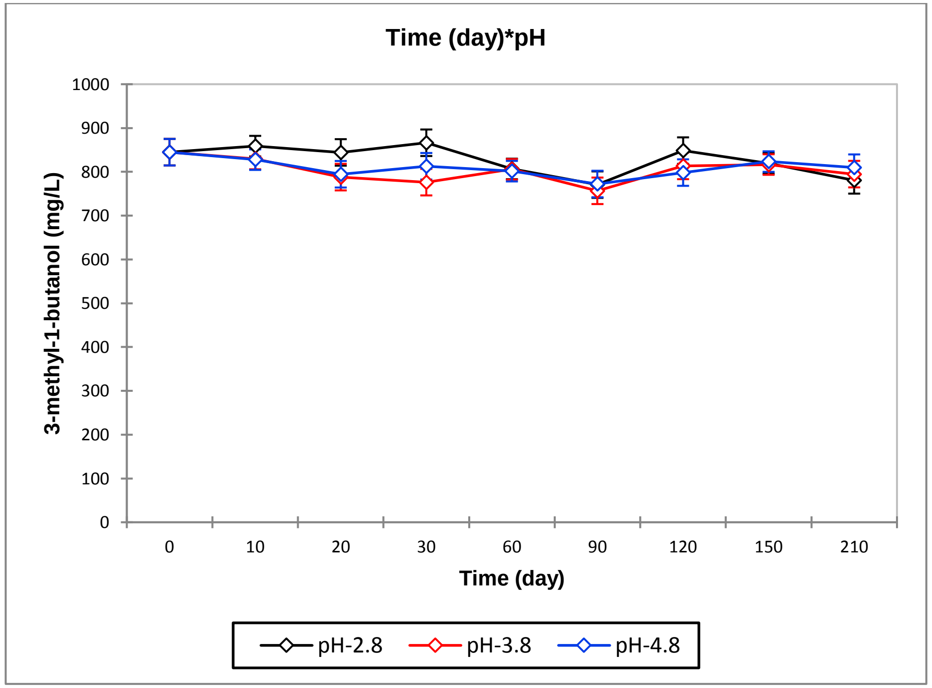
l) 2-methyl-1-butanol

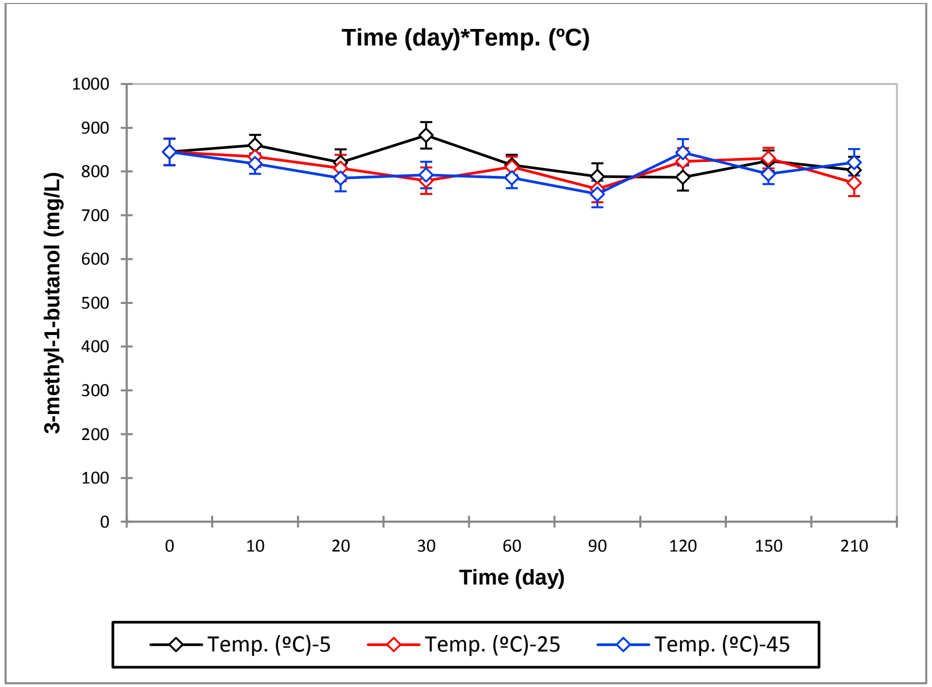


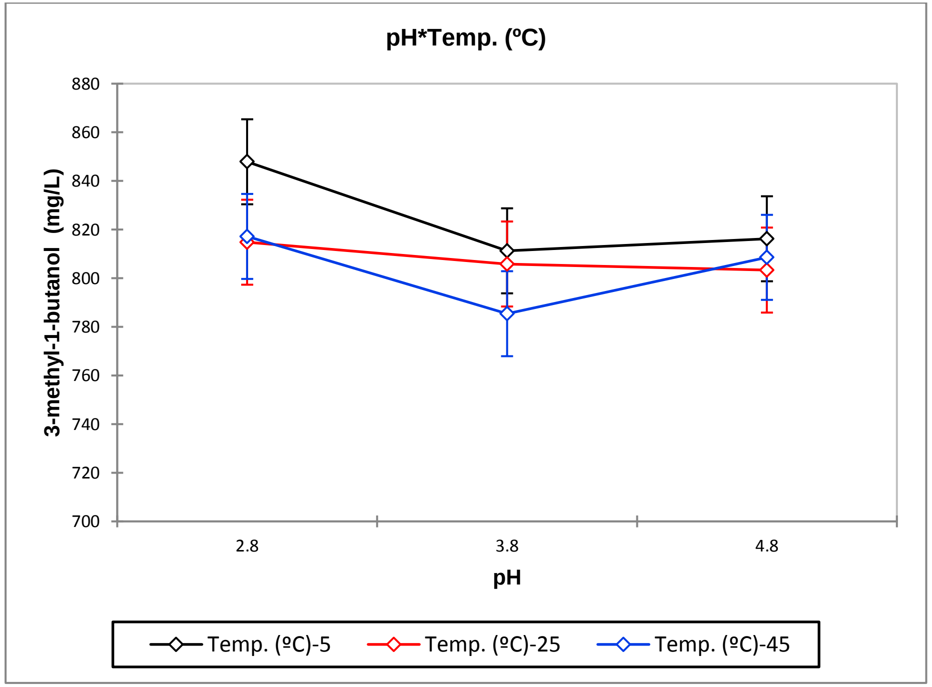




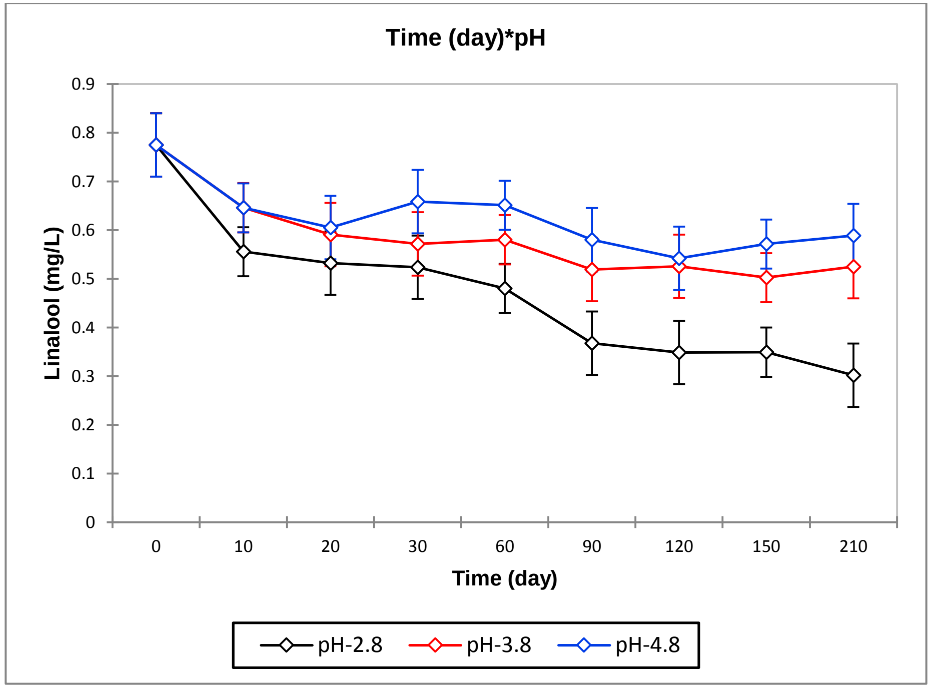
m) 3-methyl-1-butanol

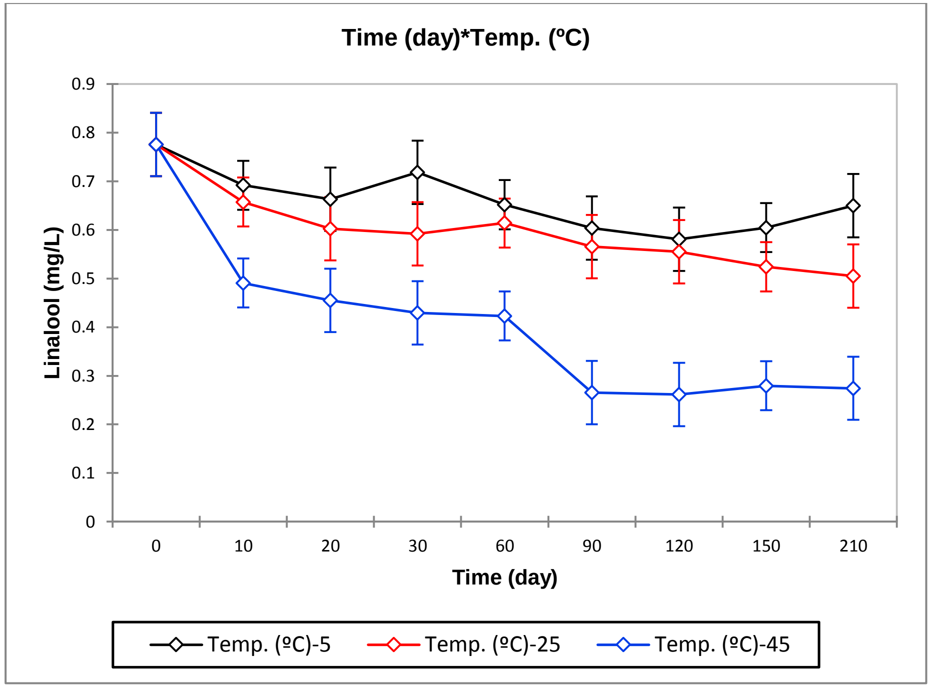


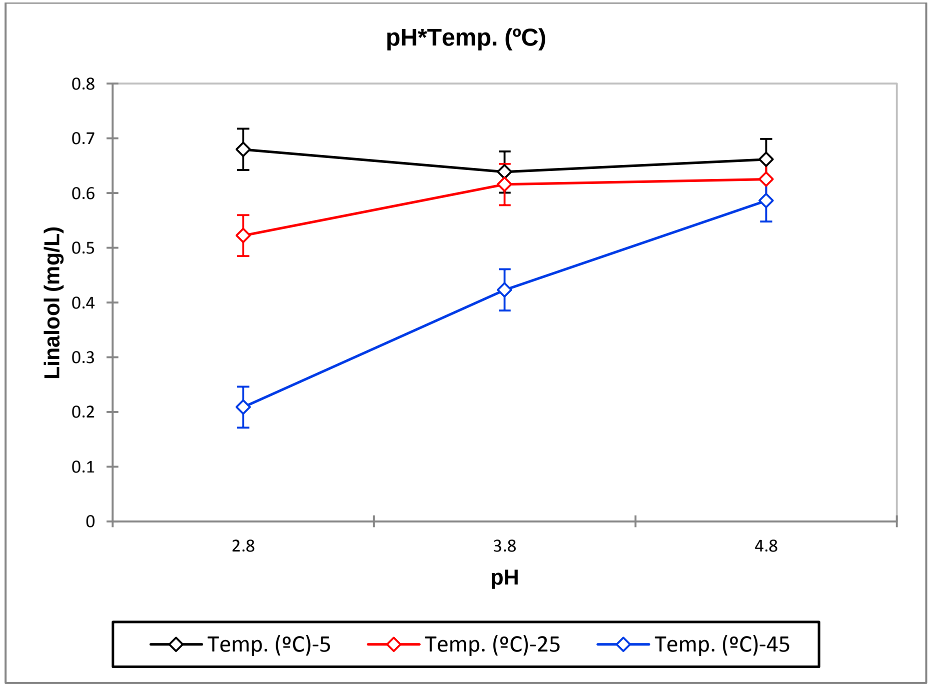




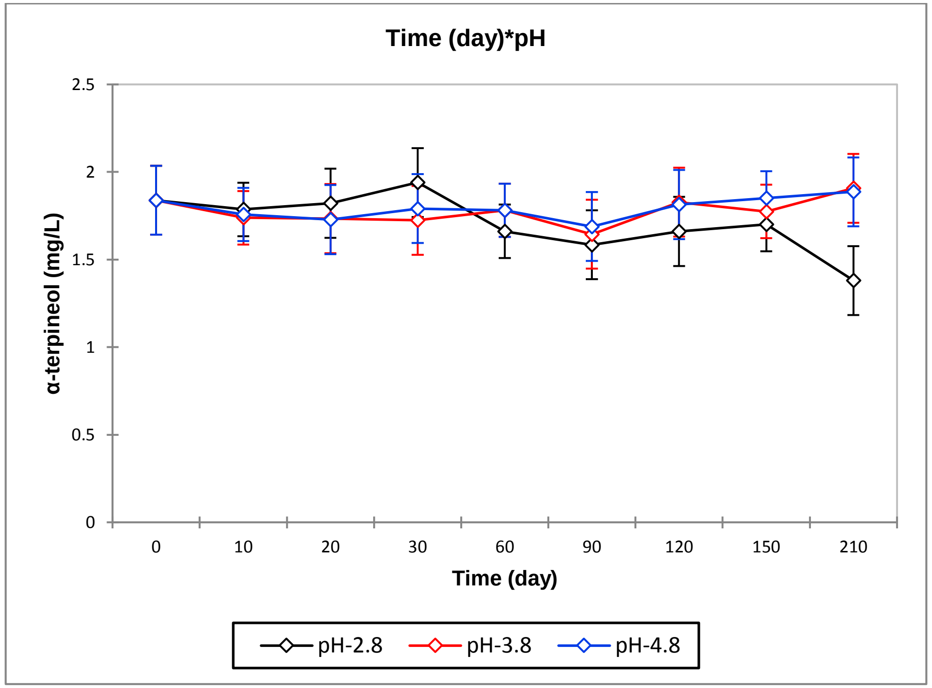
n) Linalool

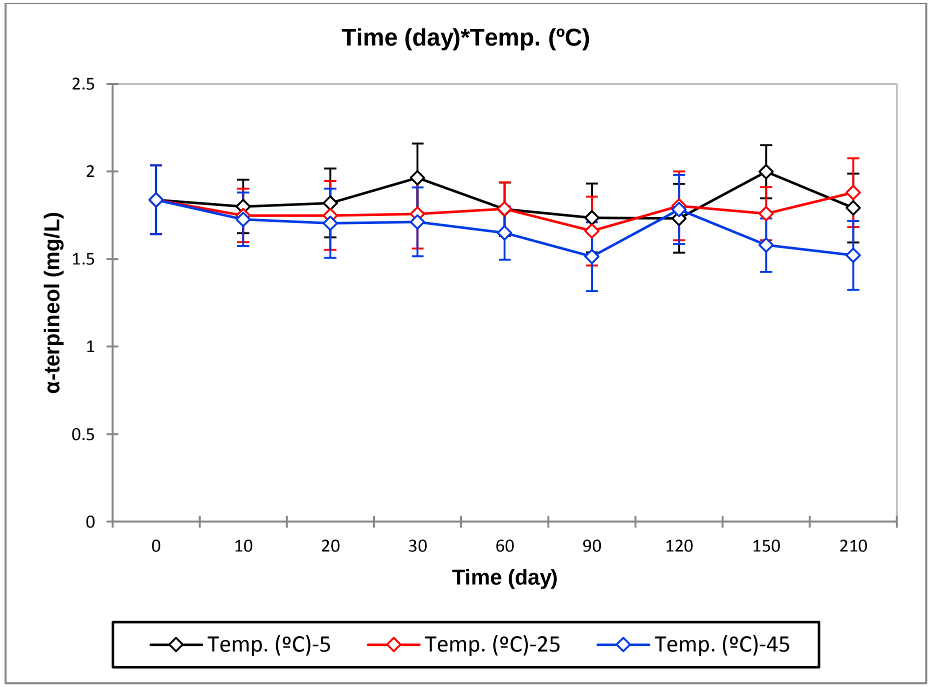

Time (day)*pH
Linalool (mg/L)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
60
90
120
150
210
Time (day)
pH-2.8
pH-3.8
pH-4.8

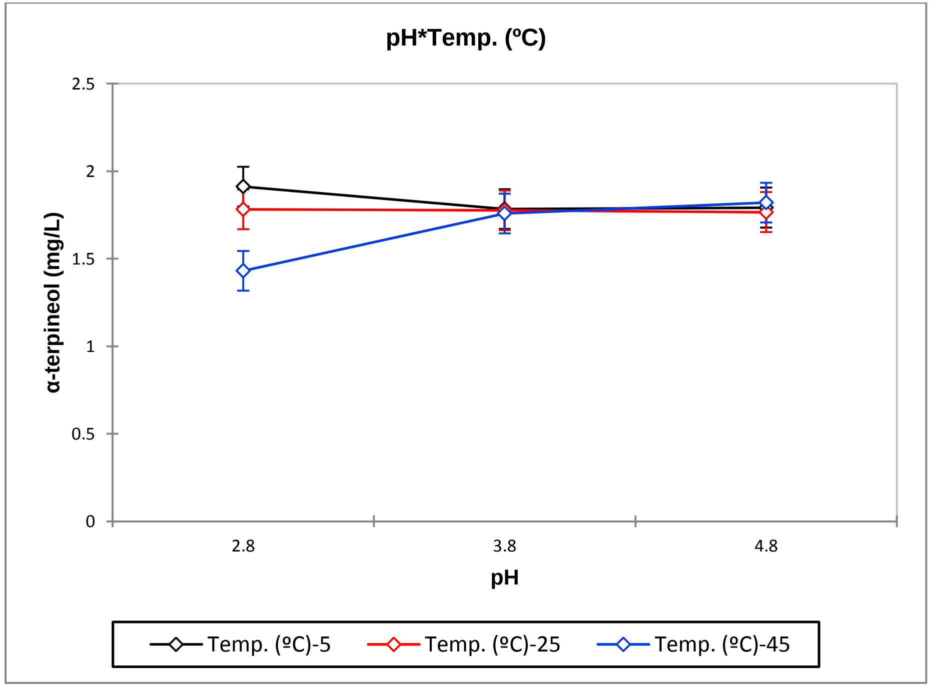

Time (day)*Temp. (ºC)
Linalool (mg/L)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
60
90
120
150
210
Time (day)
Temp. (ºC)-5
Temp. (ºC)-25
Temp. (ºC)-45


pH*Temp. (ºC)
Linalool (mg/L)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2.8
3.8
4.8
pH
Temp. (ºC)-5
Temp. (ºC)-25
Temp. (ºC)-45

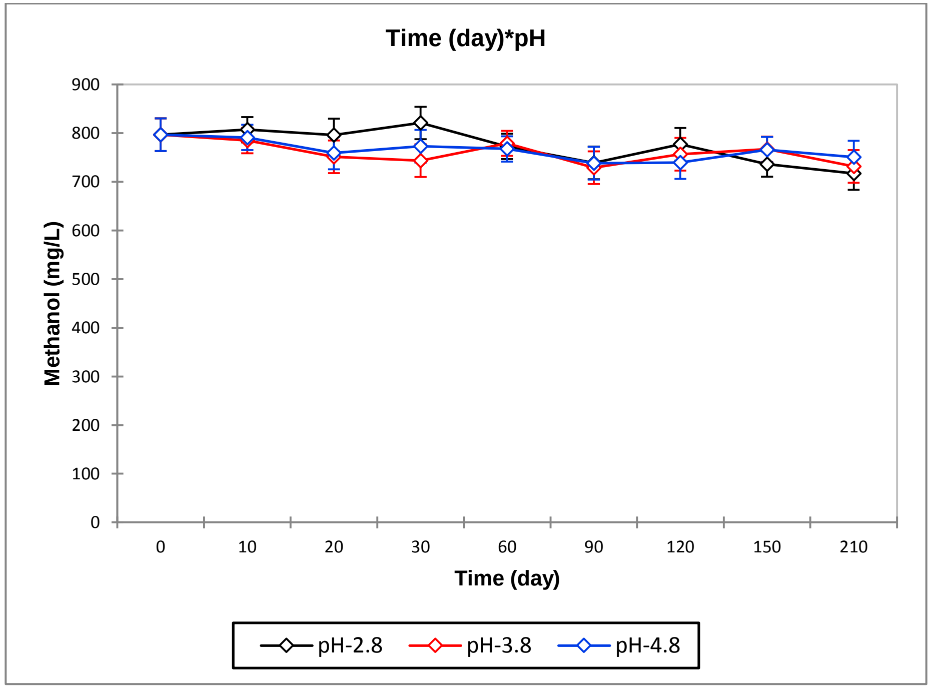
o) α-terpineol

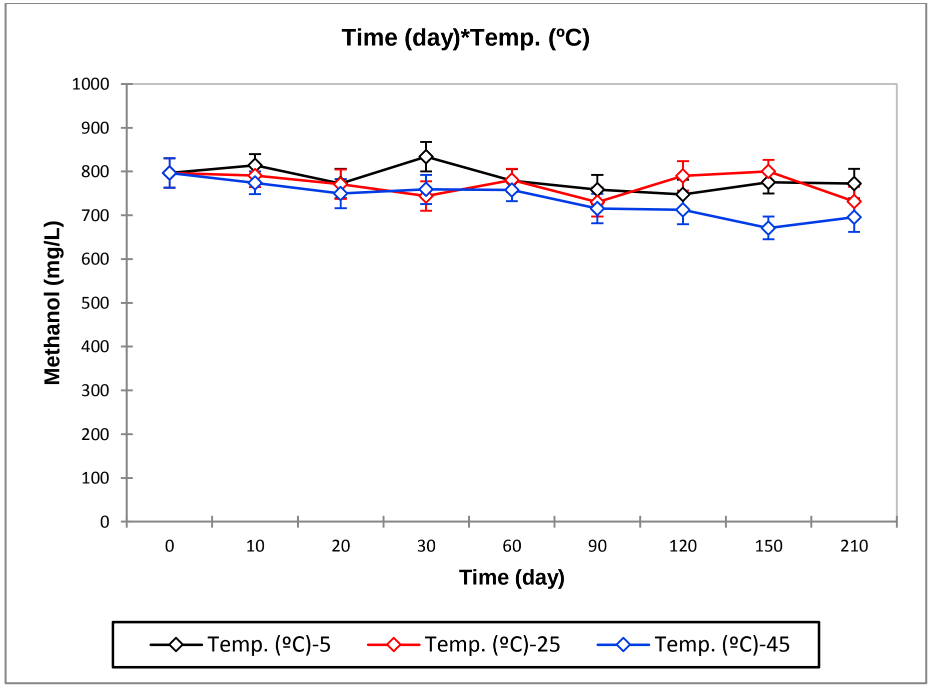


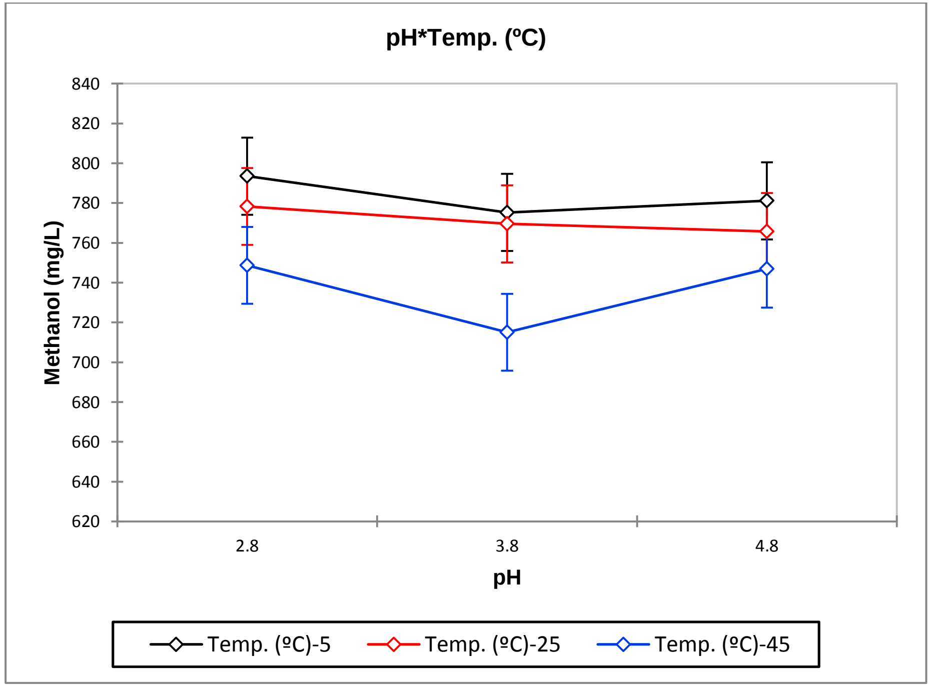




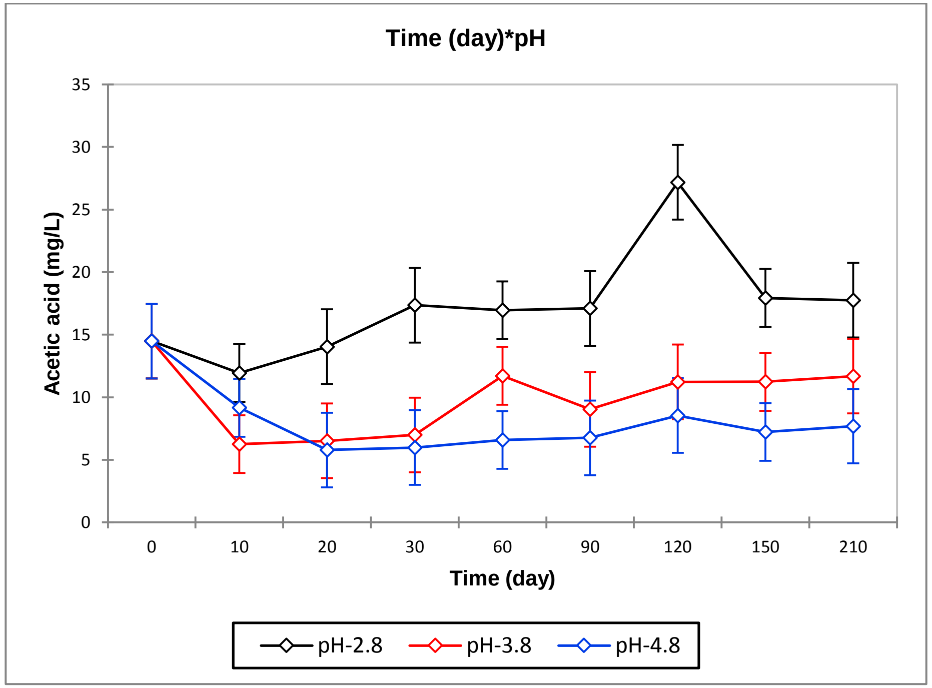
p) Methanol

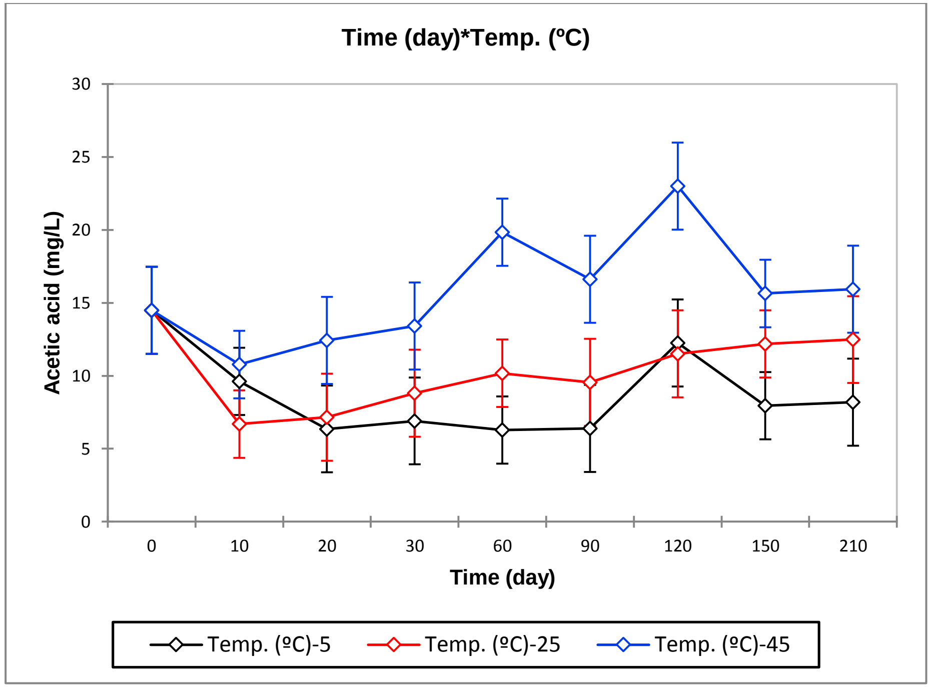

Time (day)*pH
Methanol (mg/L)
0
100
200
300
400
500
600
700
800
900
0
10
20
30
60
90
120
150
210
Time (day)
pH-2.8
pH-3.8
pH-4.8


Time (day)*Temp. (ºC)
Methanol (mg/L)
0
100
200
300
400
500
600
700
800
900
1000
0
10
20
30
60
90
120
150
210
Time (day)
Temp. (ºC)-5
Temp. (ºC)-25
Temp. (ºC)-45


pH*Temp. (ºC)
Methanol (mg/L)
620
640
660
680
700
720
740
760
780
800
820
840
2.8
3.8
4.8
pH
Temp. (ºC)-5
Temp. (ºC)-25
Temp. (ºC)-45

q) Acetic acid





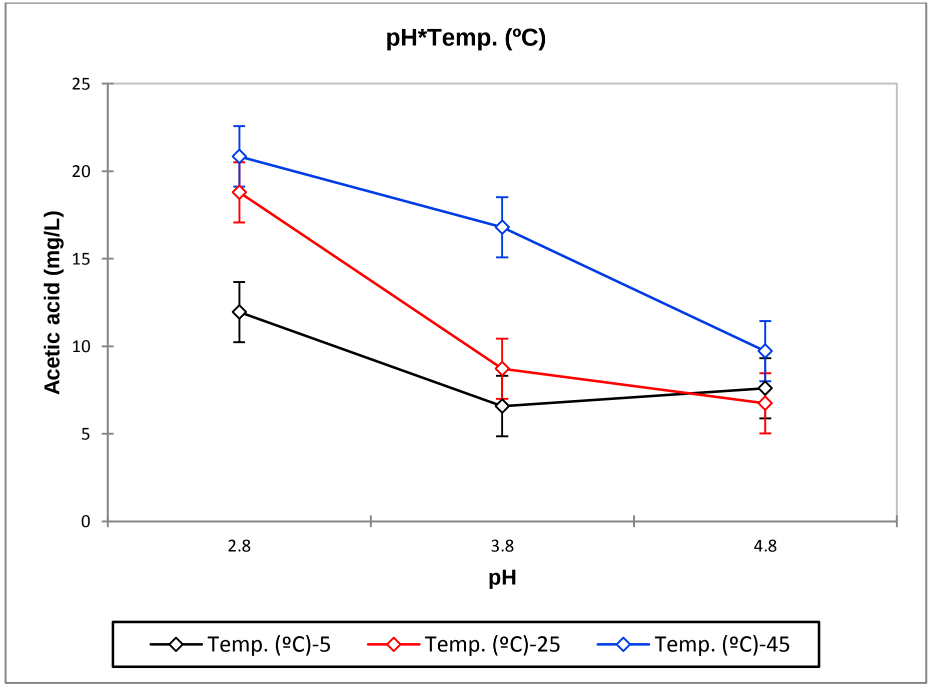
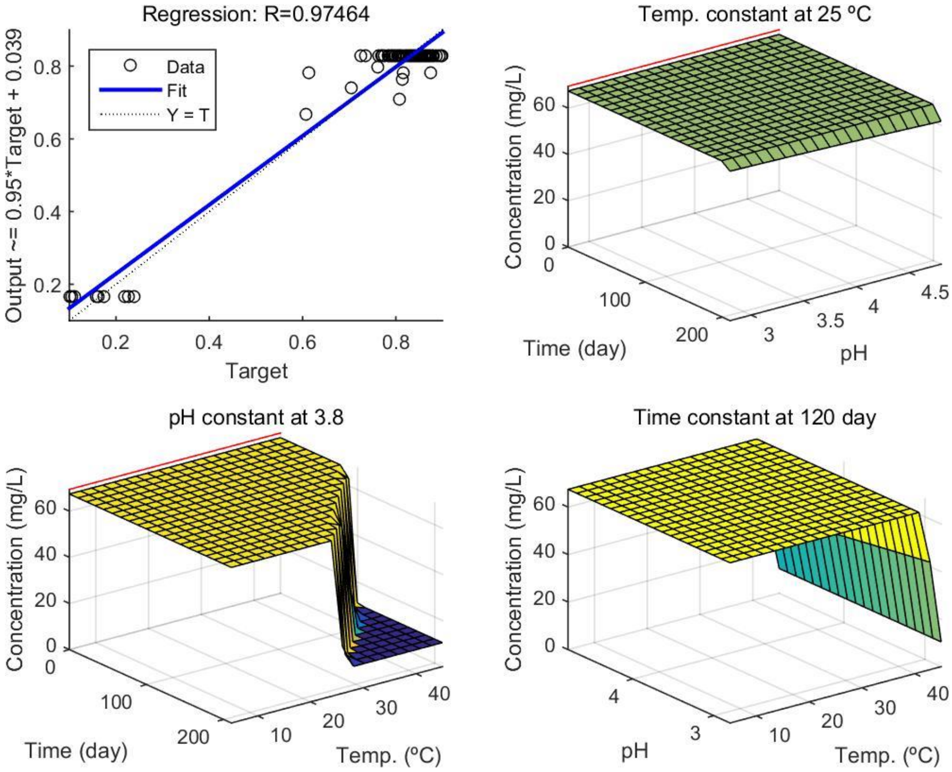
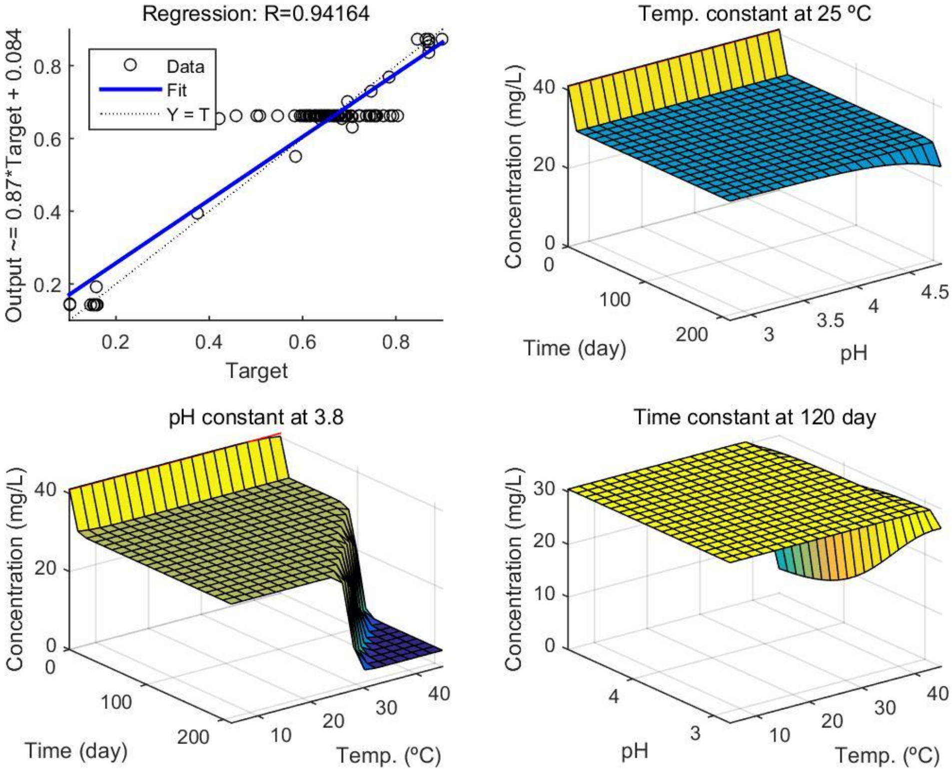


Figure S5.2. ANN models for all compounds concentration (except ethyl hexanoate plots which are shown in the manuscript): regression plot and surface diagrams of the predicted response.

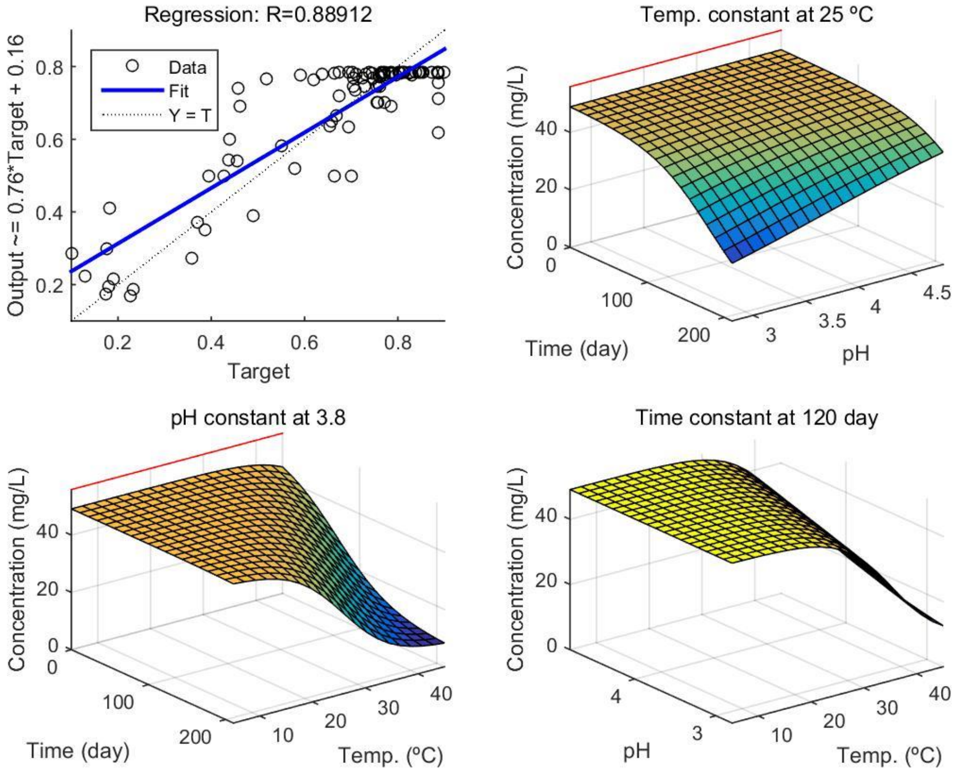
a) Acetaldehyde



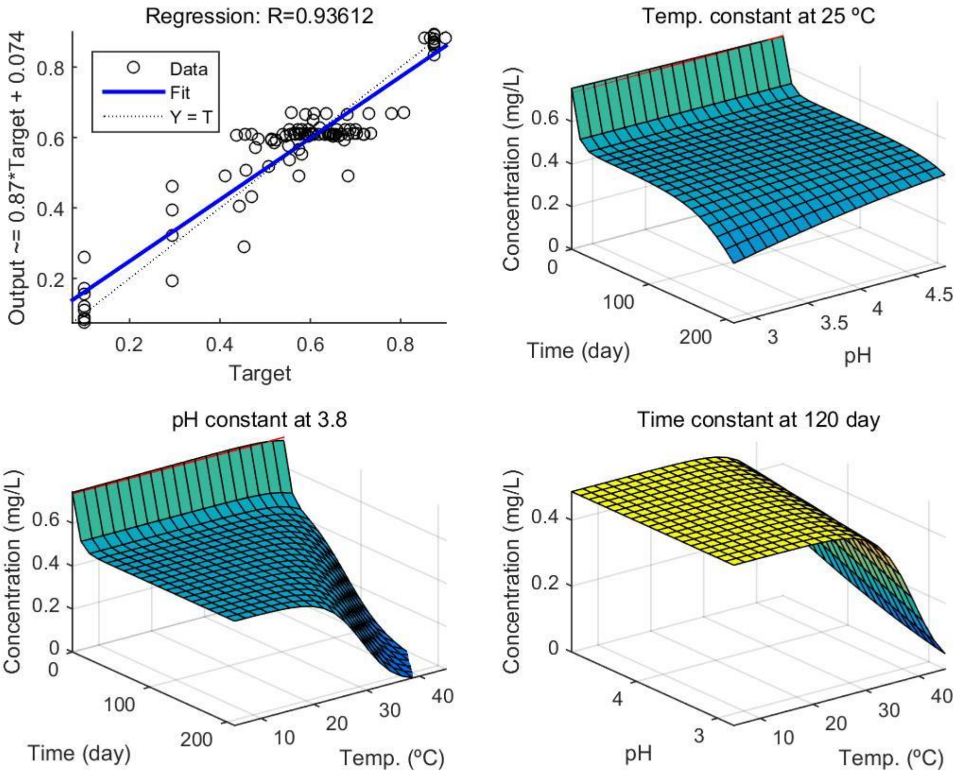
b) Acetal



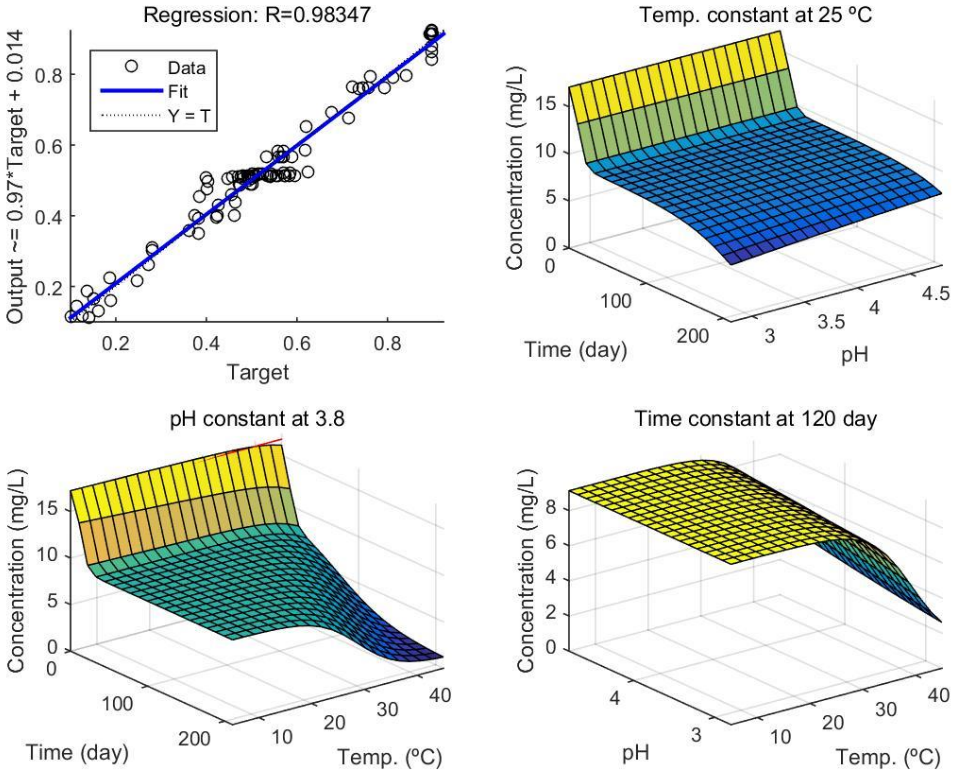
c) *Ethyl acetate*



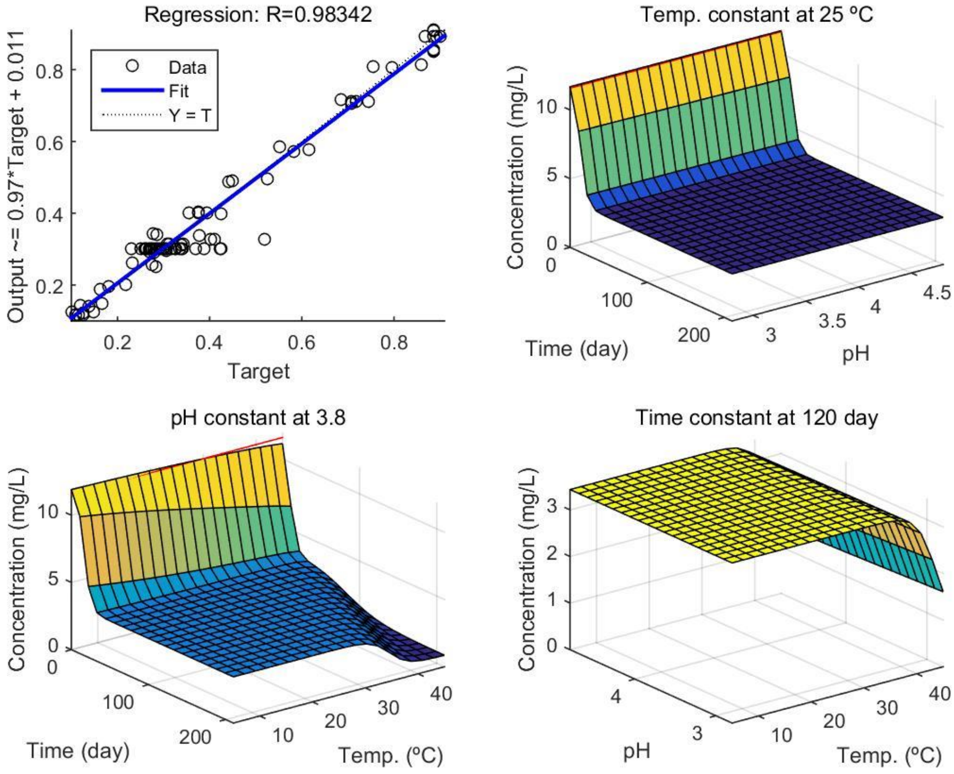
d) *Ethyl butyrate*



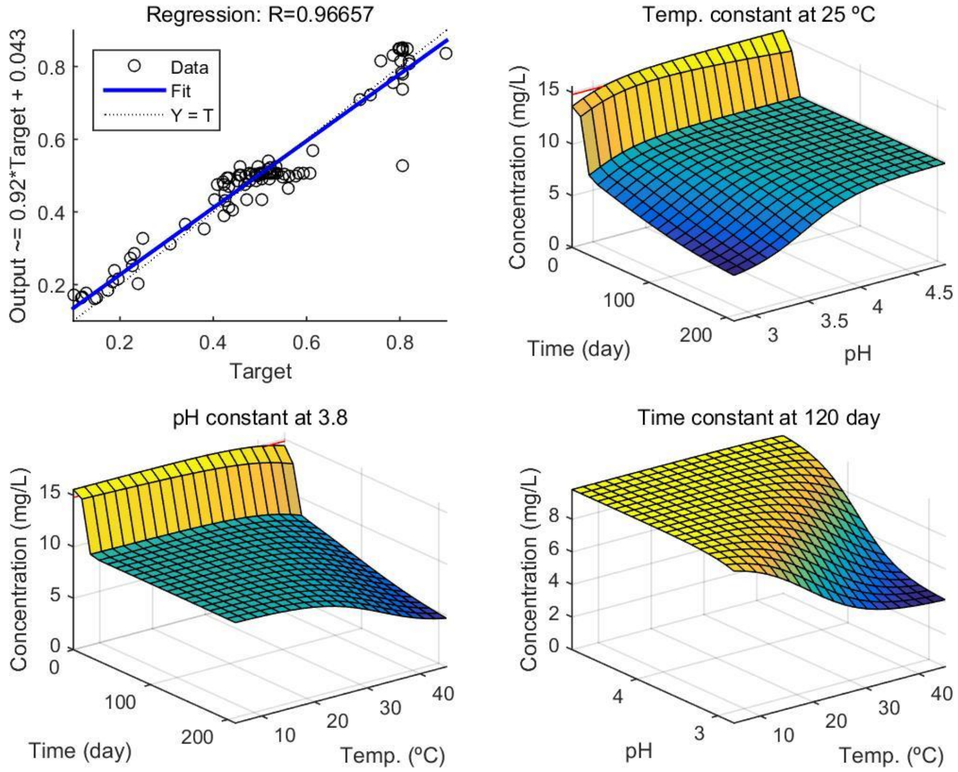
f) Ethyl octanoate



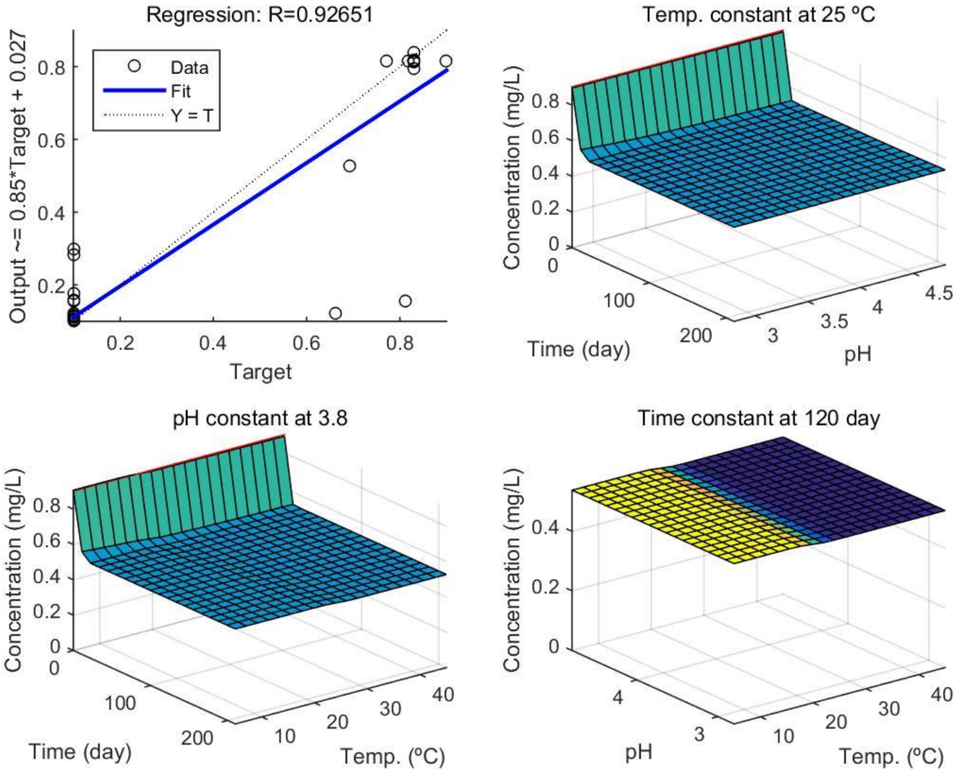
g) Ethyl decanoate



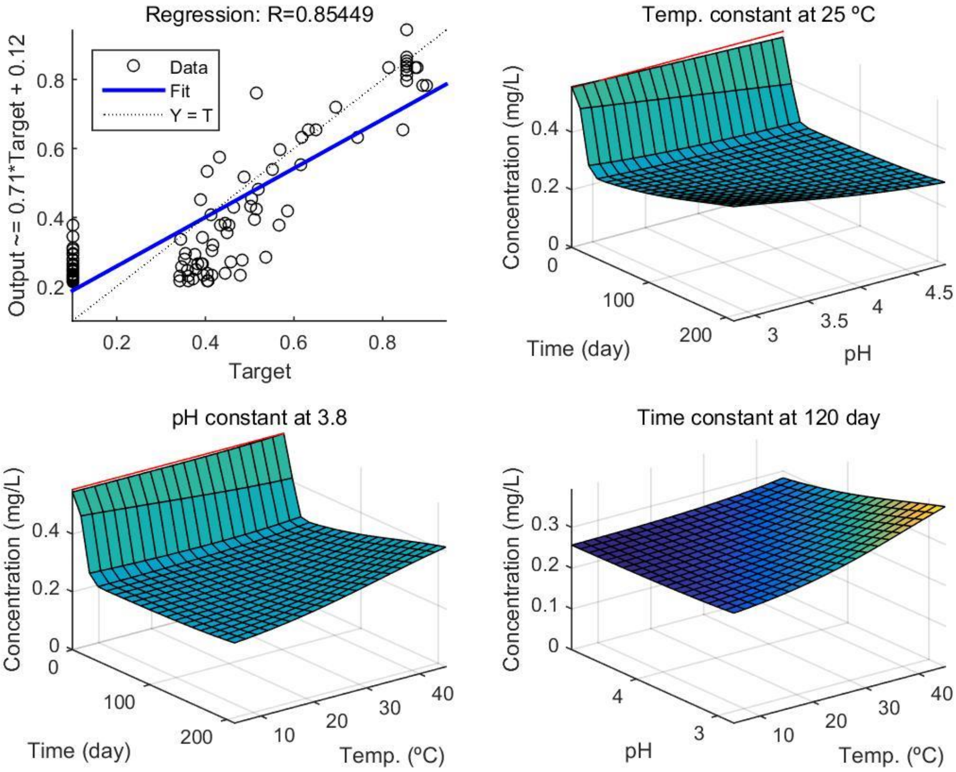
h) *Ethyl lactate*



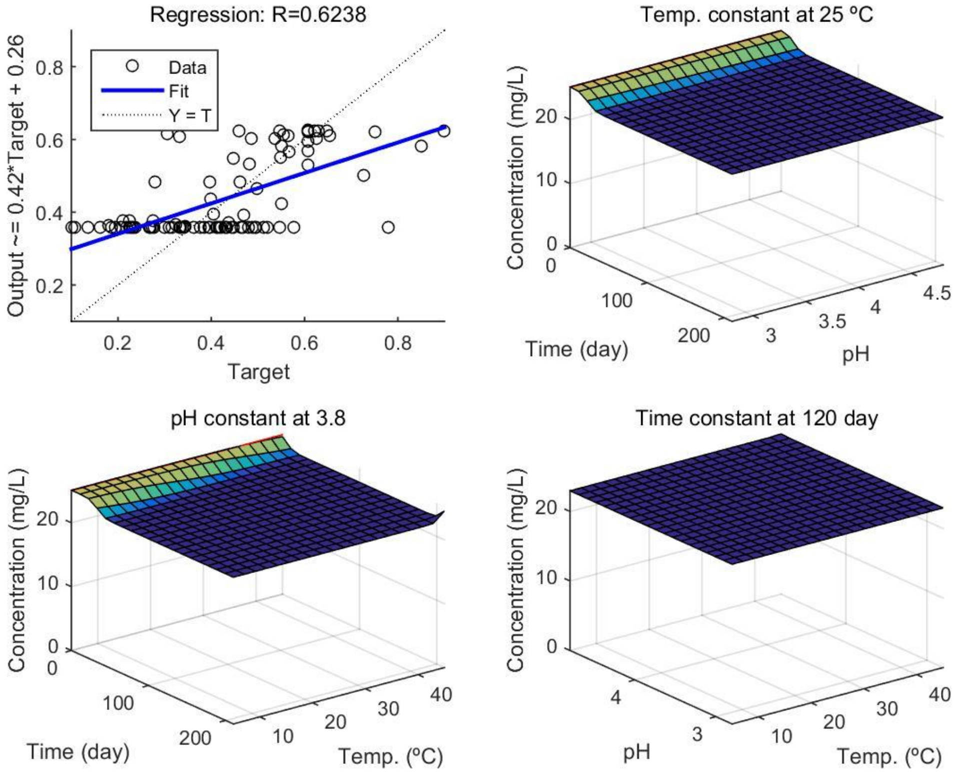
i) *Furfural*



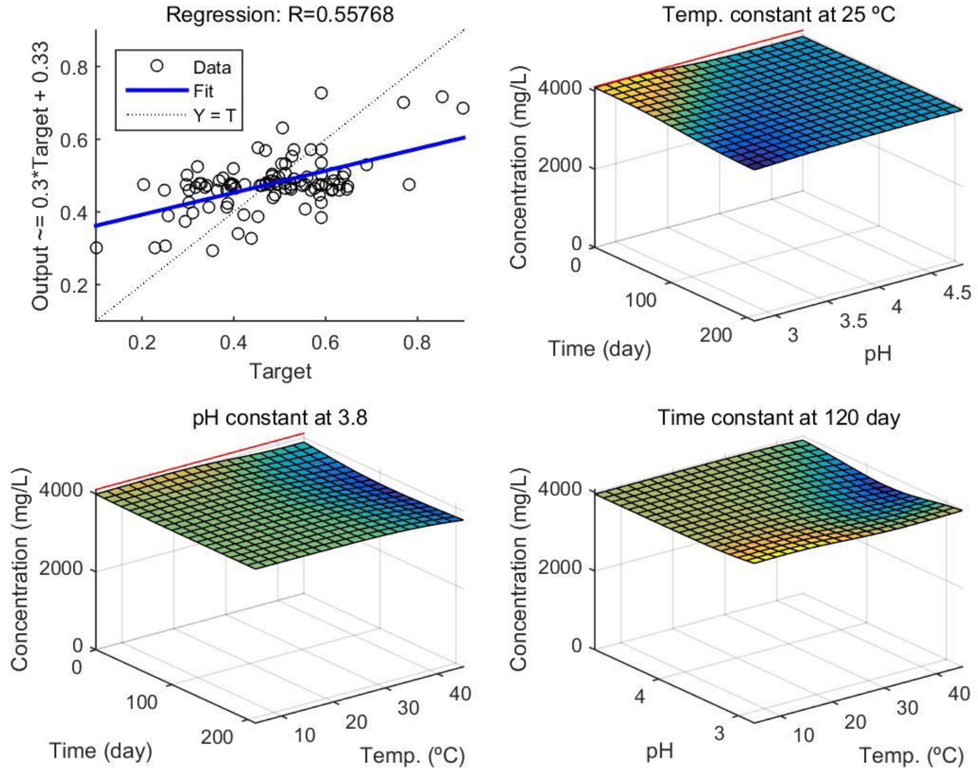
j) β-phenylethanol



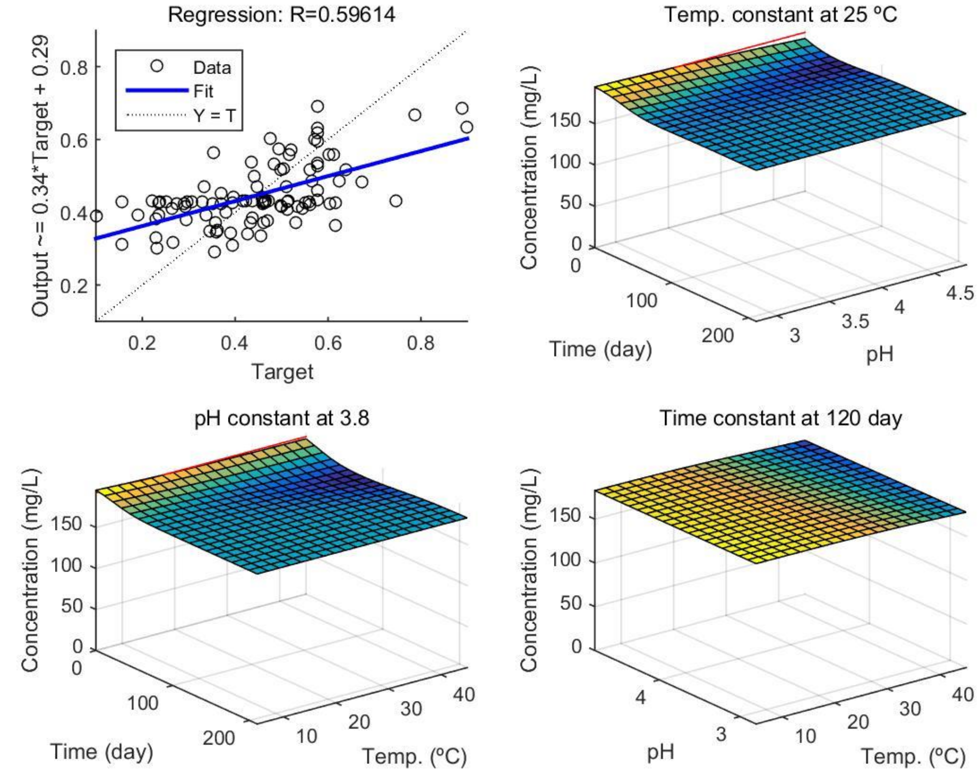
k) 1-hexanol



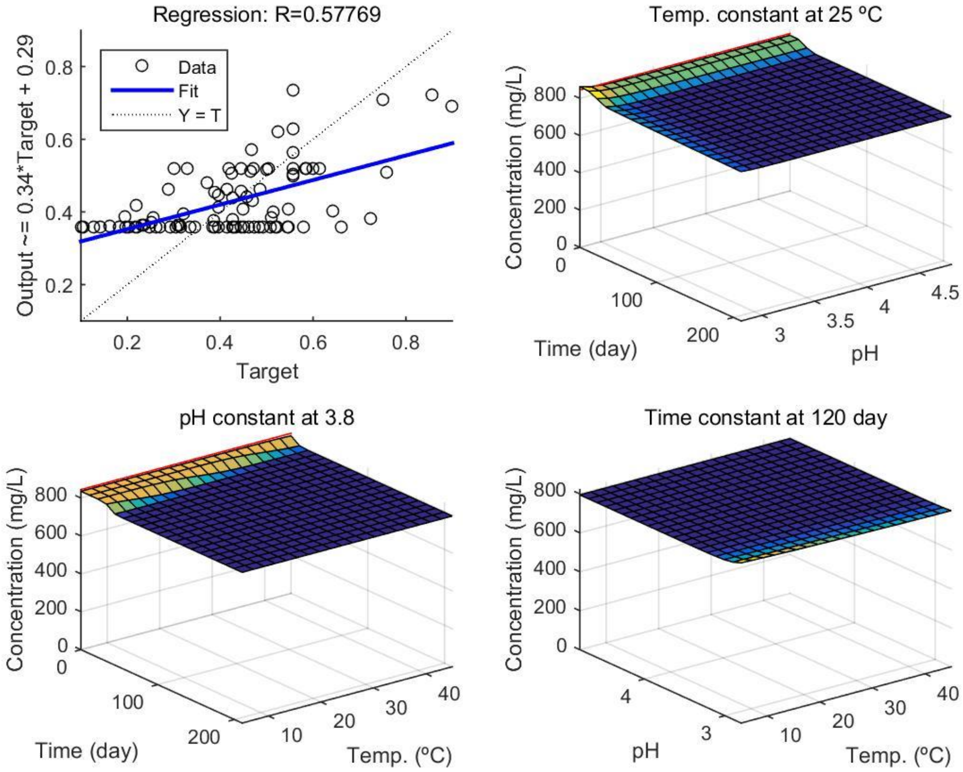
l) 1-propanol



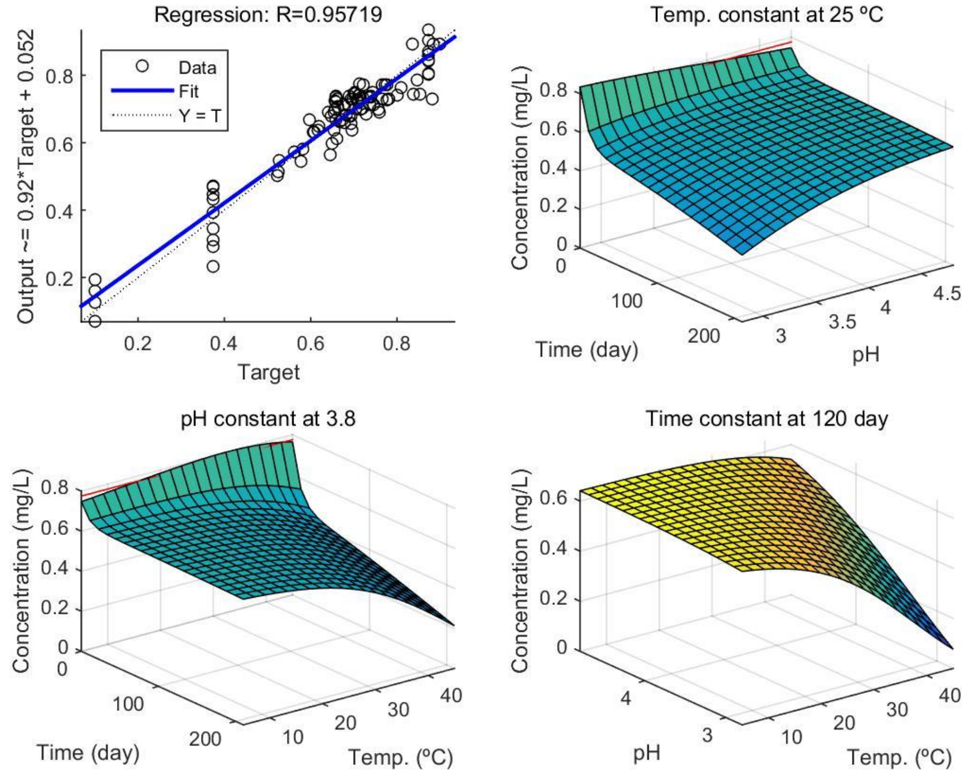
m) 2-methyl-1-butanol



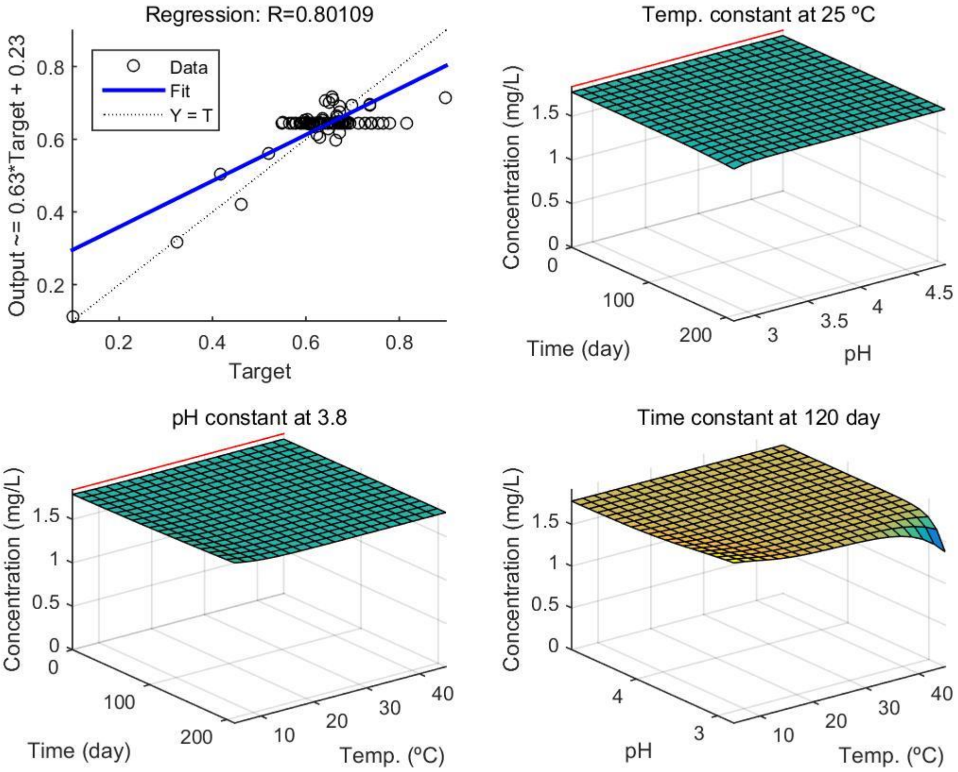
n) 3-methyl-1-butanol



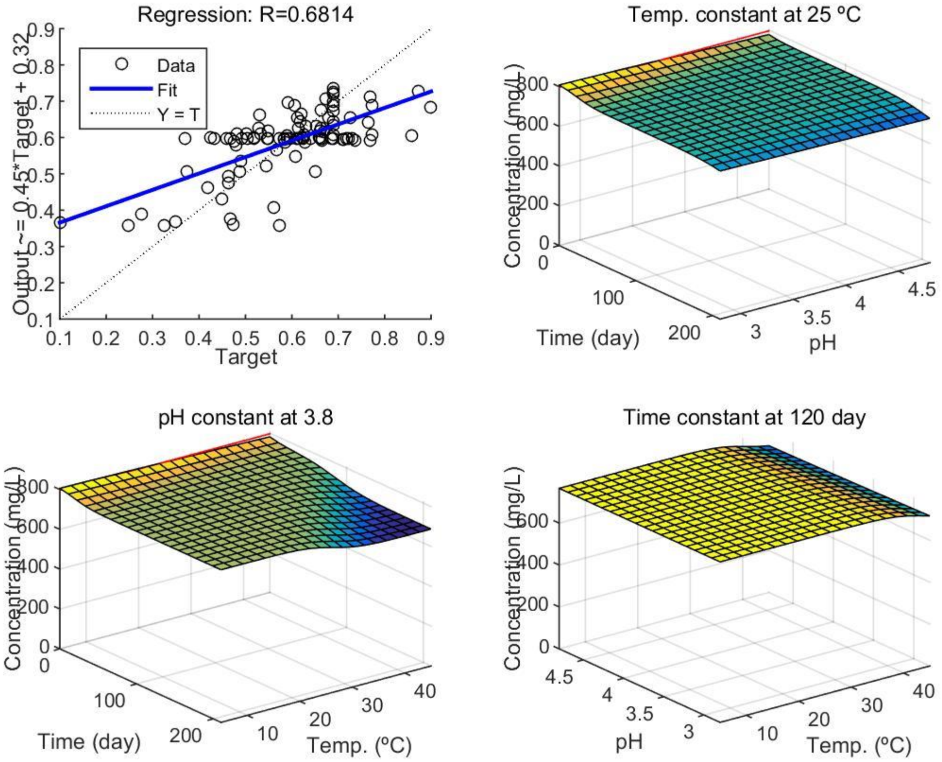
o) Linalool



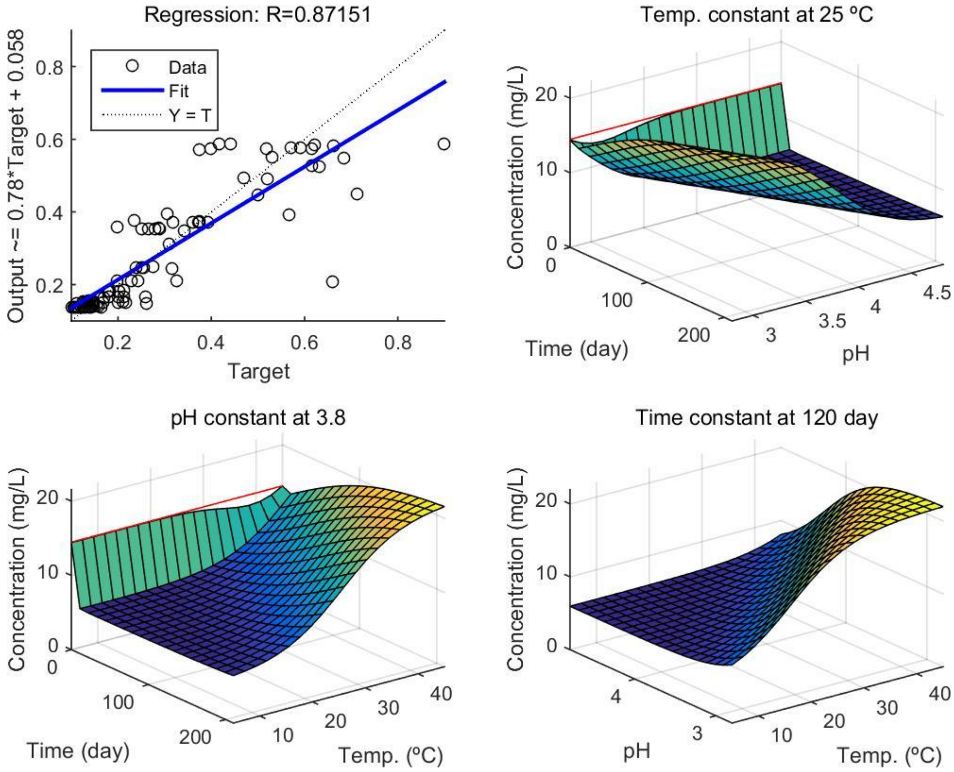
p) α-terpineol



q) Methanol



r) Acetic acid



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