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POTENTIAL OF BLANQUILLA PEAR VARIETY TO PRODUCE PEAR SPIRITS:INFLUENCE OF THE FERMENTATION
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Laura Andrea García Llobodanin
ISBN:978-84-691-8864-4/DL:T-2061-2008

Department of Chemical Engineering

Universitat Rovira i Virgili

Spain

Potential of *Blanquilla* pear variety to produce pear spirits:

Influence of the fermentation and distillation conditions in the final quality

Thesis submitted by

LAURA GARCÍA LLOBODANIN

to obtain the degree of

Doctor from Universitat Rovira i Virgili.

Tarragona, September 2008.

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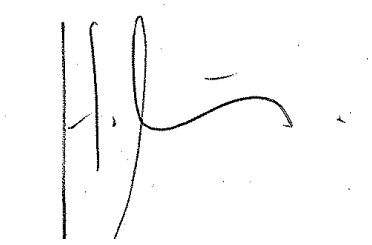
Dr. Francisco López Bonillo, associate professor of the Department of Chemical Engineering at the Universitat Rovira i Virgili

Certify: That the doctoral thesis entitled 'Potential of Blanquilla pear variety to produce pear spirits: influence of the fermentation and distillation conditions in the final quality of the spirits', submitted by Laura García Llobodanin to obtain the degree of Doctor from Universitat Rovira i Virgili, has been carried out under my supervision at the Department of Chemical Engineering of the Universitat Rovira i Virgili.

Tarragona, September 2008.



Dr. Francisco López Bonillo

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Dedicated to my parents.

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A mis tías Nilda y Editta, por demostrarme todo su cariño a través de nuestras charlas telefónicas. Aunque las circunstancias hicieron que fueran esporádicas, siempre las sentí muy cerca. A mi madrina, que desde un primer momento me apoyó a pesar de que le costaba mucho hacerse a la idea de tenerme lejos. Aunque ya no está con nosotros, siento que de alguna forma me acompaña en este logro.

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Thesis summary

According to the European Council Regulation 1576/89, fruit spirits are alcoholic beverages 'produced exclusively by the alcoholic fermentation and distillation of fleshy fruit or must of such fruit, with or without stones'. Beverages are produced according to this definition from different fruits all over the world. In Spain, the most traditional ones are produced from grape. However, in recent years, research has been carried out on other local fruits such as melon.

Fruit production in Spain is quite high, and it is the second pear producing country in the European Union. In particular, Catalonia produces more than half of the total amount of pear harvested in Spain every year. *Blanquilla* is a variety of Spanish pear, and it is the second most produced in the country (after the *Conference*). The *Blanquilla* pear is consumed locally and is also exported to other countries. Some of the pear harvest is industrialized to produce pear juices and concentrates. This context gave rise to the idea of producing a *Blanquilla* pear spirit, extremely interesting for the productive sector in the area, since the added value of the product could have an important influence on the economy of the region.

The aim of the present work, then, is to study the potential of the *Blanquilla* pear variety for producing a fruit spirit. The research focused on three main aspects of the spirit production process: the fermentation conditions, the distillation conditions and the raw material used. The influence of each of them on the quality of the distillates obtained therefrom was studied by detecting and quantifying the main flavor compounds.

The fermentation process is the first step in obtaining a fruit spirit. The conditions of the process can influence the formation of volatile compounds and, therefore, the quality of the spirit. This research focused on three main variables: the yeast employed, the fermentation temperature and the fermentation pH. Experiments were performed in two different conditions of each variable, and the distillates obtained after the respective distillations were analyzed (for their aromatic composition and ethanol content) and compared.

The distillation process also has a crucial role in the quality of the fruit spirit. The differences in the distillates obtained by distilling fermented pear juice concentrate with or without the wine lees was studied, as were the differences between distilling in a glass device, a copper alembic or a glass device with the addition of copper shavings. In these experiments, the distillates were obtained by one simple batch distillation, as a first approach to the production process.

Finally, two different raw materials were used under the same fermentation and distillation conditions: *Blanquilla* pear juice concentrate and *Blanquilla* natural pear juice. The use of juice concentrate has logistic advantages, since it is more stable than the fruit or the juice (so it can be stored for a longer period) and it occupies less space at industrial facilities.

The results showed that the fermentation yeast, temperature and pH have a significant effect on the volatile composition of the pear spirits obtained. However, the sensory evaluations performed on the spirits obtained with different yeasts and the spirits obtained at different pHs showed no significant differences among them. This means that the chemical differences detected in the distillates could not be perceived sensorially.

The aromatic compounds quantified indicate that the best quality distillates are produced by the distillations in a copper alembic. The distillations performed in presence of the fermentation lees usually give better quality distillates, though in some cases no significant differences were detected between the volatile composition of the distillates distilled in the presence or absence of the fermentation lees.

The raw material employed also affected the volatile composition of the distillates, although the results did not allow any conclusions to be drawn about quality. However, sensorial analysis showed that distillates from natural pear juice were preferred to distillates from pear juice concentrate because of their fruitier character.

In conclusion, the *Blanquilla* pear variety is suitable for the production of pear spirits. On the basis of the results obtained, the fermentation conditions did not seem to play a critical role in the quality of the pear spirit. On the contrary, distillation in a copper alembic and in the presence of the fermentation lees

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seemed to improve the quality of the distillates. In addition, natural pear juice was preferred to pear juice concentrate. Therefore, some improvement should be made in the future (i.e. the addition of the extracted aroma compounds to the pear concentrate before fermentation), if the pear juice concentrate is to be used.

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Resúmen

De acuerdo con la Regulación del Consejo Europeo (Nº 1576/89), los aguardientes de fruta son bebidas alcohólicas 'producidas exclusivamente por la fermentación alcohólica y destilación de la fruta o el mosto de dicha fruta, con o sin huesos'. Bebidas bajo esta denominación son producidas a partir de diferentes frutas en todo el mundo. En España, las más tradicionales son las de uva. Sin embargo, durante los últimos años se han realizado investigaciones con el fin de utilizar otras frutas locales tales como el melón.

La producción de frutas en España es elevada, siendo el segundo país productor de peras de la Unión Europea. Dentro de España, Cataluña produce más de la mitad del total de peras cosechadas en el estado cada año. La variedad *Blanquilla* es de origen español, y es la segunda más producida en el país (luego de la *Conference*). La pera *Blanquilla* se consume en el mercado local y también es exportada a otros países. Una parte de la misma se industrializa, produciendo zumos y concentrados. Dentro de este contexto, surge la idea de producir un aguardiente de fruta utilizando peras de la variedad *Blanquilla*. Un producto con estas características resultaría de extremo interés para el sector productivo de la zona ya que le otorgaría un valor agregado a la fruta, con el consecuente empuje económico que trae aparejado para la region.

Por lo anteriormente expuesto, el objetivo del presente trabajo es estudiar el potencial de la variedad de pera *Blanquilla* para producir aguardientes de fruta. La investigación se centró en tres aspectos fundamentales dentro del proceso productivo de los aguardientes: las condiciones de fermentación, las condiciones de destilación, y la materia prima utilizada. La influencia de cada uno de ellos en la calidad de los destilados obtenidos se determinó por medio de la detección y cuantificación de los principales compuestos volátiles.

El proceso fermentativo es la primera etapa en la obtención de un aguardiente de fruta. Las condiciones en que es llevado a cabo dicho proceso pueden influenciar la formación de compuestos volátiles, y por lo tanto, la calidad del destilado. La presente investigación se centró en tres aspectos: la levadura utilizada, la

temperatura de fermentación y el pH de fermentación. Se llevaron a cabo experimentos bajo dos condiciones diferentes de cada uno de los aspectos mencionados. Los destilados obtenidos luego de las respectivas destilaciones fueron analizados (para determinar su composición aromática y el contenido de etanol) y comparados entre sí.

El proceso de destilación también tiene un papel preponderante en la calidad de los aguardientes de fruta. Las diferencias entre los destilados obtenidos por destilación con y sin lías del zumo concentrado de pera fermentado fueron estudiadas. Por otra parte, las diferencias al destilar en un dispositivo de vidrio, un alambique de cobre, y un dispositivo de vidrio con virutas de cobre fueron analizadas. En estos experimentos, los destilados fueron obtenidos por medio de una sola destilación simple, como primera aproximación al proceso productivo.

Para finalizar, dos materias primas diferentes fueron utilizadas bajo las mismas condiciones de fermentación y destilación: zumo concentrado y zumo natural de la variedad de pera *Blanquilla*. La utilización de zumo concentrado tiene ventajas desde el punto de vista logístico, ya que es más estable que la fruta y que el zumo natural (por lo que puede ser almacenado por un período de tiempo más prolongado), y además ocupa menos espacio en las instalaciones industriales.

Los resultados obtenidos muestran que la levadura utilizada, la temperatura y el pH de fermentación producen algunas diferencias significativas en la composición de volátiles de los aguardientes de pera obtenidos. Sin embargo, no se pudo realizar ninguna afirmación acerca de cuál es el producto de mejor calidad. Por otra parte, las evaluaciones sensoriales realizadas a los aguardientes obtenidos con las diferentes levaduras y a los aguardientes obtenidos a diferentes pHs, indicaron que no habían diferencias significativas entre las dos condiciones evaluadas de cada aspecto. Esto significa que las diferencias encontradas en la composición química de los destilados, no son percibidas sensorialmente.

En lo referente al proceso de destilación, se observó que las destilaciones en alambique de cobre producen los destilados de mejor calidad (basándose en los compuestos aromáticos analizados). Las destilaciones realizadas en presencia de las lías usualmente proporcionan una mejor calidad a los destilados, aunque en algunos casos no se obtuvieron diferencias significativas cuando se comparó la

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composición volátil de los destilados producidos en presencia y ausencia de las lías.

La materia prima utilizada también afectó la composición volátil de los destilados, aunque no se pudo establecer un juicio acerca de la calidad de los mismos basándose en los resultados analíticos obtenidos. Sin embargo, en el caso en que se realizó un análisis sensorial, se obtuvo que los destilados producidos a partir de zumo natural eran preferidos frente a los producidos a partir de zumo concentrado. La razón principal fue por la presencia de un carácter frutal más acentuado.

En conclusión, se puede afirmar que la variedad de pera *Blanquilla* es adecuada para la producción de aguardientes de pera. Basándose en los resultados obtenidos, las condiciones de fermentación no parecen desempeñar un rol muy crítico en la calidad de los aguardientes de pera *Blanquilla*. Por el contrario, el proceso de destilación llevado a cabo en alambique de cobre y en presencia de las lías parece mejorar la calidad de los destilados. En cuanto a la materia prima utilizada, el zumo natural se prefirió ante el zumo concentrado para la elaboración de las bebidas alcohólicas. Por lo tanto, algunas mejoras deben realizarse en el futuro (por ejemplo la adición al zumo concentrado de extracto de aromas recuperados del proceso de concentración), si se desea utilizar el zumo concentrado como materia prima.

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Abbreviations

GC – Gas Chromatography

HPLC – High Perfomance Liquid Chromatography

ANOVA – Analysis of Variance

PCA – Principal Component Analysis

g/hL a.a. – grams per hectolitre of absolute alcohol

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Chapter 1

SCOPE & OBJECTIVES

1.1 Introduction

According to the European Council Regulation (Nº 1576/89), Article 1, Section 2, a spirit drink is defined as an alcoholic liquid intended for human consumption, having particular organoleptic qualities, a minimum alcoholic strength of 15 % (v/v), and produced either directly by the distillation of natural fermented products, with or without added flavorings, and/or by the maceration of vegetable substances, and/or the addition of other regulated substances [1]. Within the same Article, in Section 4 different categories of spirit drinks are defined: rum, whisky or whiskey, grain spirit, wine spirit, brandy or weinbrand, grape marc spirit or grape marc, fruit marc spirit, fruit spirits, cider spirit, cider brandy or perry spirit, fruit spirit drinks, juniper-flavored spirit drinks, aniseed-flavored spirit drinks and vodka, among others.

In the Mediterranean area the spirit drinks most commonly produced are derived from grape, by distillation of either the wine (wine spirits) or the residues generated during its elaboration process (grape marc spirits). The wine spirits are usually transformed to brandies due to an ageing process in oak casks. Some Protected Designation of Origin brandies such as *Cognac* and *Armagnac* in France, and *brandy de Jerez* in Spain, are very well known in the region. In South America, specifically in Chile and Peru, a wine spirit obtained from *muscat* grapes called *pisco* is a national favorite drink [2,3]. Nevertheless, back in the Mediterranean region, the most popular drinks are the ones obtained from the grape marc (grape marc spirits) [4,5,6].

Regarding other fruits, the most usual is the elaboration of fruit spirits or fruit spirit drinks. The first ones are obtained from the distillation of fermented fruit or fermented must, and the second ones are obtained by maceration of the fruit with

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ethanol of agricultural origin [1]. Two of the most commonly employed fruits for the elaboration of fruit spirits are apples and pears. To obtain a fruit spirit from apple, the first step is to ferment the fruit to elaborate the cider, which is then distilled to obtain the apple spirit. These spirits can be used for the elaboration of cider brandy (aged in oak casks). The cider brandies produced in Normandy region (France) are called *Calvados*, and they have a Protected Designation of Origin (*Appellation d'Origine Contrôlée*) [7]. Pear is also extensively used in Central Europe for the production of pear spirits. The name *Williams* is allowed by the European Council Regulation to describe pear spirits produced solely from pears of the *William* variety [1].

Other fruits are also used to produce spirits in many different countries all over the world. Usually they are elaborated from fruits cultivated in the area, and several countries have their own typical fruit spirits. The *koumaro* and *mouro* are produced in Greece from strawberry tree fruit and mulberry tree fruit respectively [8,9]. The *aguardente de medronho* is produced in Portugal, also from strawberry tree fruit [10]. In Eastern and Central Europe, plum brandies (*slivovitz*) matured under appropriate conditions are the most popular fruit brandies prepared from fresh *Wegierka* plums [11].

During the last years, some research has been developed in order to use local fruits which are not traditionally employed in the production of fruit spirits. Da Porto et al. analyzed the production technology and flavor composition of orange spirits from Tarocco variety (Italy) [12,13]. In addition, Hernández-Gómez et al. studied the development of a melon fruit spirit produced with melons from La Mancha region (Spain) [14,15,16].

As it was already mentioned, pear spirits are one of most common fruit spirits, especially in Central Europe. The variety used for its elaboration is the *Williams* or *Bartlett* (denomination employed in the United States of America). However, as far as the author knows, the use of the *Blanquilla* variety for the production of pear spirits has not been studied before.

The aim of the present research work is to develop a fruit spirit from *Blanquilla* pear, a Spanish variety which is the most important in Catalonia (Spain). To this end, different fermentation and distillation conditions are tested and different raw

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materials are used (pear natural juice and pear juice concentrate). The volatile composition of the distillates obtained is determined, and this data is used to define the best quality product. In some cases, a sensory evaluation of the spirits is performed with a panel of consumers. This allows checking for organoleptic differences in the distillates, which not always go along with the chemical differences.

1.2 Pear production in Spain

Spain has an important pear production, being the second producer country of the European Union. **Table 1.1** shows the estimate figures for the production in the European Union during the years 2005 and 2006.

Table 1.1. Pears production (1000 tons) in the European Union during 2005 and 2006.

country	2005	2006
Italy	882	966
Spain	608	535
France	230	246
Belgium	229	268
Holland	200	234
Portugal	130	175
other countries	252	236
total production in the EU	**2531**	**2660**

Source: reference [17]

Inside Spain, Catalonia Cataluña) is the main producer, producing more than half of the total amount harvested each year. **Table 1.2** shows the provisional figures for each Spanish Autonomic Community during 2006 and 2007.

Depending on the region, different pear varieties are cultivated. In Europe, two of the most common ones are the *Conference* and the *Williams* varieties. **Figure 1.1** shows a photo of each one of them. The *Conference* pear has a tapered shape with a bronze yellowish-green skin, tending to russet at the top. The flesh is creamy-white, firm, sweet and juicy. The *Williams* pear has a bell shape, and its green skin turns yellow upon later ripening, although red-skinned derivative varieties

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exist. It has a very distinctive 'pear-like' aroma, and presents a creamy and juicy flesh.

Table 1.2. Pears production (1000 tons) in the Spanish Autonomic Communities during 2006 and 2007

community	2006	2007
Galicia	19.0	15.9
Asturias	0.5	0.5
Cantabria	0.4	0.4
País Vasco	1.2	1.1
Navarra	16.4	17.1
La Rioja	51.0	49.0
Aragón	95.5	103.8
Cataluña	304.9	238.3
Baleares	0.4	0.3
Castilla y León	9.2	10.4
Madrid	0.2	0.2
Castilla La Mancha	2.5	2.0
Comunidad Valenciana	10.7	8.6
Murcia	33.8	28.5
Extremadura	24.6	25.7
Andalucía	17.5	14.4
Canarias	2.2	2.1
total Spanish production	**590**	**518.3**

Source: reference [18]

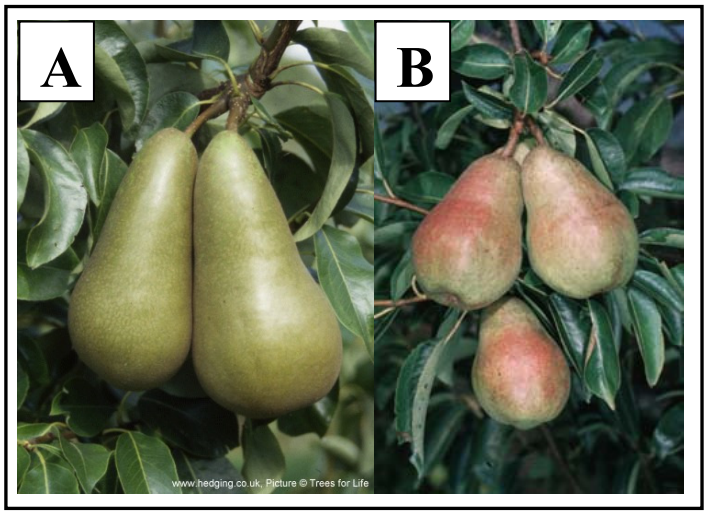

Figure 1.1. Image of (A) *Conference*, (B) *Williams (Bartlett)* pears.

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As **Table 1.3** shows, the *Conference* is the most widely produced variety in Spain, followed by the *Blanquilla*. The *Blanquilla* variety, also called *pera de Aranjuez* or *pera de agua*, is from Spanish origin and is characterized by a thin, bright-greenish skin. Its flesh is white and very juicy. **Figure 1.2** shows different photos of this pear.

Table 1.3. Estimated production (1000 tons) of different pear varieties in Spain during the years 2005 and 2006.

variety	2005	2006
Blanquilla	171.29	139.01
Conference	207.90	187.06
Ercolini-Coscia	46.16	40.30
Limonera-Gruyot	49.54	46.63
Passa Crassana	3.60	2.82
William's	46.18	46.53
others	83.22	72.69
total pears production	**607.89**	**535.04**

Source: reference [17]

Figure 1.2. Pear trees, *Blanquilla* pears before harvest, ripen *Blanquilla* pears.

The *Blanquilla* pear is consumed in the local market and is also exported to other countries. Part of it is industrialized, producing pear juice and concentrate. Within this context, the idea of producing a *Blanquilla* pear spirit results of extreme interest for the productive sector in the area. The added value of this product compared to the raw fruit could have an important influence on the economy of the region.

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1.3 Fruit alcoholic fermentation

The first step to produce a fruit spirit is the alcoholic fermentation of the fruit. During this process, the yeasts use the sugars present in the fruit (together with other nutrients needed in minor amounts) to grow and survive. Ethanol is generated as the most important primary metabolite during the alcoholic fermentation, but also other compounds are produced. Among them we can cite higher alcohols, aldehydes and fatty acid esters that, in spite of being in lower quantities, are of main importance for the aroma profile of wines and distillates [19]. Glycerol is another obtained metabolite, which gives softness to the wine. Finally, methanol is also produced during this stage, derived from the degradation of pectic substances in the raw material [20]. Although its perception threshold is quite high, 1000g/hL a.a. (grams per hectolitre of absolute alcohol), its presence in wines and spirits is extremely important from the toxicological point of view since it can cause headaches, nausea, blurred vision, irreversible blindness and even death [20,21]. Methanol's legal limit in red wines is 400 mg/L, while in white and rosé wines is 250 mg/L (Résolution Oeno 19/2004). For distilled spirits, the European Council fixes the limit in 1000 g/hL a.a. [1].

Fermentations at industrial scale are usually performed in stainless steel tanks. However, other materials can be used such as carbon steel, wood and concrete. In small industries and pilot scale fermentations, plastic tanks and glass bottles are also used. **Figure 1.3** shows several fermentation tanks used at different fermentation scales.

Nowadays, the fermentation conditions are strictly controlled in order to obtain a good quality product. A change in the fermentation conditions (such as pH, temperature and dissolved oxygen), can favor the development of some microorganisms different from the inoculated yeast [19]. These microorganisms develop other types of fermentations, producing high amounts of volatile organic acids and ethylic esters of short chain fatty acids (C_2-C_6). All these compounds have negative effects on the quality of the final product. For this reason, it is important to maintain constant and favorable fermentation conditions to assure the growth of the inoculated microorganism and therefore assure a correct alcoholic fermentation process.

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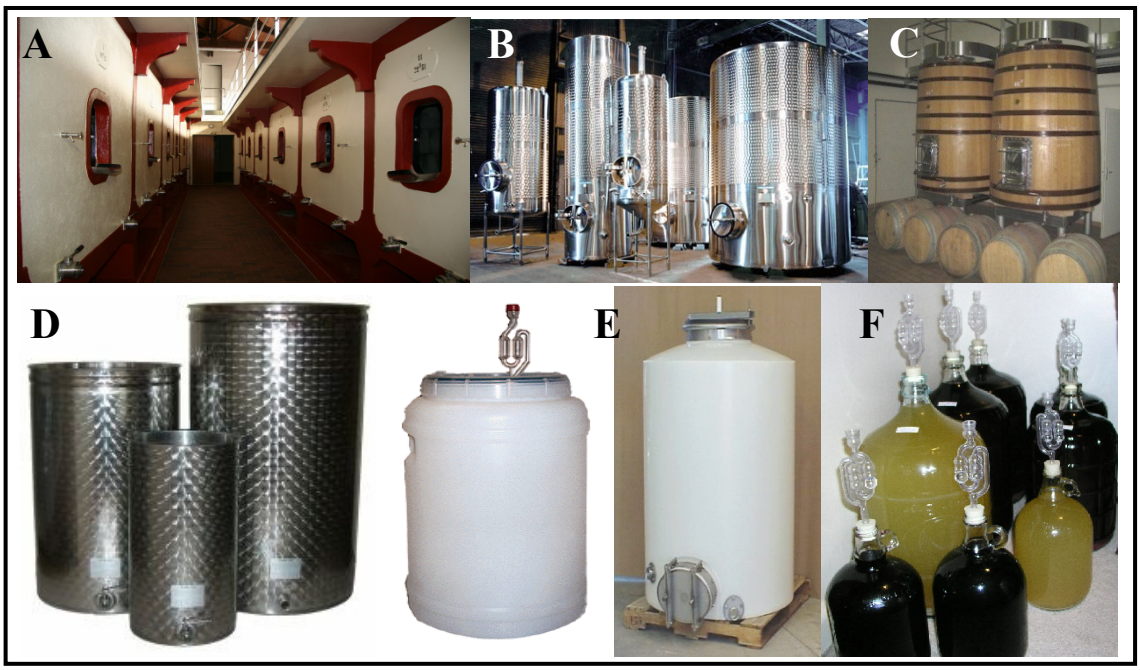

Figure 1.3. Different tanks used for the fermentation process. (A) industrial concrete tank, (B) industrial stainless steel tank, (C) industrial wood tank, (D) pilot and lab scale stainless steel tanks, (E) pilot and lab scale plastic tanks, (F) pilot and lab scale glass fermentation bottles.

The characteristics of a wine produced for further distillation are quite different from those of a table wine [22]. For example, the addition of sulphur oxide, commonly used to preserve the wine, has negative effects on the final distillate (cause it increases the concentration of acetaldehyde and diacetal). It is also recommended that the alcoholic degree of the wine should not be very high (7-10º), and the sugar content should be lower than 2 g/l. Du Plessis et al. investigated the effect of malolactic fermentation (favorable fermentation process in table wines) in wines that were subsequently distilled [23]. They found that malolactic fermentation of the wine generally had a negative effect on the final quality of the distilled product.

For all the facts previously exposed, it is important to have a controlled fermentation process, seeking to obtain a wine that is suitable for distillation.

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1.4 Fermented fruit distillation

Distillation is an ancient Asian separation technique which was introduced in Europe by the sixth century AD [22]. In 1250, the first wine distillation was held. It took place in France and the product obtained was called *eaux-de-vie*. The pot used was made of copper, and it has been progressively improved until it became the so called alembic, which is used until today for the manufacturing of alcoholic beverages. Nowadays, distillations are also held in distillation columns (usually made of stainless steel), giving the opportunity of more efficient and controlled processes. However, some favorable properties are attributed to copper, such as acting as a catalyst for favorable reactions between wine components (formation of esters and acetals) [22]. It is also thought to react with wine components such as sulphurs and fatty acids (which are negative from the product's organoleptic point of view) during the condensation process, giving insoluble products which can lately be removed from the distillate by filtration. To improve efficiency without losing the copper properties, some distillation devices couple to the traditional alembic a copper column with bubble plates and sometimes a dephlegmator. On the other hand, some stainless steel columns use copper in the final parts of the circuit (tubes, condensers, etc.) with the same aim [24]. **Figure 1.4** shows some of these distillation systems.

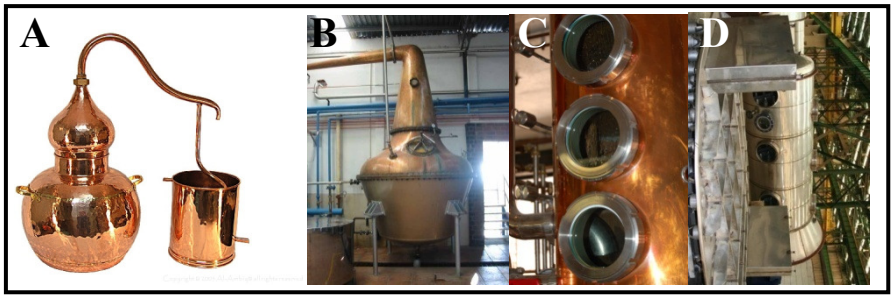

Figure 1.4. Distillation devices employed for the fabrication of fruit spirits. (A), (B) two different types of copper alembics, (C) copper distillation column, (D) stainless steel distillation column.

The concept of distillation is the physical separation of a mixture 'into two or more products that have different boiling points, by preferentially boiling the more volatile components out of the mixture' [25]. In fermented fruit distillations,

the two main components are water and ethanol. However, there are approximately 300 minority volatile compounds which will distill different depending on their boiling point, solubility in ethanol and water, and variation of ethanol content in the vapor during distillation [22]. During a batch distillation, three main fractions are collected: head, heart, and tail. In the head fraction, there is a high concentration of the most volatile and alcohol soluble compounds. Typical head products are acetaldehyde, ethyl acetate, methyl acetate and acetal. This fraction usually has an unpleasant smell and taste, so it is discarded or mixed with the succeeding batch of wine. On the other hand, the tails present high amounts of compounds with high boiling point and high solubility in water. Typical tail products are furfural, acetic acid, ethyl lactate and high molecular weight fatty acids and fatty acids esters. This fraction is also unpleasant from the organoleptical point of view. Therefore, it is discarded or mixed with the head to be added to the succeeding batch of wine. The remaining fraction, the heart of the distillation, is the fruit spirit itself. According to the European Council Regulation (Nº 1576/89), fruit spirits must have a minimum ethanol content of 37.5 % (v/v) [1]. If the distillation is performed in a traditional alembic (without using a distillation column), the ethanol degree achieved would not be sufficient. Therefore, a second distillation with the heart fraction obtained from the first distillation is performed. In this case, again there is a fractioning into head, heart and tail fractions. In some places, the first distillation is performed without fractioning, just as a way to concentrate the ethanol present in the wine. Therefore, only during the second distillation there is a fractioning in head, heart and tail, which allows the separation of negative compounds.

1.5 Fruit spirits

Fruit spirits are composed mainly by water and ethanol. However, as it was previously mentioned, there are hundreds of minor components which are responsible for the sensory character of the drink. The higher alcohols and esters are the two main groups of aromatic compounds in a spirit [26]. The compounds present in a spirit do not change much from one beverage to the other, meaning that they are not much dependent on the raw material used. Nevertheless, there are

important quantitative differences among spirits depending on the raw material employed, the handling of the raw material, and the fermentation and distillation conditions [15,27,28]. Therefore, the different stages of the process must be carefully controlled in order to obtain a good quality product.

1.6 Objectives

The main objective of the present research work is to develop a *Blanquilla* pear variety spirit.

To this end, other objectives were raised:

- To study the influence of different fermentation conditions, such as temperature, pH and yeast employed, on the distillates volatile composition and quality.
- To study the influence of the distillation equipment employed on the distillates volatile composition and quality.
- To study the influence of the presence or absence of the fermentation lees during the distillation on the distillates volatile composition and quality.
- To study the influence of the raw material employed (*Blanquilla* pear natural juice and juice concentrate) on the distillates volatile composition and quality.

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Chapter 2

INFLUENCE OF LEES AND DISTILLATION EQUIPMENT

2.1 Introduction

The lees are sediments formed mainly by yeasts, colloidal compounds, dipotassium tartrate and calcium tartrate deposited during alcoholic fermentation [1]. It is a common belief that the presence of the fermentation lees during the distillation of fruit ferments gives special attributes to the distillate, enhancing its aromatic profile. The wine lees also adsorb some sulfur compounds such as thiols and hydrogen sulfide, which are responsible for organoleptic defects because of their very low perception level and nauseous character [2].

The pot still used during the distillation process also influences the distillate composition and hence its final quality. The use of copper to build the distillation pot stills offers some advantages. It reacts with wine components such as sulfur compounds and fatty acids, improving the distillate quality. It is also a catalyst for favorable reactions between wine components [3]. Related to this, we found Cortes et al. studies on grape distillates traditionally and industrially produced. The differences in both processes go from the storage of the raw material to the system of distillation used (copper pot still for the traditional distillation and stainless steel pots with copper finals for the industrial process). They found differences in the concentrations of the volatile compounds that derive from the processing and storage of the raw material, but not in the ones derived from the system of distillation employed [4]. However, Hernandez-Gomez et al. found significant differences in the composition of melon fruit distillates produced from the same ferments distilled in glass column and in copper pot. The sensory evaluation showed that one hundred per cent of the tasters preferred the samples distilled in the copper pot [5].

In the present chapter, the influence of the fermentation lees and the equipment used during the distillation process of Blanquilla pear fermented juice are studied. To this end, a fermented juice from pear juice concentrate is distilled in presence and absence of the fermentation lees using three different equipments: a glass device (glass pot still coupled to a glass column), a copper alembic, and a glass device with the addition of 5 g/L of copper shavings to the glass pot.

2.2 Materials and methods

Pear juice preparation

Pear juice concentrate of 73 ºBrix from Blanquilla variety (donated by Indulleida S.A. Alguaire, Lleida), was diluted with water until a juice of 18º Brix was obtained. This juice was characterized by high performance liquid chromatography (HPLC) and by measuring the ºBrix, pH, amount of total sugars and density.

Fermentation process

A volume of 40 liters of pear juice (18 ºBrix) was fermented in a 100-liter stainless steel tank at room temperature. The microorganism used was a strain of *Saccharomyces cerevisiae* (Enoferm BDX, Lallemand, Switzerland). The inoculum was prepared in accordance with the instructions provided by the supplier, in a dose of 25 g of yeast/hL of pear juice. After the inoculation, ACTIFERM1, composed by thiamin and ammonium and amine nitrogen (Martin Vialatte Enologie, France), was added as a nitrogen source, again in accordance with the dose and instructions provided by the supplier. When the medium density reached 1,040 g/ml, a second nitrogen source was added: ACTIFERM2, composed by ammonium phosphate and sulphate (Martin Vialatte Enologie, France), following the same instructions. The fermentation was done in duplicate.

To monitor the process, samples were collected at different fermentation times. For each one, the temperature, the pH and the density were measured. Total and viable yeasts were counted using a Neubauer chamber. Each sample was mixed with 1/10 of its volume of methylene blue, in order to differentiate viable

(uncolored) from non viable (colored) cells. Finally, all the samples were subject to HPLC analysis.

The pH was monitored with a Crison Basic 20 pHmeter. The density was measured using a Class H Ludwig Schneider densimeter and total sugars were determined with a GAB kit for sugar analysis (GAB Sistemática Analítica S.L., Spain).

Distillation process

The pear distillates were obtained by simple batch distillation of the pear wine in the presence of its lees. Three different distillation equipments were used: a glass alembic (a glass pot still coupled to a glass column), a copper alembic and a glass alembic with the addition of 5 g/L of copper shavings to the glass pot. The operation conditions were the same in all cases: one liter of pear wine was distilled at an average flow rate of 2 mL/min, using water as the refrigerant and an electric heater as the heat source. For each equipment, the distilled fractions were collected in glass bottles and kept in the freezer until they were analyzed by GC. The first four fractions were of 25 mL each, and the following ones were of 50 mL each until a total distilled volume of 500 mL had been collected. The distillations in each equipment were performed in duplicate.

A second series of distillations was carried out under the same conditions described above, but without the lees of the pear fermented juice. According to previous results, the first distillation fraction was fixed in 5 mL, the second one was 20 mL, the third one was 275 mL, the fourth one was 50 mL and the fifth one was 150 mL. The distillations in each equipment were performed in duplicate.

HPLC analysis

HPLC analysis was used to characterize the pear juice and the pear fermented juice, and also to monitor the fermentation process. The HPLC equipment was an Agilent 1100 Series with HP Chemstation software (Agilent, Waldbron, Germany) for data acquisition. Sugars, glycerol, and ethanol were measured using a Refractive Index Detector (Agilent, Waldbron, Germany). The column was a Transgenomic ICSepICE COREGEL – 87H3, at an oven temperature of 50 ºC. The injection volume was 20 µl. The mobile phase was a solution of pH = 2.20 prepared with concentrated H_2SO_4 (95-97 %) in Milli-Q water. The flow rate was

0.6 mL/min. All the samples and the mobile phase were filtered before the analysis using cellulose acetate filters (Teknokroma) with a pore size of 0.45 μm. Samples were analyzed in duplicate.

GC analysis

Gas chromatography was used to quantify the methanol in the fermented pear juice (since HPLC analysis gives less exact concentration values), and also to characterize the different samples collected during the distillations. The method used by Cortés et al. for determining volatile compounds in *orujos* was adapted to determine the volatile composition, and the methanol and ethanol content in each sample [4]. The equipment used was an Agilent 6890N with a Flame Ionization Detector, automatic injector and HP Chemstation software (Agilent, Waldbron, Germany) for the data analyses. The column was a Teknokroma TR – MetaWax capillary column (polyethyleneglycol stationary phase; 30 m × 0.25 mm × 0.5 μm). The injection volume was 1μl in split 1:5 mode at an injector temperature of 250 ºC. The carrier gas was helium at a column flow rate of 1.1 mL/min. The oven temperature was programmed at 40 ºC for 6 minutes, then increased to 80 ºC at a rate of 1.5 ºC/min. and from 80 to 200 ºC at 3 ºC/min. The detector temperature was 260 ºC, with a H_2 flow rate of 40 ml/min. and an air flow rate of 350 mL/min. Helium was used as the auxiliary gas, at a flow rate of 25 mL/min.

The internal standard was 4-methyl-2-pentanol (Fluka) for all the compounds except ethanol, for which it was acetonitrile (J.T. Baker) [6]. A solution containing these two standards in a concentration of 1.0 g/L and 100 g/l respectively was prepared and mixed at a ratio of 1/10 for each sample. Each sample was injected by duplicate.

Statistical analysis

One-way analysis of variance (ANOVA) was used to ascertain if the type of equipment employed and the presence of lees during the distillation cause any significant difference in the composition of the heart fraction (significant at 5% level).

To compare the different distillation equipments, a first series of ANOVA tests was applied to each compound of the heart fraction from the wine distilled with lees in the different equipments. Multiple comparison of pairs was carried out to

isolate the value or values that differ, using the least significant difference (LSD) at the 5% significance level. The same procedure was used for the heart fractions of the wines distilled without lees in the different distillation equipments.

A second series of ANOVA tests was applied to each compound of the heart fractions from the pear fermented juice distilled with and without lees in each of the distillation equipments. These tests show whether there is any significant difference between the distillations with and without lees.

Principal components analysis (PCA) of all the data was performed to determine the degree of differentiation among the different distillation equipments used and the presence or absence of lees during the distillation.

All the statistical analyses were performed by means of SPSS statistical package (version 13.0).

2.3 Results and discussion

Fermentation process

The pear juice prepared had 18 ºBrix, a pH of 4.75 and a density of 1.090 g/mL. **Figure 2.1** shows the total microorganism growth and the total sugar consumption (average of two fermentations). There was a lag period of about 24 hours, after which the yeasts grew using the sugars present in the medium.

After 65 hours of fermentation, the microorganisms reached the stationary phase. At the beginning of this period, sugars were still being consumed (as a source of carbohydrates for the living cells), but after 90 hours of fermentation their concentration was practically constant at 6 g/L. After 150 hours of fermentation, the yeast concentration slowly started to decrease, probably due to cell lysis. The number of non-viable microorganisms was counted between the sixty-fifth hour and the end of the fermentation. It remained almost constant throughout the process, at values that ranged from 1.8×10^8 to 2.8×10^8 cells/mL. **Figure 2.1** also shows the different sugar concentrations of the pear juice and their consumption during the fermentation process (average of two injections). These data (obtained by HPLC) confirm the results found using the GAB kit for total sugars analysis, but they also provide some new information. Fructose is the main

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sugar. Its concentration in the pear juice is 125 g/L, followed by glucose (30 g/L) and finally sucrose (5 g/L).

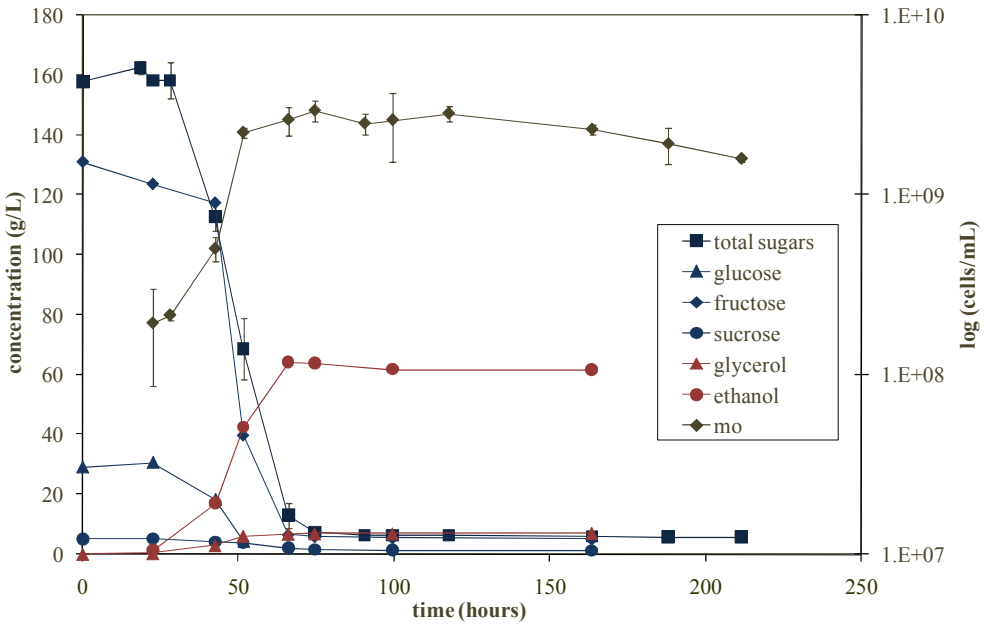

Figure 2.1. Total microorganisms growth, sugars consumption, and ethanol and glycerol formation during the fermentation process.

Glucose was the most rapidly consumed sugar. It reached a concentration of less than 0.1 g/L (not detectable) in less than 65 hours of fermentation. This agrees with the fact that *Saccharomyces cerevisiae* strains are mostly glucophilic, and they utilize glucose faster than other sugars such as fructose or sucrose [7]. Fructose and sucrose were not completely consumed during the fermentation, and reached a concentration of 5.1 g/L and 1.3 g/L, respectively, by the end of the process.

The formations of glycerol and ethanol during the fermentation are also shown in **Figure 2.1**. Ethanol was produced during the microorganism growth phase, and reached a value of 62 g/L (8 alcoholic degrees). This value is within the alcoholic degree range suggested by Lèauté for wines which will undergo a subsequent distillation process [3]. Glycerol concentration increased during the microorganism growth phase, going from 0.1 to 6.7 g/L in the first 65 hours of fermentation. After that, its value remained constant until the end of the fermentation.

The GC analyses of the pear wine revealed a methanol concentration of 3.8 ± 0.1 mg/L.

The density decreased during the fermentation, and reached a constant value of 1.02 g/mL after 65 hours, which is the time at which microorganisms reached the stationary phase.

The temperature was between 20 and 25 ºC, except for the period between 50 and 65 hours of fermentation, when it was 28 ºC. The pH decreased from 4.75 to 4.52 during the fermentation process.

Since both fermentations showed the same behavior throughout the process, only one of them was used to perform all the distillations.

Distillation process

In every distillation process, the head and the tail (corresponding to the beginning and the end of the distillation respectively) must be discarded. The main objective of this separation is to ensure that the heart fraction has a low concentration of toxic and sensorially negative compounds, and acceptable concentrations of ethanol and compounds which can impart a favorable aroma and flavor to the spirit. In order to define the optimum heart fraction, the compound profiles during the distillation processes and their total amount in the distillates must be determined.

Figure 2.2 shows the concentration profiles of the different compounds during each distillation process for the wine distilled with lees (each result is the mean of two distillations and two GC injections). In **Figure 2.2-A**, it can be seen that methanol concentration increased from values around 10 - 15 mg/L in the first fraction to values around 40 – 55 mg/L in the middle of the distillation, and then remained constant or slowly decreased until the end of the process. Léauté suggests that because of its low boiling point (65.5ºC), and high solubility in water and ethanol, methanol distils in the head and heart of the distillate only [3]. However, studies made by Hernández-Gómez et al. on melon fruit distillates found methanol in all the distillation fractions. They indicated that this behavior is only to be expected due to the formation of azeotropic mixtures [5]. Apostolopoulou et al. also found methanol in all the fractions (heads, hearts and

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tails) of traditional Greek distillates [8]. Finally, Glatthar et al. found the same behavior for pear distillates [9]. So our results are in agreement with these last publications. As far as ethanol is concerned, the first fraction contained the highest concentration (around 700 g/L). Then, it rapidly decreased until it reached a constant value of 15 – 17 g/L in the last four fractions.

In **Figure 2.2-B** the profiles of the total higher alcohols (1-propanol, 2-methyl-1-propanol, 1-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 1-hexanol and phenethyl alcohol) and total esters (methyl acetate, ethyl acetate, ethyl decanoate and ethyl-2-trans-4-cis-decadienoate) are shown. For all the equipments tested, the first fraction contained the highest concentration of higher alcohols. This concentration decreased from the second fraction on until it reached a value around 40 mg/L in the last fraction. Each of the higher alcohols except for the phenethyl alcohol followed this decreasing tendency. Phenethyl alcohol however, shows a quite opposite behavior. Its concentration increased to a maximum value in the sixth or seventh fraction, and then slowly decreased until the end of the distillation (see **Annex I**). This behavior is expected since phenethyl alcohol has a high boiling point (higher than water) and is partially soluble in water, so it distils mainly during the middle and the end of the distillation. At the same time, the rest of the higher alcohols also showed the expected behavior since they have a relatively low boiling point and are soluble in alcohol, but at the same time are completely or partially soluble in water, so they distill at the beginning and in the middle fractions of the distillate [3].

The behavior of esters was similar to that of higher alcohols, but the total concentration decreased more drastically, reaching a non detectable value (less than 1 mg/L) after the sixth fraction. Esters can be divided into two groups. On the one hand, ethyl and methyl acetate, which are negative compounds when present in high concentrations (i.e.: the maximum concentration of ethyl acetate permitted by 'The Regulating Council for the Specific Denomination of Galician Orujo' is 300 g/hL a.a.), and are similar to acetaldehyde (low boiling point and soluble in alcohol). They are expected to distil at the beginning of the distillation.

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POTENTIAL OF BLANQUILLA PEAR VARIETY TO PRODUCE PEAR SPIRITS:INFLUENCE OF THE FERMENTATION
AND DISTILLATION CONDITIONS IN THE FINAL QUALITY OF THE SPIRITS
Laura Andrea García Llobodanin
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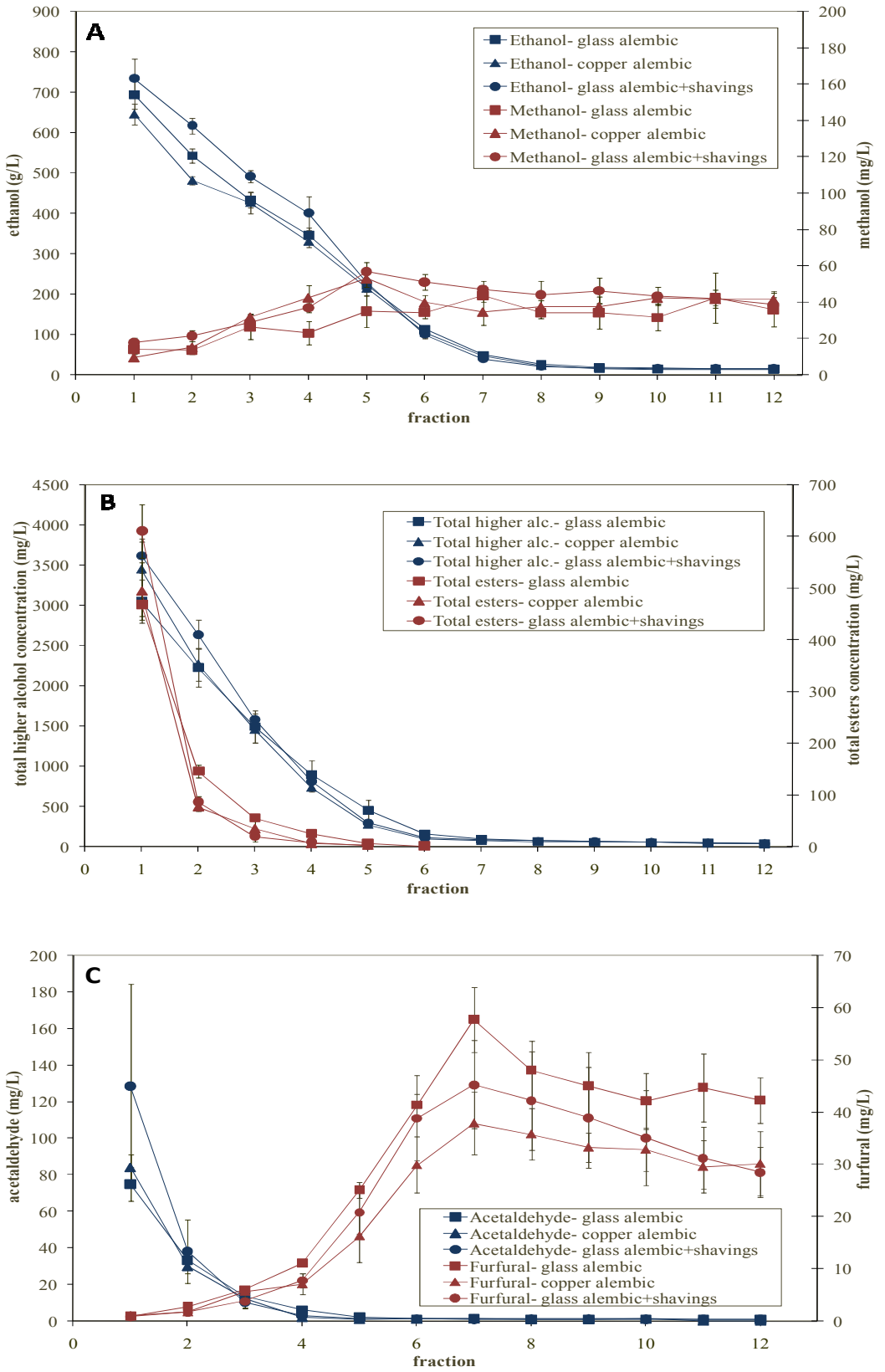

Figure 2.2 Concentration profiles of the different compounds during the distillation processes of the wine distilled with lees. (**A**) ethanol and methanol; (**B**) total higher alcohols and total esters; (**C**) acetaldehyde and furfural.
Fractions 1-4: 25 mL each; 5-12: 50 mL each.

On the other hand, ethyl decanoate and ethyl-2-trans-4-cis-decadienoate, which are favorable compounds derived from fatty acids. They have relatively high boiling points and are completely or partially soluble in ethanol, so they are expected to distil between the beginning and the middle of the distillation [3]. This expected behavior is observed in all the distillations performed (see **Annex I**).

Figure 2.2-C shows that for all the equipments used, the highest acetaldehyde concentration was found in the first fraction, decreasing dramatically in the subsequent ones until it reached a constant value of around 1 mg/L. This behavior agrees with the fact that acetaldehyde has a low boiling point (21ºC) and is soluble in ethanol, so it is expected to distil in the first fractions [3]. On the other hand, the behavior of furfural is quite the opposite. Its concentration was very low in the first fractions and increased until it reached a maximum in the seventh fraction. After that, it slowly decreased until the end of the distillation. This behavior is coherent with the fact that it has a high boiling point (167ºC) and is also very soluble in water, so its concentration is expected to increase from the middle of the heart to the tails [3].

In order to define the best separation volume for the heads and tails, a mass balance was applied to each compound, to determine the mass present in the total 500 mL distilled with each equipment. The mass is related to the total ethanol volume in order to obtain the concentration in grams per hectoliter of absolute alcohol of each compound. **Table 2.1** shows these results.

Ethanol: Ethanol content is obviously of utmost importance in alcoholic drinks. During the first distillation of wine, the alcoholic content of the heart should be around 28 % (v/v) [3]. If we consider all the distillation fractions, this value was not reached with any of the three distillation equipments. So, it is essential that the last fractions of distillate (which have the lower alcoholic content) be eliminated.

Methanol: According to the European Council Regulation (Nº 1576/89), the limit of methanol in fruit spirits is 1000 g/hL a.a. The values obtained in the three distillation equipments tested were much lower than this. This may be due to the fact that the concentration of pectic substances in our fermentation medium is very low because the pear juice used was obtained from pear concentrate (which

is depectinized as part of its production process). Therefore, since the methanol produced during fermentation is derived from the degradation of pectic substances, it could be the cause of the low methanol concentration in our distillates [10, 11].

Table 2.1. Mean concentrations (g/hL a.a.) and standard deviations of the main volatile compounds in the pear wine distilled with its lees, for each distillation process (glass alembic, copper alembic, glass alembic with copper shavings).

compound	glass alembic	copper alembic	glass al. w/shav.*
ethanol (%v/v)	18.9 ± 1.0	17.7 ± 1.0	20.0 ± 1.2
methanol	17.6 ± 3.5	21.4 ± 2.2	21.2 ± 2.4
acetaldehyde	4.0 ± 0.5	4.0 ± 0.5	5.0 ± 2.0
furfural	18.9 ± 2.1	14.3 ± 2.3	14.4 ± 3.1
acetal	0.3 ± 0.1	0.3 ± 0.1	0.6 ± 0.1
methyl acetate	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
ethyl acetate	16.7 ± 1.0	15.2 ± 2.2	16.4 ± 1.4
phenethyl alcohol	27.4 ± 5.3	28.9 ± 3.5	27.2 ± 3.8
1-hexanol	1.2 ± 0.5	1.4 ± 0.5	1.4 ± 0.5
1-butanol	3.9 ± 2.2	4.0 ± 2.1	4.4 ± 0.8
2-methyl-1-butanol	23.8 ± 2.0	24.2 ± 1.2	22.1 ± 3.8
3-methyl-1-butanol	134.7 ± 18.6	140.8 ± 13.6	136.8 ± 6.8
1-propanol	26.3 ± 3.6	27.6 ± 2.7	26.9 ± 0.8
2-methyl-1-propanol	37.7 ± 4.1	38.8 ± 2.7	37.2 ± 1.0
total higher alcohols	**255.0 ± 36.3**	**265.7 ± 26.3**	**256.0 ± 17.5**
ethyl decanoate	1.3 ± 0.1	1.3 ± 0.3	1.1 ± 0.1
ethyl-2-trans-4-cis-decadienoate	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1

*al. w/shav.= alembic with shavings

Acetaldehyde: Acetaldehyde is formed from the fermented raw materials and its concentration increases during the distillation process [12]. It can provide the beverage with a fruity character when present in low concentrations, but for higher ones it provides a sharp smell [13]. The official limits adopted by the European Council (Nº 1576/89) for fruit distillates are 73-500 g/hL a.a., much higher than the concentration found in our distillates (4 – 5 g/hL a.a.) [12].

Furfural: Furfural is produced by the degradation of fermentable sugars (pentoses) caused by heating in acid conditions and/or the Maillard reaction [8]. It

has a smell that is reminiscent of bitter almonds and it is toxic (Reference Dose: 3μg/kg bw/day), so its presence in beverages is not desired. Its concentration in pear brandy is around 2 g/hL a.a., which is considerably lower than the concentration obtained in our distillates [14]. This agrees with the results found by Cortés et. al. in industrial (0.5 to 2.0 g/hL a.a.) and homemade (up to 8.5 g/hL a.a.) Galician *orujos* [4]. These results confirm the need to remove the last fractions of distillate in order to obtain a better quality product.

Esters: They are in the fruit or are formed during the fermentation of the raw material. Long chain esters (C6-C12) contribute to the aromatic character of the spirits providing a pleasant smell, so their presence in the final product is highly desirable [15, 16]. Ethyl 2-trans-4-cis decadienoate, in particular, is one of the most important aroma compounds in pears, imparting to all its derivatives (such as pear distillates) a very characteristic and pleasant pear-like aroma [17]. The concentration of ethyl 2-trans-4-cis decadienoate in pear brandy ranges between 5.0 and 5.3 g/hL a.a. [14]. This value is well above the concentration found in our distillates. Cortés et al. found that the mean concentration of ethyl decanoate in *orujos* is 13.3 g/hL a.a. for industrial samples, and 33.7 g/hL a.a. for homemade samples [4]. This concentration is also much higher than the one found in our distillates. However, Souflero et. al. found concentrations between 0.8 and 2.0g/hL a.a. in samples of blackberry distillate (*mouro*) [12]. In addition, the concentration commonly found in pear brandy is between 1.0 and 1.5 g/L a.a., which is in good agreement with our results [14]. On the other hand, short chain esters usually originate from bacterial spoilage and have a negative influence on the sensory quality of the spirits, giving nuances of dissolvent, glue or rancid butter. For example, concentrations higher than 180 g/hL a.a. of ethyl acetate add acidic character and even solvent nuances to the spirits [8, 12]. In our distillations, the concentrations of these types of esters are quite low.

Higher alcohols: Higher alcohols are formed during the fermentation process. They make an important contribution to the aroma profile of distillates, imparting a flavoring aroma and essential character [12]. For this reason, the European Council Regulation (Nº 1576/89) demands a minimum total amount of these compounds of 140 g/hL a.a. However, high amounts can have a negative effect on the distillate flavor, giving a pungent smell and taste [5]. For this reason, the

'Regulating Council for the Specific Denomination of Galician Orujo' fixes the maximum amount for the sum of higher alcohols at 600 g/hL a.a. [4]. Our distillates respect the requirements of the European legislation, and at the same time are in agreement with the values that are commonly found in pear brandy (155 – 246 g/hL a.a.) [8]. Within the higher alcohols, the concentration of isoamyl alcohols (2-methyl-1-butanol and 3-methyl-1-butanol) must be controlled since they can give disagreeable odors [13]. Their perception threshold is 6 g/hL a.a., which is much lower than the values recorded for our distillates. However, commercial samples of pear distillate show a concentration of 2-methyl-1-butanol of 67 g/hL a.a. [12]. Another source shows that pear brandy has a 2-methyl-1-butanol concentration of 30 – 40 g/hL a.a., while 3-methyl-1-butanol ranges from 110 to 120 g/hL a.a. [14]. These data reveal that the concentration of 2-methyl-1-butanol in our samples is well below the commercial standards while the concentration of 3-methyl-1-butanol (135 – 141 g/hL a.a.) is some way above the observed range in commercial samples.

Phenethyl alcohol: It derives from L-phenylalanine through the metabolic reaction of the yeast during carbonic anaerobiosis [12, 16]. When present in low concentrations, phenethyl alcohol provides the distillates with a pleasant floral aroma resembling that of a rose. Because it is a typical tail component, it should be present in distillates in low concentrations; so it is an indicator of good (or bad) tail fraction separation [4, 8]. Soufleros et al. state that the distillation technique and the type of alembic used seem to play a significant role in the phenethyl alcohol concentration in distillates [12]. The influence of the distillation system on the phenethyl alcohol concentration was confirmed by Cortés et al. during their study of Galician *orujos* [4]. However, they believe that this is related to how the tail fraction is used and not to the material of the distillation equipment. In our distillates, the distillation equipments used did not seem to affect the total phenethyl alcohol concentration. The fractions collected and the distillation method we used (simple batch distillation) were the same for all the equipments tested, so our results support the statement made by Cortés et al. [4].

The phenethyl alcohol concentration of commercial samples of pear brandy range between 0.5 – 2.0 g/hL a.a. [14]. These values are much lower than the ones obtained in our distillates. Soufleros et al. found concentrations between 0.0 and

12.7 g/hL a.a. in blackberry distillates (*mouro*) [12]. Apostolopoulou et al. found, for *tsipouro*, a phenethyl alcohol concentration between 3.0 and 7.2 g/hL a.a. in industrial samples and between 1.0 and 9.9 g/hL a.a. in homemade samples [8]. Cortés et al. studied homemade and industrial orujos, and found phenethyl ethanol concentrations of 0.0 – 18.7 g/hL a.a. and 1.2 – 5.9 g/hL a.a. respectively [4]. All these results agree with the fact that the phenethyl alcohol concentration in our samples is too high, even compared to homemade fruit distillates. For this reason, the last fractions need to be separated if the concentration is to be closer to the concentrations of commercial samples.

Table 2.2. Mean concentrations (g/hL a.a.) and standard deviations of the main volatile compounds in the heart fraction of the wine distilled with its lees for each distillation process (glass alembic, copper alembic, glass alembic with copper shavings).

compound	glass alembic	copper alembic	glass al w/shav.*
ethanol (%v/v)	22.9 ± 0.3	26.5 ± 3.9	24.1 ± 0.9
methanol	21.2 ± 2.8^{a}	18.5 ± 2.2^{a}	21.8 ± 1.5^{a}
acetaldehyde	3.3 ± 0.6^{a}	1.9 ± 0.2^{b}	3.0 ± 0.9^{a,b}
furfural	14.8 ± 2.9^{a}	11.3 ± 1.1^{a}	11.5 ± 1.5^{a}
ethyl acetate	8.0 ± 0.4^{a}	2.2 ± 0.1^{b}	3.2 ± 0.1^{c}
phenethyl alcohol	18.3 ± 0.7^{a}	20.8 ± 3.3^{a}	20.4 ± 1.4^{a}
1-hexanol	1.3 ± 0.6^{a}	1.2 ± 0.5^{a}	1.5 ± 0.4^{a}
1-butanol	3.4 ± 2.1^{a}	2.9 ± 1.6^{a}	4.7 ± 1.2^{a}
2-methyl-1-butanol	15.7 ± 0.9^{a}	14.1 ± 0.9^{a}	18.3 ± 1.3^{b}
3-methyl-1-butanol	159.3 ± 3.1^{a}	138.1 ± 11.4^{b}	140.1 ± 9.0^{b}
1-propanol	35.7 ± 1.5^{a}	33.1 ± 2.6^{a}	35.6 ± 1.8^{a}
2-methyl-1-propanol	38.7 ± 2.3^{a}	31.4 ± 2.5^{b}	36.4 ± 2.4^{a}
total higher alcohols	**272.4 ± 11.2**	**241.6 ± 22.8**	**257.0 ± 17.5**
ethyl decanoate	0.7 ± 0.1^{a}	0.6 ± 0.1^{a}	0.6 ± 0.1^{a}
ethyl-2-trans-4-cis-decadienoate	1.4 ± 0.4^{a}	1.4 ± 0.2^{a}	1.2 ± 0.0^{a}

Different superscripts indicate significant differences ($p \leq 0.05$) between parameter values.
*al. w/shav.= alembic with shavings

On the basis of the previous results, it was decided to remove the first fraction of each distillation (25 ml) and the four last ones (total volume of 200 mL). The remaining fractions were put together, as the heart of the distillate (total volume of 275 mL). All the heart fractions (of the three equipments tested) were analyzed by GC. **Table 2.2** shows the results of these GC analyses for each distillation process

of the pear wine distilled with lees (distillations and GC analyses were done in duplicate).

The same distillations were repeated using the pear wine without the lees. From the previous results we could see that it was not necessary to separate such a big volume as head fraction. Therefore, we decided that the first fraction was 5 mL. The profiles obtained for the compounds analyzed were the same as for those of the distillations of pear wine with lees, although there are some quantitative differences. **Figure 2.3** shows these results.

The heart fraction is defined in the same way as for the distillations with lees (volume that goes from 25 mL to 300 mL of distillate), in order to have comparable results. **Table 2.3** shows the results of the GC analyses of the hearts of each distillation process for these fermented pear juice distillates.

Table 2.3. Mean concentrations (g/hL a.a.) and standard deviations of the main volatile compounds in the heart fraction of the wine distilled without its lees for each distillation process (glass alembic, copper alembic, glass device with copper shavings).

compound	glass alembic	copper alembic	glass al w/shav.*
ethanol (%v/v)	24.2 ± 1.5	22.6 ± 0.2	24.5 ± 0.1
methanol	30.2 ± 1.6^{a}	23.8 ± 2.9^{b}	29.2 ± 3.8^{a,b}
acetaldehyde	3.5 ± 0.8^{a}	3.3 ± 0.7^{a}	2.3 ± 0.2^{b}
furfural	17.1 ± 0.6^{a}	10.9 ± 1.3^{b}	16.3 ± 1.3^{a}
ethyl acetate	10.0 ± 3.2^{a}	3.6 ± 0.9^{b}	3.5 ± 0.5^{b}
phenethyl alcohol	18.4 ± 0.8^{a}	21.9 ± 1.6^{b}	19.4 ± 2.3^{a,b}
1-hexanol	4.4 ± 0.2^{a}	4.9 ± 0.3^{b}	5.3 ± 0.5^{b}
1-butanol	6.8 ± 0.6^{a}	7.2 ± 0.2^{a}	8.3 ± 0.2^{b}
2-methyl-1-butanol	16.7 ± 2.8^{a,b}	16.0 ± 0.9^{a}	19.0 ± 0.9^{b}
3-methyl-1-butanol	168.7 ± 6.0^{a}	170.0 ± 11.5^{a}	152.7 ± 9.6^{b}
1-propanol	35.9 ± 1.1^{a}	38.6 ± 2.2^{a}	37.6 ± 2.0^{a}
2-methyl-1-propanol	37.9 ± 1.5^{a}	37.4 ± 2.3^{a}	38.7 ± 0.6^{a}
total higher alcohols	**284.5 ± 12.9**	**290.9 ± 18.6**	**275.6 ± 15.6**
ethyl decanoate	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ethyl-2-trans-4-cis-decadienoate	0.5 ± 0.6^{a}	0.7 ± 0.1^{a}	0.6 ± 0.1^{a}

Different superscripts indicate significant differences ($p \leq 0.05$) between parameter values.
*al. w/shav.= alembic with shavings

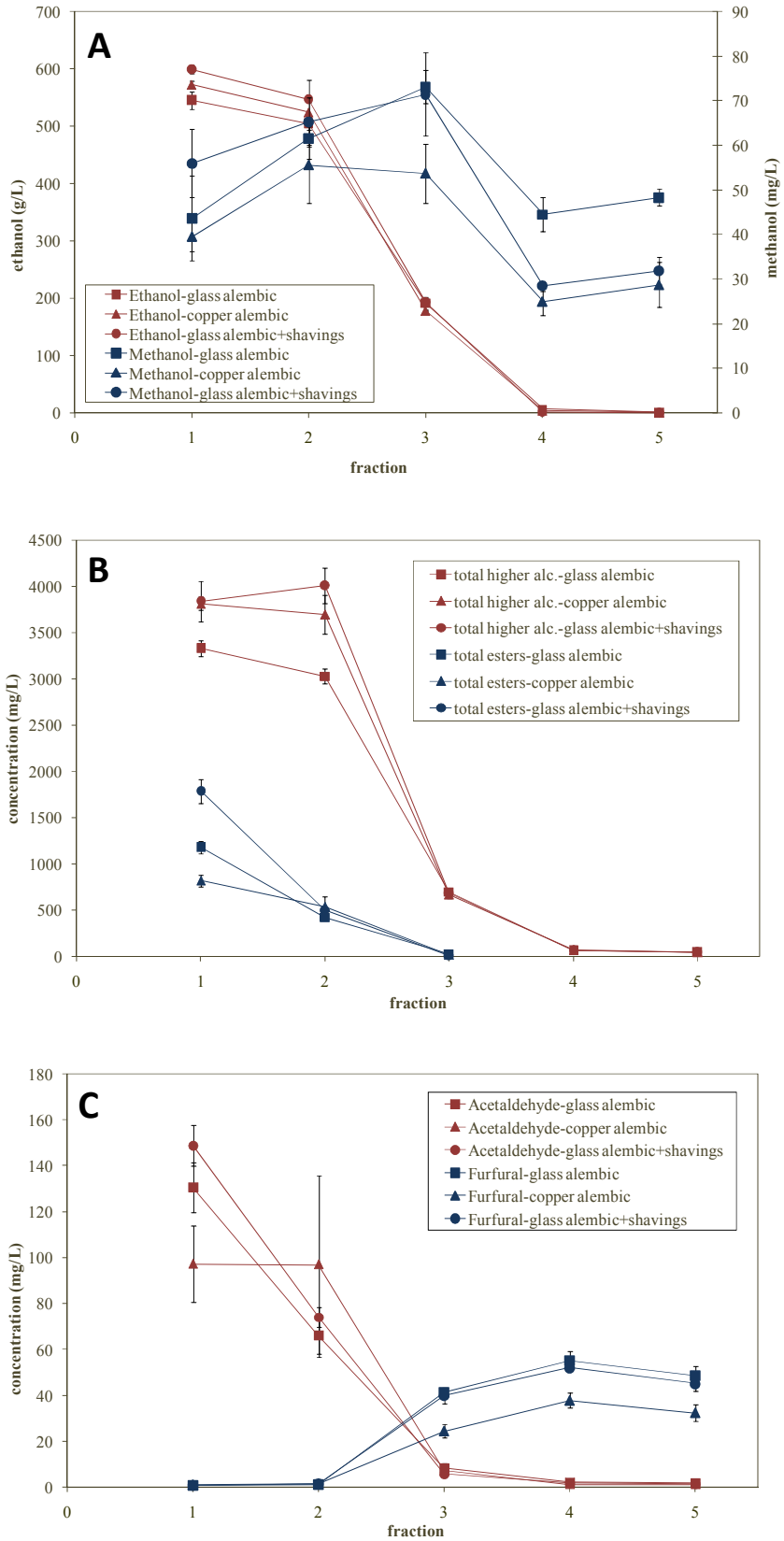

Figure 2.3. Concentration profiles of the different compounds during each distillation process for the pear wine distilled without lees. (**A**) ethanol and methanol; (**B**) total higher alcohols and total esters; (**C**) acetaldehyde and furfural. Fraction 1: 5 mL; 2: 20 mL; 3: 275 mL; 4: 50 mL; 5: 150 mL.

Statistical analysis

The first series of ANOVA tests for the heart fraction of the fermented pear juice distilled with lees (**Table 2.2**) showed that the concentrations of ethyl acetate and 3-methyl-1-butanol in the heart of the distillates were significantly lower ($p < 0.05$) when distilled in the presence of copper (copper alembic and glass alembic with copper shavings). In addition, the concentrations of 2-methyl-1-propanol were significantly lower ($p < 0.05$) when distilled in the copper alembic. The concentrations of 2-methyl-1-butanol and acetaldehyde were also lower when distilled in the copper alembic, though this difference is not significant compared to the glass alembic and the glass alembic with copper shavings respectively. For the rest of the compounds, no significant differences were detected.

For the fermented juice distilled without lees (**Table 2.3**), the concentration of ethyl acetate was significantly lower ($p < 0.05$) for the distillations in the presence of copper. On the contrary, the concentration of 1-hexanol was significantly higher ($p < 0.05$) when distilled in the same devices. The concentration of 1-butanol was significantly lower ($p < 0.05$) for the copper alembic and the glass alembic. However, the concentration of 3-methyl-1-butanol was significantly higher ($p < 0.05$) for these devices. Finally, the furfural concentration was significantly lower ($p < 0.05$) for the distillation in the copper alembic.

Considering that ethyl acetate and furfural are negative compounds for a distillate, and higher alcohols have no major influence in this case (since their concentration is within the accepted range for the three equipments tested), the copper alembic seems to be the best equipment for performing the distillations, either with or without lees.

The second series of ANOVA tests focused on comparing the distillations with and without lees for each equipment. For the distillation in the glass alembic, the concentrations of methanol, 1-butanol, 3-methyl-1-butanol and 1-hexanol were significantly lower ($p < 0.05$) when the distillation was carried out in the presence of lees. On the contrary, ethyl decanoate and ethyl-2-trans-4-cis-decadienoate concentrations significantly increased in the presence of lees ($p < 0.05$). In the case of the copper alembic, acetaldehyde, ethyl acetate, 1-propanol, 2-methyl-1-propanol, 1-butanol, 3-methyl-1-butanol and 1-hexanol concentrations diminished when the distillation was carried out in the presence of lees ($p < 0.05$). On the

other hand, ethyl decanoate and ethyl-2-trans-4-cis-decadienoate concentrations significantly increased in the presence of lees ($p < 0.05$). Finally, for the glass alembic with copper shavings, methanol, 2-methyl-1-propanol, 1-butanol, 1-hexanol and furfural concentrations significantly decreased when the distillation was carried out in the presence of lees ($p < 0.05$). On the contrary, ethyl-decanoate and ethyl-2-trans-4-cis-decadienoate concentrations significantly increased in the presence of lees ($p < 0.05$). The compounds that were not mentioned in the previous analysis underwent no significant changes in their concentration.

From this series of ANOVA tests, it can be concluded that the compounds that are considered to be negative for the quality of the distillates (methanol, ethyl acetate, furfural) diminished or did not change their concentrations when they were distilled in the presence of lees, for all the equipments tested. In addition, the positive compounds (ethyl decanoate and ethyl-2-trans-4-cis-decadienoate) increased their concentrations in the presence of lees for all the equipments tested. So it can be assumed that distillation in the presence of lees leads to a better quality product.

Table 2.4 shows the PCA results. Four principal components explain 84.4% of the variance. Most of the volatile compounds give important loads to the first two principal components, while phenethyl alcohol and 2-methyl-butanol give it to the third and fourth component respectively.

Figure 2.4 represents two plots of the different principal components. PC1 clearly differentiates the distillations with lees from the ones without the lees (**Figure 2.4-A**). Ethyl decanoate and ethyl-2-*trans*-4-*cis*-decadienoate have a negative load in PC1 so their concentration increases as the component becomes more negative. This means that the distillates that were distilled with the lees have a more intense fruity aroma compared to the ones distilled without the lees. The rest of the compounds present in PC1 have a positive load, so their concentration is lower for the distillates distilled with lees. Considering that methanol is a toxic compound, distillation in presence of the lees results advantageous. These results are in agreement with the ones obtained from the ANOVA tests, which indicated that distilling the ferments from *Blanquilla* pear juice concentrate in presence of the

lees will improve their quality. **Figure 2.4-B** shows that PC2 differentiates the distillations in glass column from the ones in copper alembic and glass column with shavings. PC3 also has a small contribution on this differentiation.

Table 2.4. Principal Component Analysis results for the volatile compounds analyzed in the heart fraction of the different distillates.

Principal Component	compound	loading	variance explained (%)	total variance (%)
PC1	ethyl decanoate	-0.945	47.03	47.03
	1-hexanol	0.942		
	ethyl-2-*trans*-4-*cis*-decadienoate	-0.939		
	1-butanol	0.880		
	methanol	0.743		
PC2	3-methyl-1-butanol	0.796	16.73	63.76
	acetaldehyde	0.785		
	ethyl acetate	0.721		
	2-methyl-1-propanol	0.676		
	1-propanol	0.596		
	furfural	0.504		
PC3	phenethyl alcohol	0.914	12.24	76.00
PC4	2-methyl-1-butanol	0.913	8.35	84.35

The loads of the volatile compounds in PC2 are all positive. This means that the concentrations of ethyl acetate and furfural (both of them negative compounds) in the distillates produced in the glass column are expected to be higher than the ones produced in the other pot stills. This also confirms the results of the ANOVA tests, showing that the presence of copper seems to be positive for the distillates quality.

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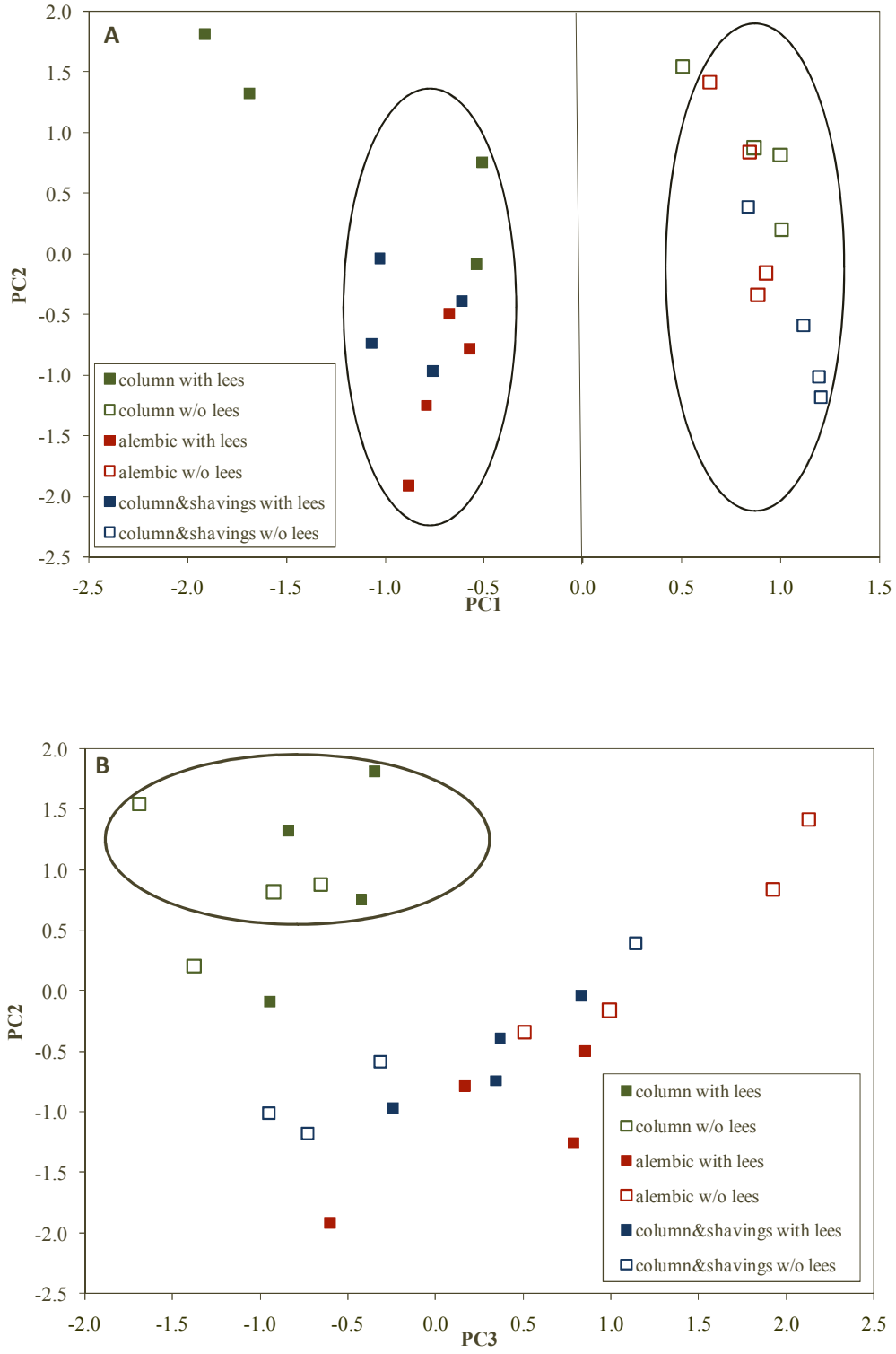

Figure 2.4. Plot of the principal components 1,2 and 3 (PC1, PC2 and PC3) for the PCA analysis of the main volatile compounds in the heart fraction of the different distillates. (**A**) PC1 vs. PC2; (**B**) PC3 vs. PC2. w/o: without.

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Chapter 3

INFLUENCE OF FERMENTATION CONDITIONS: TEMPERATURE

3.1 Introduction

The fermentation temperature is one of the most important conditions in the fermentation process. It can influence the formation and retention of aroma compounds in the medium, affecting the quality of the final ferment and hence, the distillate produced from it [1]. An example of this is the production of acetaldehyde by yeasts, which strongly depends on the fermentation temperature, among other fermentation conditions. Because yeasts have a profound effect on the formation of aroma compounds during the fermentation, the influence of temperature on their growth properties is of main importance [2]. Charoenchai et al. studied the effect of temperature on the growth rates and cell biomass of wine yeasts [3]. They found that for both strains of *Saccharomyces Cerevisiae* studied, growth rate increased with increasing temperatures. In addition, cell biomass produced during fermentation increased substantially between 10ºC and 15ºC, remaining constant between 15ºC and 25ºC. On the other hand, they found that several strains of non- *Saccharomyces* yeasts exhibited growth rates which were faster than those of *S. Cerevisiae*, though *S. Cerevisiae* gave the highest cell biomass in the temperature range 15ºC to 25ºC and one of the highest at lower temperatures. These results suggest that working at temperatures lower than 15ºC could result in fermentations with a dominant specie different from the inoculated strain of *Saccharomyces Cerevisiae*. Therefore, although low temperatures (10 – 15 ºC) are associated to the retention of flavor volatiles, they are not a common practice because of this important drawback [4]. In addition, faster growth rates of other genera of yeasts can compromise the dominance of *S. Cerevisiae* during the first stage of the fermentation. These facts strongly influence the ferment quality

because different yeasts produce quantitative difference on the main aroma compounds [2].

In the present chapter the influence of the fermentation temperature in ferments and distillates obtained from Blanquilla pear juice concentrate is studied. To this end, fermentations are carried out at two different temperatures (17ºC and 25ºC), keeping the rest of conditions fixed. The fermentation yeast used is a strain of *Saccharomyces Cerevisiae*.

3.2 Materials and methods

Pear juice preparation

Pear juice concentrate of 73 ºBrix from Blanquilla variety (donated by Indulleida S.A. Alguaire, Lleida), was diluted with water until a juice of 18º Brix was obtained. This juice was characterized by high performance liquid chromatography (HPLC) and by measuring the ºBrix, pH, amount of total sugars and density.

Fermentation process

Fermentations were conducted in a 5 L Labfors 3 fermenting reactor (Infors, Switzerland) which was filled up to 3 L with the pear juice. The temperature was fixed at 17.0 ± 0.5 ºC for the first set of experiments and 25.0 ± 0.5 ºC for the second set. The microorganism used was *Saccharomyces cerevisiae* (Enoferm BDX, Lallemand, Switzerland). The inoculum was prepared in accordance with the instructions provided by the supplier, in a dose of 25 g of yeast/hL of pear juice. When the medium density reached 1.040 g/mL, 300 mg/L of di-ammonium hydrogen phosphate (Scharlau) was added as a nitrogen source. Fermentations at each temperature were performed in duplicate.

To monitor the process, samples were collected at different fermentation times. For each one, the pH was monitored with a Crison Basic 20 pH meter. Total yeasts were counted using a Neubauer chamber. The density was measured using a Class H Ludwig Schneider densimeter and the total sugar content was determined using a GAB kit for sugar analysis (GAB Sistemática Analítica S.L.,

Spain). Finally, all the samples were subject to HPLC analysis. The final ferments were also subject to GC analysis.

Distillation process

To perform the distillations, the two fermentations realized at each temperature were put together. The pear distillates were obtained from simple batch distillation of the fermented pear juices (obtained from fermenting at 17 ºC and 25 ºC) in the presence of their lees, in a 3 L copper alembic. The operation conditions were the same in all cases: 1 L of the fermented pear juice was distilled at a flow rate of 2 mL/min, using water as the refrigerant and an electric heater as the heat source. The distillations were performed in duplicate. Based on the results obtained in **Chapter 2,** the first fraction (fraction 1) was of 5 mL, the second fraction (fraction 2) was of 20 mL, the third fraction (fraction 3) was of 275 mL, the next one (fraction 4) was of 50 mL and the last fraction (fraction 5) was of 150 mL. The distilled fractions were collected in glass bottles and kept in the freezer until they were analyzed using GC. After the analyses, fractions 2 and 3 are put together and kept as the heart of the distillate.

The same procedure is repeated distilling the pear ferments in absence of the wine lees.

HPLC analysis

HPLC analysis was used to characterize the pear juice, the pear fermented juices, and also to monitor the fermentation process. The HPLC equipment was an Agilent 1100 Series with HP Chemstation software (Agilent, Waldbron, Germany) for data acquisition. Sugars, glycerol, and ethanol were measured using a Refractive Index Detector (Agilent, Waldbron, Germany). The column was a Transgenomic ICSepICE COREGEL – 87H3, at an oven temperature of 50 ºC. The injection volume was 20 µl. The mobile phase was a solution of pH = 2.20 prepared with concentrated H_2SO_4 (95-97 %) in Milli-Q water. The flow rate was 0.6 mL/min. All the samples and the mobile phase were filtered before the analysis using cellulose acetate filters (Teknokroma) with a pore size of 0.45 µm. Samples were analyzed in duplicate.

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GC analysis

Gas chromatography was used to analyze the fermentation products, and also to characterize the different samples collected during the distillations. The method used by Cortés et al. for determining volatile compounds in *orujos* was adapted to determine the volatile composition, and the methanol and ethanol content in each sample [5]. The equipment used was an Agilent 6890N with a Flame Ionization Detector, automatic injector and HP Chemstation software (Agilent, Waldbron, Germany) for the data analyses. The column was a Teknokroma TR – MetaWax capillary column (polyethyleneglycol stationary phase; 30 m × 0.25 mm × 0.5 μm). The injection volume was 1μL in split 1:5 mode at an injector temperature of 250 ºC. The carrier gas was helium at a column flow rate of 1.1 mL/min. The oven temperature was programmed at 40 ºC for 6 minutes, then increased to 80 ºC at a rate of 1.5 ºC/min. and from 80 to 200 ºC at 3 ºC/min. The detector temperature was 260 ºC, with a H_2 flow rate of 40 mL/min. and an air flow rate of 350 mL/min. Helium was used as the auxiliary gas, at a flow rate of 25 mL/min.

The internal standard was 4-methyl-2-pentanol (Fluka) for all the compounds except ethanol, for which it was acetonitrile (J.T. Baker) [6]. A solution containing these two standards in a concentration of 1.0 g/L and 100 g/L respectively was prepared and mixed at a ratio of 1/10 for each sample. Each sample was injected in duplicate.

Statistical analysis

One-way analysis of variance (ANOVA) was used to ascertain if there are significant differences (at 5% level) among the fermentation products obtained at the two different temperatures.

ANOVA tests are also applied to the hearts and the different fractions of the distillates (produced from the different ferments distilled with and without the fermentation lees). In these cases, multiple comparison of pairs was carried out to isolate the value or values that differ, using the least significant difference (LSD) at the 5% significance level.

Principal components analysis (PCA) was also performed to the aroma compounds in the heart fractions, to determine the degree of differentiation among the different distillates.

All the statistical analyses were performed by means of SPSS statistical package (version 13.0).

3.3 Results and discussion

Fermentation process

The pear juice prepared had 18 ºBrix, a pH of 4.46 and a density of 1.092 g/mL. **Figure 3.1** shows the total microorganisms' growth, the different sugars consumption and the ethanol and glycerol formation for the fermentations at 17ºC and 25ºC. The results shown are for one fermentation at each temperature, the duplicate gave similar results in both cases (see **Annex II**). Fructose was the main sugar, followed by glucose and sucrose (see **Figure 3.1-A**). As expected, fermentations at 17ºC went more slowly than at 25ºC. After 100 hours of fermentation at 25ºC the total sugars concentration remained almost constant at 4.0 to 5.0 g/L, while 200 hours of fermentation at 17ºC are needed to reach the same constant value. The total sugars concentration determined by the GAB method was in agreement with the results obtained by HPLC for the three main sugars in the juice (see **Annex II**). Sugars consumption is directly related to the microorganisms' growth, so a higher growth rate is expected at 25ºC. This was confirmed in our experiments (see **Figure 3.1-B**). At 25ºC the maximum microorganisms' concentration was reached in around 50 hours, while for fermentations at 17ºC it took around 120 hours. This is not only due to the more slowly growth rate at lower temperatures, but also because of the longer lag phase. The microorganisms at 17ºC showed a lag phase of almost 48 hours while at 25ºC it was of less than 24 hours. However, the maximum microorganisms concentration reached was about the same for both fermentation temperatures. These results are in agreement with the ones found by Charoenchai et al., who found that growth rate increased with the fermentation temperature but the cell biomass remained constant for fermentations between 15ºC and 25ºC [3].

Ethanol and glycerol are metabolites from yeasts, so their production is expected to be directly related to sugars consumption and yeasts growth. **Figure 3.1-B** shows how the ethanol concentration increased dramatically during the microorganisms exponential growth phase. During the first part of the stationary

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phase, the ethanol concentration kept increasing at both temperatures, but after some time it remained constant and then slowly decreased towards the end of the fermentation processes. Glycerol was produced in a lesser amount, but the qualitative behavior was the same one than ethanol at both temperatures.

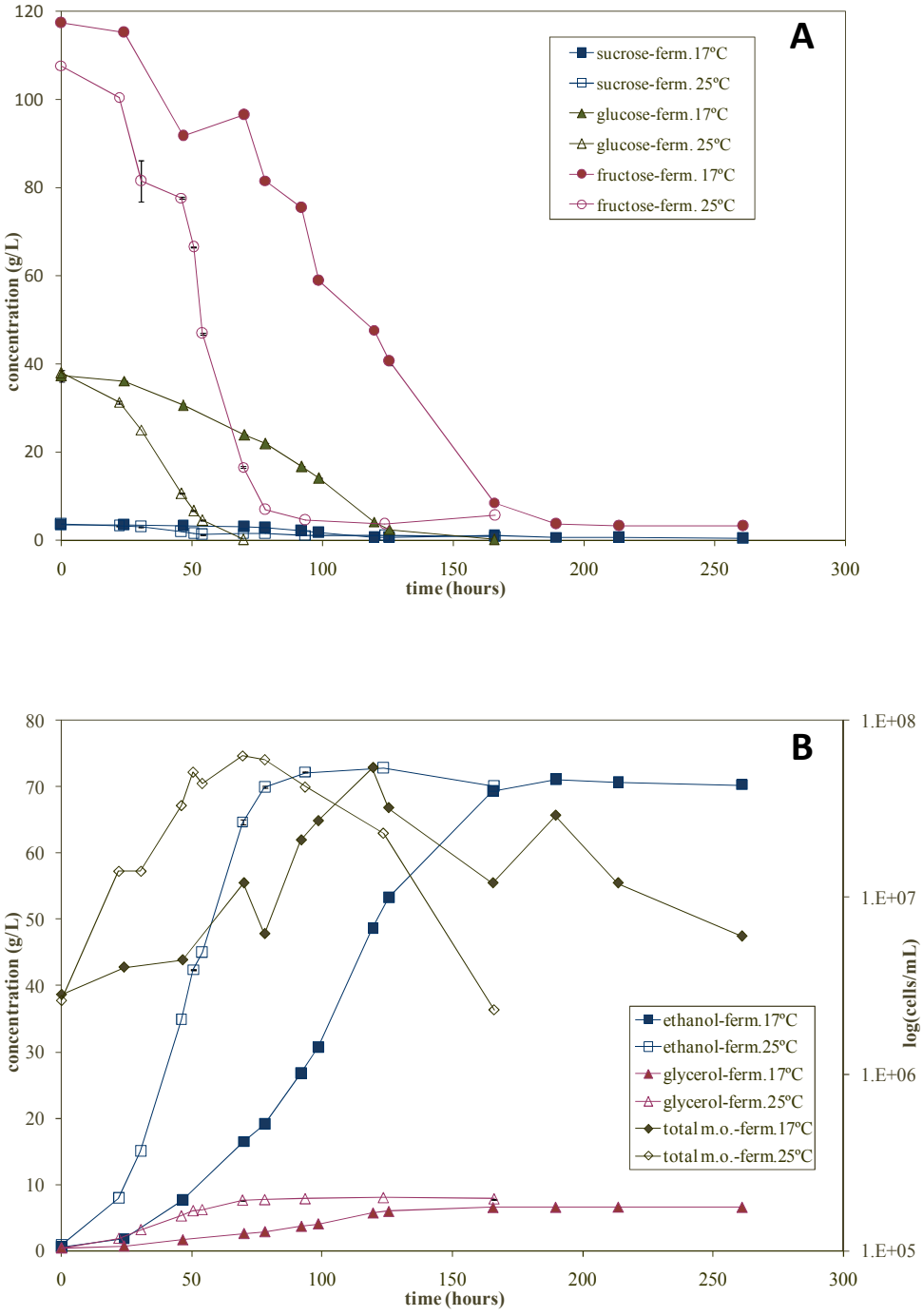

Figure 3.1. Concentration profiles during pear juice fermentations at 17ºC and 25ºC. (**A**) sucrose, glucose, fructose and total microorganisms' concentrations. (**B**) ethanol, glycerol and total microorganisms' concentrations. m.o. = microorganisms.

Table 3.1 shows the concentrations (obtained by GC analyses) of the main volatile compounds in the pear juice fermented at 17ºC and 25ºC, and also the results of the ANOVA tests. The compounds concentrations did not show a significant difference with the fermentation temperature except for the methyl acetate, which showed a significantly higher concentration when fermented at 25ºC.

Table 3.1. Mean concentrations (g/hL a.a.) and standard deviations of the main volatile compounds in fermented pear juice produced at 17ºC and 25ºC.

compound	fermentation at 17ºC	fermentation at 25ºC
acetaldehyde	4.9 ± 2.0^{a}	4.2 ± 2.7^{a}
furfural	14.3 ± 3.0^{a}	12.1 ± 3.1^{a}
methanol	14.2 ± 2.8^{a}	16.4 ± 1.0^{a}
ethanol (%v/v)	7.4 ± 1.0^{a}	8.1 ± 0.3^{a}
1-propanol	66.0 ± 3.8^{a}	55.2 ± 10.1^{a}
2-methyl-1-propanol	90.9 ± 17.0^{a}	131.2 ± 53.3^{a}
2-methyl-1-butanol	15.0 ± 3.6^{a}	15.5 ± 5.4^{a}
3-methyl-1-butanol	141.1 ± 42.3^{a}	179.7 ± 80.5^{a}
1-hexanol	38.4 ± 10.2^{a}	32.0 ± 15.6^{a}
phenethyl alcohol	30.2 ± 5.4^{a}	26.5 ± 6.7^{a}
total higher alcohols	**381.6 ± 82.3**	**440.1 ± 171.6**
methyl acetate	10.7 ± 5.1^{a}	20.2 ± 2.3^{b}
ethyl acetate	21.9 ± 16.9^{a}	40.9 ± 13.4^{a}
ethyl-2-*trans*-4-*cis*-decadienoate	20.9 ± 9.6^{a}	18.9 ± 7.4^{a}
total esters	**53.5 ± 31.6**	**80.0 ± 23.1**

Different superscripts indicate significant differences ($p \leq 0.05$) between parameter values.

Distillation process

As expected, the profiles of the different volatile compounds were qualitatively the same ones described in **Chapter 2**. **Figure 3.2** shows these results (each point is the mean of two distillations and two GC injections). Acetaldehyde shows a considerable higher concentration in the first two fractions of the distillates from juices fermented at 25ºC compared to the ones fermented at 17ºC (see **Figure 3.2-A**). However, this difference disappears from the third fraction on. The statistical tests showed that for the first fraction, the difference is significant only between the distillates from juice fermented at 25ºC distilled with lees and the distillates from juice fermented at 17ºC also distilled with lees.

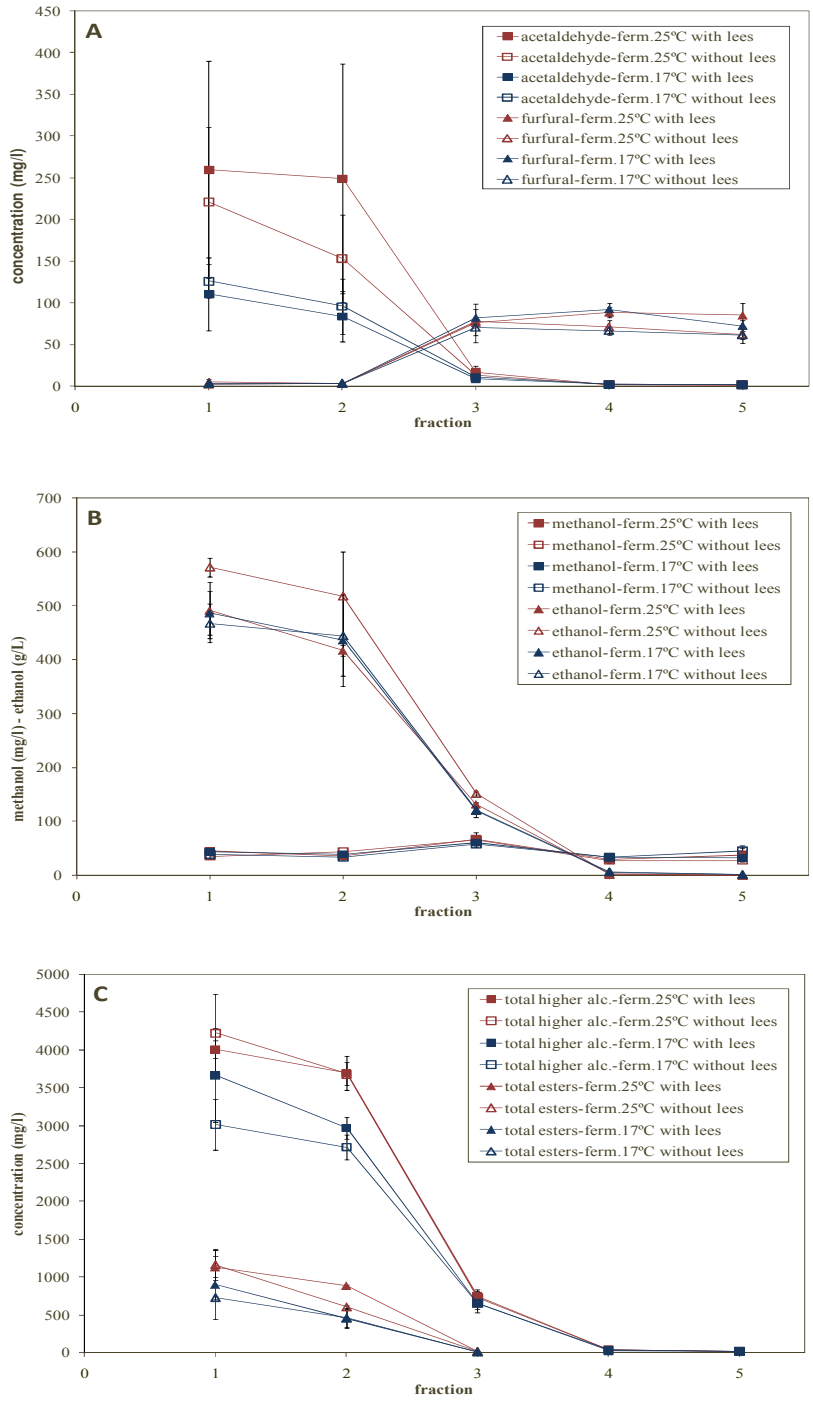

Figure 3.2. Concentration profiles of the different compounds during the distillation with and without lees of the juices fermented at 25ºC and 17ºC. (A) methanol and ethanol; (B) total higher alcohols and total esters; (C) acetaldehyde and furfural.
Fraction 1: 5mL, fraction 2: 20 mL, fraction 3: 275 mL, fraction 4: 50 mL, fraction 5: 150 mL.

In the second fraction, the concentration of acetaldehyde in the distillations with lees of the juice fermented at 25ºC was significantly different from the concentration in the distillations of the juice fermented at 17ºC, distilled either

with or without lees. No significant differences were observed in the concentrations of furfural, methanol and ethanol in any of the fractions, for all the distillations performed.

Total higher alcohols (1-propanol, 2-methyl-1-propanol, 1-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 1-hexanol and phenethyl alcohol) and total esters (methyl acetate, ethyl acetate, and ethyl 2-*trans*-4-*cis*-decadienoate) showed the same tendency as acetaldehyde (see **Figure 3.2-C**). Statistical analyses on the first fractions revealed that the concentration of total higher alcohols in the distillations without lees of the juice fermented at 17ºC, were significantly different from the others. On the contrary, total esters did not show any significant difference. If we consider the total higher alcohols and total esters concentrations in the second fractions, we can find a significant difference between the distillates from the juices fermented at 25ºC and the ones fermented at 17ºC, distilled either with or without lees, for both types of compounds. In addition, there was a significant difference between the higher alcohols concentration of the distillates from pear juices fermented at 17ºC distilled with and without lees. The total esters concentration also showed a significant difference for the pear juices fermented at 25ºC distilled with and without lees.

The rest of the fractions showed no differences in the concentrations of any of the compounds analyzed.

Table 3.2 shows the concentrations of the main volatile compounds in the heart fraction (mixture of the second and third distillation fraction) for the different distillates, and the ANOVA test results. For each fermentation temperature, there was no significant difference among the hearts obtained by distilling with and without the fermentation lees. However, if we compare the hearts obtained from the pear juices fermented at 17 ºC and 25 ºC, we can see a significant difference in the concentrations of 1-propanol, 1-butanol, 1-hexanol and phenethyl alcohol either for the distillations performed with or without lees. In addition, there is a significant difference in the concentration of furfural in the heart of the ferment obtained at 17ºC distilled with lees, compared to the ones obtained at 25ºC and distilled without the lees. Finally, there is also a significant difference between the concentration of 2-methyl-1-propanol in the distillate obtained from the

fermentation at 17ºC distilled without the lees compared to the one obtained at 25ºC and distilled with the lees.

Table 3.2. Mean concentrations (g/hL a.a.) and standard deviations of the main volatile compounds in the heart fraction of the distillates from fermented pear juice produced at 17ºC and 25ºC.

compound	D1	D2	D3	D4
acetaldehyde	8.1 ± 0.1^{a}	10.5 ± 2.1^{a}	13.0 ± 1.7^{a}	12.5 ± 8.0^{a}
furfural	50.1 ± 2.3^{a}	39.5 ± 1.9^{a,c}	38.7 ± 15.0^{a,c}	36.2 ± 3.9^{b,c}
methanol	42.3 ± 4.1^{a}	36.8 ± 3.2^{a}	34.4 ± 12.9^{a}	32.7 ± 4.8^{a}
ethanol (%v/v)	16.5 ± 0.6	15.8 ± 0.6	20.9 ± 3.9	21.3 ± 1.8
1-propanol	143.2 ± 16.3^{a}	126.8 ± 10.8^{a}	76.1 ± 27.9^{b}	65.6 ± 19.7^{b}
2-methyl-1-propanol	129.8 ± 8.1^{a,c}	123.1 ± 8.6^{a}	167.8 ± 43.7^{b,c}	149.8 ± 31.8^{a,c}
1-butanol	19.3 ± 1.1^{a}	18.1 ± 0.6^{a}	12.8 ± 1.3^{b}	12.0 ± 0.8^{b}
2-methyl-1-butanol	13.5 ± 1.4^{a}	14.9 ± 1.8^{a}	15.8 ± 3.6^{a}	15.9 ± 2.4^{a}
3-methyl-1-butanol	236.2 ± 5.6^{a}	224.2 ± 8.1^{a}	237.3 ± 51.2^{a}	200.8 ± 36.9^{a}
1-hexanol	34.6 ± 0.7^{a}	31.9 ± 0.3^{a}	24.6 ± 4.1^{b}	24.1 ± 3.9^{b}
phenethyl alcohol	5.2 ± 0.6^{a}	4.2 ± 0.5^{a}	7.4 ± 1.5^{b}	6.8 ± 0.5^{b}
total higher alcohols	**581.8 ± 33.8^{a}**	**543.2 ± 30.7^{a}**	**541.8 ± 133.3^{a}**	**475.0 ± 92.1^{a}**
ethyl acetate	19.9 ± 2.4^{a}	24.2 ± 5.2^{a}	32.4 ± 28.5^{a}	21.7 ± 12.4^{a}

Different superscripts indicate significant differences ($p \leq 0.05$) between parameter values.
D1: distillate from the pear juice fermented at 17ºC distilled with the lees; D2: distillate from the pear juice fermented at 17ºC distilled without the lees; D3: distillate from the pear juice fermented at 25ºC distilled with the lees; D4: distillate from the pear juice fermented at 25ºC distilled without the lees.

From these results, we can see that the significant differences found for the total higher alcohols and total esters concentrations in the second fraction of the distillates from pear juices fermented at 25ºC and 17ºC (see **Figure 2-C**), was not maintained in the heart fractions. This was probably because the volume of fraction 2 was very small compared to the volume of fraction 3, so the diluting effect when both fractions were mixed is quite important. This could also explain the facts that no significant differences were found in the total higher alcohols concentration for the hearts from pear juice fermented at 17 ºC and distilled with and without lees, and in the total esters concentration for the hearts from pear juice fermented at 25 ºC and distilled with and without lees.

PCA of the heart fractions divided the compounds into two different components, which explained 82.6% of the variance. **Table 3.3** shows these results. PC1 clearly differentiated the distillates from fermented juices at different temperatures, as **Figure 3.3** shows. The first three compounds of PC1, which correspond to the higher loads in the component, showed a significant difference in the ANOVA test (see **Table 2**). So the PCA results confirmed the results obtained by this test. To a lesser extent, PC1 also separated the distillations with lees performed at 17 ºC from the distillations without lees performed at the same temperature. This was due to a lower concentration of all of the compounds that form PC1 in the distillates from juices fermented at 17 ºC distilled without lees. However, these differences were very small (see **Figure 3**) and they were not significant in any case (see **Table 2**).

Table 3.3. Principal Components Analysis results for the volatile compounds analyzed in the heart fraction of the different distillates.

principal component	compound	loading	variance explained (%)	total variance (%)
PC1	1-hexanol	0.955	45.32	45.32
	1-propanol	0.952		
	1-butanol	0.877		
	furfural	0.866		
	methanol	0.855		
	3-methyl-1-butanol	0.719		
PC2	2-methyl-1-propanol	0.957	36.75	82.07
	phenethyl alcohol	0.855		
	ethyl acetate	0.830		
	2-methyl-1-butanol	0.701		
	acetaldehyde	0.697		

In spite of the differences found, no statement can be made about the quality of the distillates. Higher alcohols are positive if their concentration is not too high (i.e.: not higher than 600 g/hL a.a. for Galician Orujos, according to the 'Regulating Council for the Specific Denomination of Galician Orujo'). In our case, the concentrations of 1-propanol, 1-butanol and 1-hexanol are significantly higher in the distillates from the juice fermented at 17ºC. However, phenethyl alcohol concentration is significantly lower in these cases, and the total higher alcohols concentration is not significantly different in any of the cases. In

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addition, negative compounds such as furfural, methanol and ethyl acetate did not show any significant difference in their concentrations among the different heart fractions.

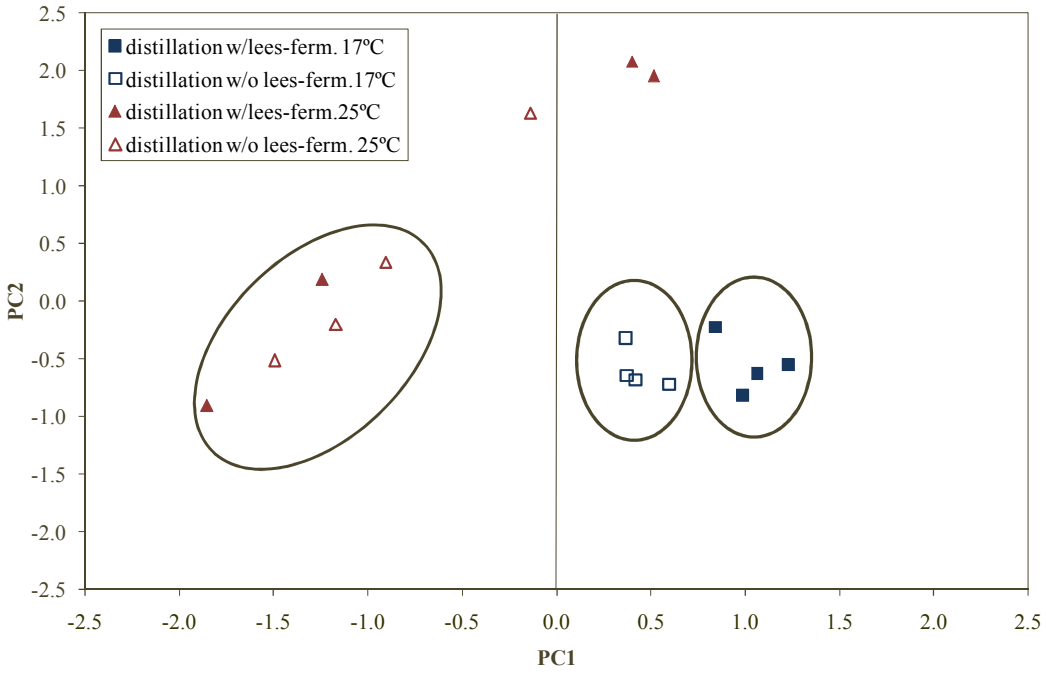

Figure 3.3. Plot of the principal components 1 and 2 (PC1 and PC2) for the PCA analysis of the main volatile compounds in the heart fraction of the different distillates.
w/: with; w/o: without; ferm: fermentation.

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Chapter 4

INFLUENCE OF FERMENTATION CONDITIONS: YEASTS

4.1 Introduction

The main part of the aroma compounds present in a fermented juice (and consequently present in the spirit derived from it), is formed during fermentation under the influence of yeast [1]. The yeast employed, among other fermentation variables, influence the formation of these compounds. In fact, the formation of higher alcohols by yeast occurs independently of the raw materials used to produce the beverage. Fatty acid esters are also an important group of aroma compounds synthesized by yeast. In addition, methanol (a very toxic compound for human beings), is released by the pectin-methyl-esterase present in yeasts (among others) [2]. Different types of yeast have different pectin-methyl-esterase activities, so the yeast employed for the fermentation process will have influence on the amount of methanol released.

Nowadays, *Saccharomyces cerevisiae* is the recommended and most widely used yeast specie for alcoholic fermentation of fruits [3, 4]. Different strains of this specie are commercially available, and they will usually produce individual ester and alcohol profiles even when fermenting similar media [5]. Schel et al. compared the concentration of different volatile compounds produced by three different strains of *Saccharomyces cerevisiae* (one laboratory and two commercial strains) in pear, plum and cherry mashes and spirits [3]. They found that one of the commercial strains produced lower amounts of acetaldehyde in all the mashes, and lower amounts of 1-propanol in plum and cherry mashes. In addition, the mashes fermented with the laboratory strain released the highest amount of methanol. On the other hand, the spirits produced with mashes fermented with the laboratory strain, presented higher concentrations of 1-propanol in all the spirits,

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higher concentrations of ethyl acetate in plum spirits and lower concentrations of 3-methyl-1-butanol in pear and cherry spirits. Arrizon et al. also compared the production of volatile compounds by two different strains of *Saccharomyces cerevisiae* during the elaboration of a prickly pear distilled beverage. They found that the yeast strain had influence on the amount of acetaldehyde and *n*-propanol produced [6].

The aim of this chapter is to study the influence of the fermentation yeast in the composition of distillates from Blanquilla pear juice. Two different strains of *Saccharomyces cerevisiae* were used to ferment the pear juice, which was then distilled and analyzed by GC and sensory analysis.

4.2 Materials and methods

Pear juice preparation

Pear concentrate of 73 ºBrix from Blanquilla variety (donated by Nufri S.A., Mollerussa, Lleida), was diluted with water until a juice of 18º Brix was obtained. This juice was characterized by HPLC and by measuring the ºBrix and the pH.

Fermentation process

Fermentations of the pear juice were carried out in 5 L bottles under semi-anaerobic conditions. A volume of 3 L of juice was put in each bottle and it was inoculated with a dose of 20 g/hL of yeast, according to the instructions provided by the supplier. Two different yeast strains of *Saccharomyces cerevisiae* were used, *Siha Aktiv6* and *Uvaferm CGC62* (both purchased from Begerow GmbH & Co., Langenlonsheim, Germany). The bottles inoculated with the *Siha* strain were kept in a room at 15 ºC and the bottles inoculated with the *Uvaferm* strain were kept in a room at 20 ºC (corresponding to the optimum fermentation conditions according to previous experiments). Fermentations were performed in duplicate.

To monitor the process, samples were collected at different fermentation times. For each sample, the temperature was measured and the pH monitored. Finally, all the samples were subject to HPLC analysis.

HPLC analysis

HPLC analysis was used to characterize the pear juice and the pear fermented juice, and also to monitor the fermentation process. The HPLC equipment was a Bischoff Modell 2200 with a Bischoff Model 728 Autosampler (Bischoff, Leonberg, Germany) and a ERC7510 Refraction Index detector (ERC, Altegolfsheim, Germany). A McDAcq15 Integrator (Bischoff, Leonberg, Germany) was used for data acquisition. The column was an Aminex HPX-87H (Biorad, Munich, Germany). The mobile phase was a solution of H_2SO_4 0.1 N at a flow rate of 0.6 mL/min, with a column temperature of 50 ºC and an injection volume of 20 µL. All the samples and the mobile phase were filtered before the analysis using cellulose acetate filters with a pore size of 0.45 µm. Samples were analyzed in duplicate.

Distillation process

The fermented juices were distilled in presence of its lees in a 10 L copper pot still (Jacob-Carl, Göppingen, Germany) fitted with a column of three bubble plates and a dephlegmator (Holstein, Markdorf, Germany). For each distillation, 3 L of the fermented juice were used. The first 285 mL were collected divided in eight different fractions. The first two fractions were of 20 mL each, the third one was of 15 mL, the fourth and fifth ones were of 80 mL, the next two were of 20 mL and the last one was of 30 mL. The fractions corresponding to the head were separated from the rest based on the acetaldehyde concentration, by means of a detaching test kit (Schliessmann, Schwäbisch Hall, Germany). The fractions corresponding to the tail were separated by organoleptic analysis. The remaining fractions were put together to form the heart. The head, heart and tail of each distillation were analyzed using GC, and their ethanol concentration was determined using a density-meter DMA48 (Paar Physica, Graz/Strassburg) [7].

GC analysis

Gas chromatography was used to determine the concentrations of different compounds in the head, heart and tail fractions. The method used by Cortés et al. for determining volatile compounds in *orujos* was adapted to determine the volatile composition, and the methanol and ethanol content in each sample [8]. The equipment used was an Agilent 6890N with a Flame Ionization Detector, automatic injector and HP Chemstation software (Agilent, Waldbron, Germany)

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for the data analyses. The column was a Teknokroma TR – MetaWax capillary column (polyethyleneglycol stationary phase; 30 m × 0.25 mm × 0.5 μm). The injection volume was 1μL in split 1:5 mode at an injector temperature of 250 ºC. The carrier gas was helium at a column flow rate of 1.1 mL/min. The oven temperature was programmed at 40 ºC for 6 minutes, then increased to 80 ºC at a rate of 1.5 ºC/min. and from 80 to 200 ºC at 3 ºC/min. The detector temperature was 260 ºC, with a H_2 flow rate of 40 mL/min. and an air flow rate of 350 mL/min. Helium was used as the auxiliary gas, at a flow rate of 25 mL/min.

The internal standard was 4-methyl-2-pentanol (Fluka) for all the compounds except ethanol, for which it was acetonitrile (J.T. Baker) [9]. A solution containing these two standards in a concentration of 1.0 g/L and 100 g/L respectively was prepared and mixed at a ratio of 1/10 for each sample. Each sample was injected by duplicate.

Statistical analysis

One-way analysis of variance (ANOVA) was used to ascertain if there are significant differences (at 5% level) among the *Blanquilla* pear spirits obtained with the two different yeast strains.

Principal components analysis (PCA) was also performed to the spirits to determine the degree of differentiation between the different distillates.

All the statistical analyses were performed by means of SPSS statistical package (version 14.0).

Sensory evaluation

All the heart fractions were diluted with demineralized water to an ethanol content of 40% (v/v). The spirits obtained were tested for their flavour quality using order-of-precedence tests. Sensory evaluation was conducted with a panel of 9 consumers whom were asked to evaluate separately the smell and the taste of the spirits. The results were analyzed using the Friedmann statistic test [10].

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4.3 Results and discussion

Fermentation process

The pear juice prepared had 18 ºBrix and a pH of 4.25. **Figure 4.1** shows the fructose and glucose consumption, and the ethanol and glycerol formation during the fermentation process for both yeasts (each value is the mean of two fermentations and two HPLC injections). As it can be seen, the profiles of all the compounds monitored were the same for both yeasts.

The temperature was constant between 15 – 17 ºC for the fermentations with the *Siha* strain and between 20 – 22 ºC for the fermentations with the *Uvaferm* strain. The pH slowly increased during the first week for all the fermentations, and then remained stable at 4.45 for the *Siha* strain and 4.40 for the *Uvaferm* strain.

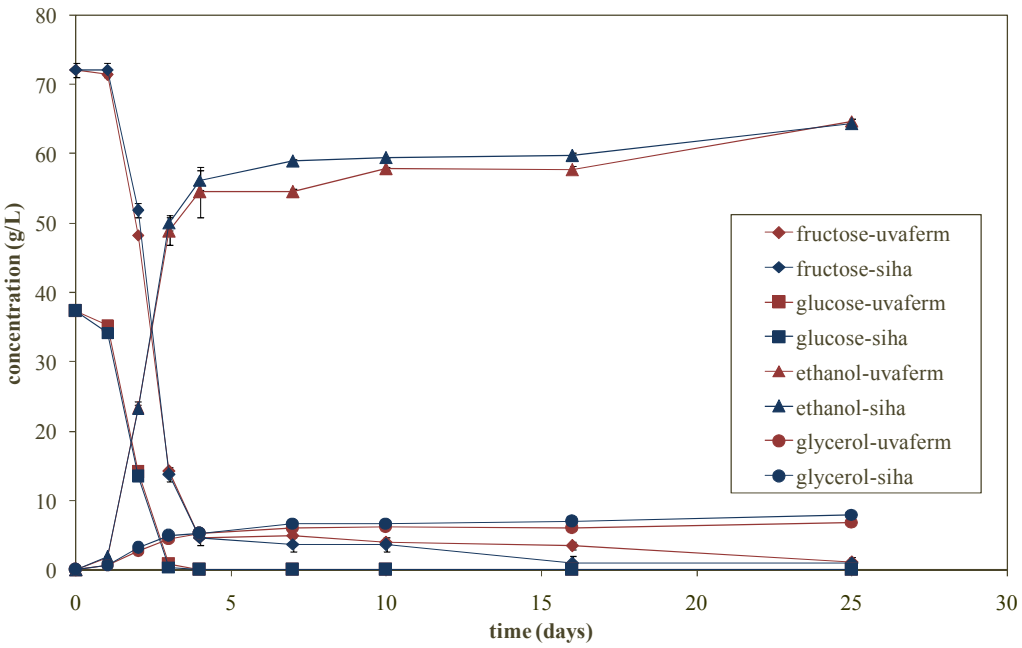

Figure 4.1. Concentration profiles of fructose, glucose, ethanol and glycerol during Blanquilla variety pear juice fermentations with *Uvaferm CGC62* and *Siha Aktiv6* yeast strains.

Distillation process

Based on the detaching test for acetaldehyde, the first fraction (20 mL) of each distillation was separated as the head fraction. On the other hand, the organoleptic analysis showed that fractions 6, 7 and 8 (corresponding to the last 70 mL

distilled) had long chain fatty acids aroma and only residual smell of pear in all the distillations performed, so these fractions were put together conforming the tail. The middle fractions (195 mL) are also put together, being the heart of the distillate. **Figure 4.2** shows the aromatic composition of several compounds in the three different distillation fractions analyzed (head, heart and tail).

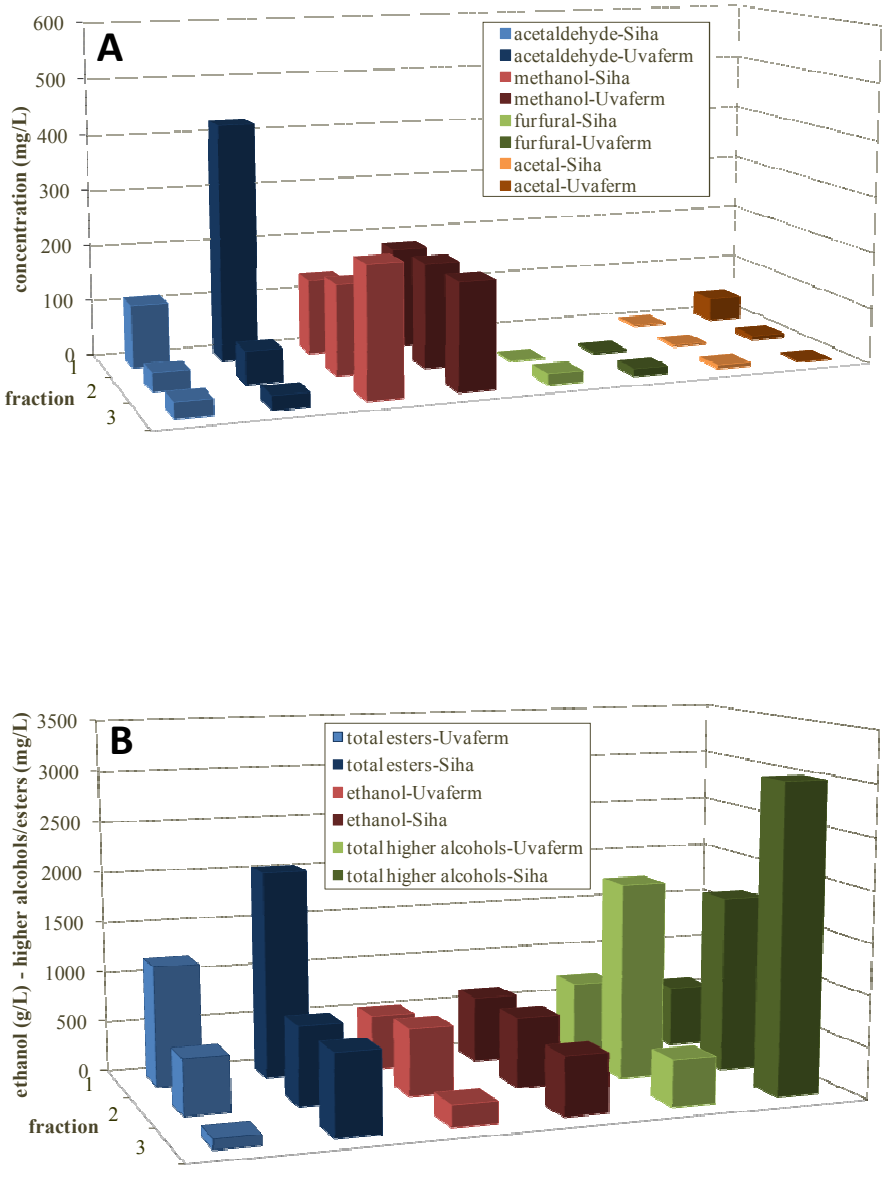

Figure 4.2. Concentration profiles of different compounds during the distillations of fermented *Blanquilla* concentrated pear juice. (**A**) acetaldehyde, methanol, furfural and acetal; (**B**) total higher alcohols, total esters and ethanol.
Siha, distillates from pear juice fermented with the *Siha* strain; Uvaferm, distillates from pear juice fermented with the *Uvaferm* strain. Fraction 1: head, 2: heart, 3: tail.

The different concentrations were determined by densitometry in the case of the ethanol and GC for the rest of the compounds. As it was described in **Chapter 2**, based on Léauté's work on simple batch distillations in alembic, the distillation profile of a compound depends on its boiling point and on its solubility in water and ethanol [11]. As **Figure 4.2-A** shows, acetaldehyde, acetal, methanol and furfural presented the same distillation profiles than the simple batch distillations in copper and glass alembic (see **Chapter 2**). However, the total higher alcohols (1-butanol, 2-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 1-propanol, 2-methyl-1-propanol, phenyl alcohol) and the total esters (methyl acetate, ethyl acetate, ethyl hexanoate, ethyl lactate, ethyl decanoate, ethyl-2-trans-4-cis-decadienoate) showed a quite different behaviour (see **Figure 4.2-B**). This is probably because of the distillation system employed. In this set of distillations a column with three plates and a dephlegmator was used, so part of the volatized products were condensed and returned to the system. This means that very volatile compounds (like acetaldehyde or acetal) still distil at the very beginning of the distillation, because the temperatures in the column are not low enough to condense them. However, compounds with a higher boiling point but soluble in ethanol (like higher alcohols and short chain fatty acids esters), which distil earlier in a simple batch distillation because of their solubility in ethanol, in the system employed for these experiments they will condense and return to the system until the temperature is high enough to avoid their condensation. Only then they will distil. These results were confirmed by the distillation profile of each compound (see **Annex III**). The esters with very low boiling point (methyl acetate, ethyl acetate) had the same profile than in a simple batch distillation. As the boiling point of the compound increased, it distiled towards the heart and tail of the system even if they are very soluble in ethanol. Therefore, this distillation system concentrates some of the main aromatic compounds in the middle fractions, increasing their concentration in the spirit. Similar differences in the volatile compounds profiles were obtained by Hernández-Gómez et al. for melon distillates produced in copper alembic and glass column filled with Raschig rings [12,13].

It should be noticed that the concentration of acetal slightly increased towards the end of the distillation in the ferments from *Siha* strain. This could be due to its

formation during the distillation process [16]. In addition, the volatiles with higher boiling points and very soluble in water (like furfural) still distil towards the end of the fermentation. Methanol is an isolated case because it forms azeotropic mixtures and therefore, it distils during the whole distillation process.

It was also observed that the concentration of the total higher alcohols in the fermentations with *Siha* strain kept increasing until the end of the distillation, while their concentration for the fermentations with the *Uvaferm* strain dramatically decreased from the second to the third fraction. The same behaviour was observed with the total esters amount, although the difference is less pronounced than for the total higher alcohols. This fact was probably related to the ethanol amount present in the different fractions. As it was mentioned before, the distillation behaviour of the compounds depend in part on their ethanol and water solubility. Higher alcohols and esters are very soluble in ethanol, and in most of the cases their solubility is higher in ethanol than in water. Therefore, the higher concentration of ethanol present in the last fraction of the distillates from *Siha* strain respect to the distillates from *Uvaferm* strain, implied a higher amount of higher alcohols and esters in that fraction.

Table 4.1 shows the concentrations of the different volatile compounds in the hearts obtained from the fermented juices distilled with the two different yeast strains (*Siha* and *Uvaferm*). Both types of spirits had a concentration of total higher alcohols higher than 140 g/hL a.a., which is the lowest amount accepted by the European Council Regulation (Nº 1576/89). In addition, they showed low concentrations of toxic and/or aromatically unpleasant compounds such as methanol, furfural, methyl acetate and ethyl lactate. However, the concentration of ethyl acetate, a typical head product which supplies nuances of dissolvent or glue to the spirit if present in high amounts [14], was quite high. This was especially observed in the spirits produced from the juices fermented with *Siha*. Cortés et al. indicated that it degrades the spirits characteristics if present in concentrations higher than 50 g/hL a.a., so our spirits produced with the *Siha* strain would be in the limit [14].

Table 4.1 also shows the results of the ANOVA tests, which evidenced significant differences between both types of distillates for several of the compounds

analysed. The spirits from pear juice fermented with *Uvaferm* had a significantly higher concentration of acetaldehyde, acetal, furfural and phenethyl alcohol. However, these concentrations were very low in both types of spirits, being far below the usual values found in bibliography for fruit spirits [8,15,16]. Therefore, these small differences could be attributed to small differences in the cut-off points of the heads and the tails rather than to real differences in the distillates from the two yeast strains [17].

Table 4.1. Concentrations (g/hL a.a.) and standard deviations of the different volatile compounds present in the distilled spirits from *Blanquilla* pear fermented with the yeasts *Siha Aktiv6* and *Uvaferm CGC62*.

compound	*Siha*	*Uvaferm*
ethanol (%v/v)	680.2 ± 3.3	656.9 ± 3.1
methanol	18.8 ± 2.9^{a}	22.5 ± 8.1^{a}
acetaldehyde	3.9 ± 0.9^{a}	6.9 ± 2.3^{b}
acetal	0.4 ± 0.0^{a}	0.9 ± 0.1^{b}
furfural	0.1 ± 0.1^{a}	0.4 ± 0.1^{b}
phenethyl alcohol	0.0 ± 0.0^{a}	0.2 ± 0.0^{b}
1-hexanol	2.2 ± 1.4^{a}	0.4 ± 0.3^{b}
1-butanol	0.6 ± 0.2^{a}	0.4 ± 0.2^{a}
2-butanol	13.9 ± 16.0^{a}	0.0 ± 0.0^{a}
2-methyl-1-butanol	16.9 ± 4.6^{a}	23.2 ± 0.7^{b}
3-methyl-1-butanol	96.5 ± 29.3^{a}	109.3 ± 5.3^{a}
1-propanol	34.9 ± 5.8^{a}	59.0 ± 4.6^{b}
2-methyl-1-propanol	35.4 ± 7.3^{a}	39.0 ± 0.8^{a}
total higher alcohols	**200.4 ± 64.6**	**231.5 ± 11.9**
methyl acetate	0.3 ± 0.0^{a}	0.4 ± 0.3^{a}
ethyl acetate	52.8 ± 4.0^{a}	28.9 ± 1.0^{b}
ethyl lactate	8.2 ± 2.8^{a}	3.6 ± 0.5^{b}
ethyl hexanoate	1.6 ± 0.4^{a}	0.3 ± 0.1^{b}
ethyl decanoate	20.4 ± 8.6^{a}	12.7 ± 0.4^{a}
ethyl-2-trans-4-cis-decadienoate	5.5 ± 1.6^{a}	6.6 ± 2.3^{a}
total esters	**88.8 ± 17.4**	**52.5 ± 4.6**

Different superscripts indicate significant differences ($p \leq 0.05$) between parameter values.
Siha, spirits from Blanquilla pear concentrate fermented with the *Siha* strain; *Uvaferm*, spirits from Blanquilla pear concentrate fermented with the *Uvaferm* strain.

The concentrations of 2-methyl-1-butanol and 1-propanol were significantly higher in the spirits from pear juice fermented with *Uvaferm*. However, 1-propanol did not reach the perception threshold (100 g/hL a.a.) in any case, and 2-

methyl-1-butanol is present in concentrations usually found in fruit spirits in both cases [14]. On the other hand, 1-hexanol, ethyl acetate, ethyl lactate and ethyl hexanoate showed significantly lower concentrations in the spirits from pear juice fermented with *Uvaferm*. 1-hexanol has a positive organoleptical effect when present at concentrations between 0.5 – 10 g/hL a.a., so its low concentration in this case turns to be negative [14]. Ethyl hexanoate supplies a fruity aroma to the spirits, so its presence is beneficial [8]. Its perception threshold is very low, 0.23 mg/l, so it affects the aromatic profile of both types of spirits. According to the concentrations found, its effect should be more notorious in the spirits from the pear juice fermented with *Siha*. Finally, ethyl acetate and ethyl lactate are both negative compounds which give unpleasant properties to the spirits. The influence of ethyl acetate in our spirits was already discussed in a previous paragraph. Ethyl lactate supplies a smell of rancid butter to the beverages but it has a quite high perception threshold, 250 mg/L (about 50 g/hL a.a.) [8]. This concentration was not reached in any of our spirits, so its presence should not affect the spirits quality. The higher concentration of ethyl lactate in the spirits from Siha strain, together with the presence of 2-butanol could be linked to the lactic acid bacteria contamination [14]. However, as it was seen, none of these concentrations was high suggesting that the contamination occurred towards the end of the fermentation process and it probably will not affect the quality of the distillates.

According to the results obtained by the GC analyses and the ANOVA tests, the spirits from *Blanquilla* pear juice fermented with *Siha* have a significantly higher concentration of 1-hexanol, ethyl hexanoate and ethyl acetate, in amounts that could influence the spirits quality. As it was mentioned, 1-hexanol and ethyl hexanoate have a positive effect on the spirit. However, the ethyl acetate has a negative effect and its concentration is quite high in the pear spirits from *Siha* strain. In conclusion, considering only the compounds analyzed by GC, we could not make any statement about which product has a better quality.

PCA analysis was performed to determine if it was possible to differentiate both types of spirits. Three Principal Components (PC1, PC2, and PC3) explained 96.35 % of the total variance. **Table 4.2** summarizes the obtained results, and **Figure 4.3** plots PC1, PC2 and PC3 for the different spirits.

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Table 4.2. Principal Component Analysis results for the volatile compounds in the spirits from *Blanquilla* concentrated pear juice fermented with the yeasts *Siha Aktiv6* and *Uvaferm CGC62*.

Principal Component	compound	loading	variance explained (%)	total variance (%)
PC1	ethyl lactate	0.976	51.89	51.89
	1-propanol	-0.961		
	1-hexanol	0.960		
	ethyl acetate	0.940		
	ethyl decanoate	0.912		
	phenethyl alcohol	-0.847		
	acetal	-0.828		
PC2	2-butanol	-0.990	27.96	79.85
	3-methyl-1-butanol	0.975		
	2-methyl-1-propanol	0.972		
	2-methyl-1-butanol	0.951		
	ethyl hexanoate	-0.765		
	furfural	0.739		
	1-butanol	-0.644		
PC3	methanol	0.928	16.51	96.35
	methyl acetate	-0.916		
	ethyl-2-trans-4-cis-decadienoate	0.681		
	acetaldehyde	-0.613		

PC1 is the principal component which clearly differentiated the pear spirits obtained when the *Siha* strain was used from the pear spirits obtained when the *Uvaferm* strain was used. According to the PCA results, the *Siha* strain produced higher amounts of ethyl lactate, 1-hexanol, ethyl acetate and ethyl decanoate. On the other hand, it produced lower amounts of 1-propanol, phenethyl alcohol and acetal.

PC2 differentiated both distillates from the fermentations with *Siha*. This was probably because of their very different concentration in 2-butanol (see **Table**

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4.1). On the other hand, PC3 differentiated both distillates from the fermentations with *Uvaferm*, probably because of the different concentration in methanol.

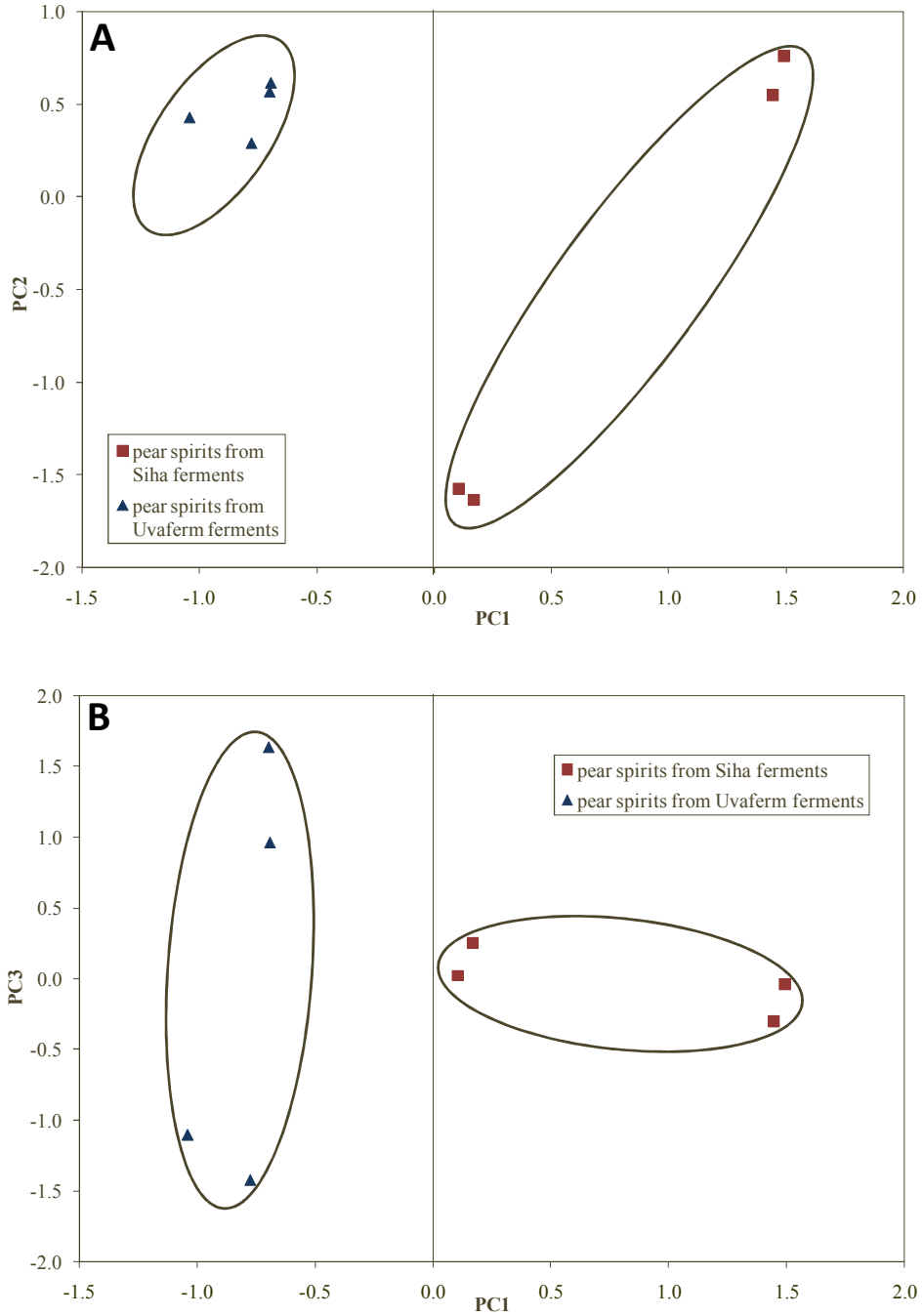

Figure 4.3. Plot of the Principal Components 1,2 and 3 (PC1, PC2, PC3) obtained from the Principal Components Analysis performed to the main volatile compounds in the pear spirits. (A) PC1 vs. PC2; (B) PC1 vs. PC3.
Siha: pear spirits from *Blanquilla* pear juice fermented with *Siha* strain; *Uvaferm*: pear spirits from *Blanquilla* pear juice fermented with *Uvaferm* strain.

All of the compounds which contributed to PC1 (except for the ethyl decanoate) showed significant differences in the ANOVA tests of the spirits from pear juice fermented with *Siha* and the spirits from pear juice fermented with *Uvaferm*.

Sensory evaluation

The spirits with a better punctuation in the order of preference test were the ones obtained from the fermentations with the *Siha* strain. This was observed both for the smell and the taste parameters. However, the statistical analysis (Friedmann-test) indicated that the difference was not significant in any of the cases ($p<0.05$). In addition, the pairs comparison also showed no significant difference in the samples smell. On the contrary, the pairs comparison for the taste showed that one of the spirits obtained from the fermentations with *Uvaferm* could be distinguished from the rest. This sample showed a very slight yeast growth on the surface by the time of distilling. Although no important difference was detected between the samples when doing the chemical analysis of the *Uvaferm* spirits (**Table 4.1** shows a quite low standard deviation in all the compounds analyzed from the experiments performed with *Uvaferm*), the superficial microorganisms growth may have developed some atypical compounds which could be detected in the sensory analysis.

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POTENTIAL OF BLANQUILLA PEAR VARIETY TO PRODUCE PEAR SPIRITS:INFLUENCE OF THE FERMENTATION
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Laura Andrea García Llobodanin
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Chapter 5

INFLUENCE OF FERMENTATION CONDITIONS: pH

5.1 Introduction

The pH of the medium at which the fermentation process takes place, plays an important role in the final composition of the ferments, and in the spirits produced from them. The pH, just as it was described for the temperature (see **Chapter 3**), may affect the growth rate of the different microorganisms [1]. Hernández-Gómez et al. found that adjusting the pH of melon juice from 5.2 to 3.8, successfully contained bacterial growth during the fermentation [2,3]. This was also observed with other melon substrates such as melon mash and melon mash without the skin. In addition, they noticed that the fermentation of the melon mash at pH 3.8 was faster than at pH 4.8. However, this behavior is not observed independently of the yeast strain or pH range. Charoenchai et al. found that several *Saccharomyces cerevisiae* strains showed no significant increase or decrease in their growth rates over the range pH 3.0 to 4.0 [1].

The microbiological composition of the fruit ferments during the fermentation process strongly affects their aromatic profile. Most of the volatile compounds are formed during the fermentation, and the prevailing microorganisms together with other fermentation conditions, will determine which compounds are produced and in which amount [4]. To cite some examples, the variation of the fermentation pH caused significant differences in the concentration of total higher alcohols in melon wines and melon spirits [3]. It also diminished the concentrations of methanol, 2-butanol and 1-propanol in grappa low wines (wines obtained from grape marc for the production of grappa) [5].

The present chapter studies the influence of the fermentation pH in the aromatic composition of pear fermented juices and their spirits. Two different sets of

experiments were performed, both using concentrated juice of *Blanquilla* pear variety as the raw material. In the first set, the juice diluted in water was fermented at the native pH (4.25) and at an adjusted pH of 3.20; and then it was distilled using a 10 L copper pot coupled to a column with three plates and a dephlegmator. The second set of experiments was performed at the native pH (4.10) and at an adjusted pH of 3.27; and then it was double distilled in a 20 L copper alembic. The aromatic profile of all the spirits was determined by GC, and their organoleptic characteristics were compared by sensory analysis.

5.2 Materials and methods

Experimental Set 1

Pear juice preparation

Pear concentrate of 73 ºBrix from *Blanquilla* variety (donated by Nufri S.A., Mollerussa, Lleida), was diluted with water until a juice of 18º Brix was obtained. This juice was characterized by HPLC and by measuring the ºBrix and the pH.

Fermentation process

The pear juice prepared was divided into two fractions. The pH of one of them was adjusted to a value of 3.20 using a mixture of malic and lactic acids (Säure-kombination, Schliessmann Schwäbisch Hall, Germany). The other fraction was left at its native pH. Fermentations of both pear juices were carried out in 5 L bottles under semi-anaerobic conditions. A volume of 3 L of juice was put in each bottle and it was inoculated with a dose of 20 g/hL of yeast, according to the instructions provided by the supplier. The yeast used was a strain of *Saccharomyces cerevisiae*, the *Siha Aktiv6* (Begerow GmbH & Co., Langenlonsheim, Germany). The inoculated bottles were kept in a room at 15 ºC. Fermentations were performed in duplicate. After the fermentations finished (total sugars concentration lower than 4 g/L), the carafes were kept closed at room temperature for some days before distilling (in order to enhance the aromatic profile of the ferments).

To monitor the process, samples were collected at different fermentation times. For each sample the pH was measured, and finally they were all subject to HPLC analysis.

HPLC analysis

HPLC analysis was used to characterize the pear juice and the pear fermented juice, and also to monitor the fermentation process. The HPLC equipment was a Bischoff Modell 2200 with a Bischoff Model 728 Autosampler (Bischoff, Leonberg, Germany) and a ERC7510 Refraction Index detector (ERC, Altegolfsheim, Germany). A McDAcq15 Integrator (Bischoff, Leonberg, Germany) was used for data acquisition. The column was an Aminex HPX-87H (Biorad, Munich, Germany). The mobile phase was a solution of H_2SO_4 0.1 N at a flow rate of 0.6 mL/min, with a column temperature of 50 ºC and an injection volume of 20 µL. All the samples and the mobile phase were filtered before the analysis using cellulose acetate filters with a pore size of 0.45 µm. Samples were analyzed in duplicate.

Distillation process

The fermented juices were distilled in presence of its lees in a 10 L copper pot still (Jacob-Carl, Göppingen, Germany) fitted with a column of three bubble plates and a dephlegmator (Holstein, Markdorf, Germany). For each distillation, 3 L of the fermented juice were used. The first 285 mL were collected divided in eight different fractions. The first two fractions were of 20 mL each, the third one was of 15 mL, the fourth and fifth ones were of 80 mL, the next two were of 20 mL and the last one was of 30 mL. The fractions corresponding to the head were separated from the rest based on the acetaldehyde concentration, by means of a detaching test kit (Schliessmann, Schwäbisch Hall, Germany). The fractions corresponding to the tail were separated by organoleptic analysis. The remaining fractions were put together to form the heart. The head, heart and tail of each distillation were analyzed using GC, and their ethanol concentration was determined using a density-meter DMA48 (Paar Physica, Graz/Strassburg) [6].

GC analysis

The method used by Cortés et al. for determining volatile compounds in *orujos* was adapted to determine the volatile composition and the methanol content in all

the distillates fractions [7]. The equipment used was an Agilent 6890N with a Flame Ionization Detector, automatic injector and HP Chemstation software (Agilent, Waldbron, Germany) for the data analyses. The column was a Teknokroma TR – MetaWax capillary column (polyethyleneglycol stationary phase; 30 m × 0.25 mm × 0.5 μm). The injection volume was 1μL in split 1:5 mode at an injector temperature of 250 ºC. The carrier gas was helium at a column flow rate of 1.1 mL/min. The oven temperature was programmed at 40 ºC for 6 minutes, then increased to 80 ºC at a rate of 1.5 ºC/min. and from 80 to 200 ºC at 3 ºC/min. The detector temperature was 260 ºC, with a H_2 flow rate of 40 mL/min. and an air flow rate of 350 mL/min. Helium was used as the auxiliary gas, at a flow rate of 25 mL/min.

The internal standard was 4-methyl-2-pentanol (Fluka) for all the compounds except ethanol, for which it was acetonitrile (J.T. Baker) [8]. A solution containing these two standards in a concentration of 1.0 g/L and 100 g/L respectively was prepared and mixed at a ratio of 1/10 for each sample. Each sample was injected by duplicate.

Statistical analysis

One-way analysis of variance (ANOVA) was applied to ascertain if there are significant differences (at 5% level) among the *Blanquilla* pear spirits obtained at the two different fermentation pHs.

Principal components analysis (PCA) was also performed to the pear spirits, to determine the degree of differentiation between the different distillates.

All the statistical analyses were performed by means of SPSS statistical package (version 14.0).

Sensory evaluation

All the heart fractions were diluted with demineralized water to an ethanol content of 40% (v/v). The spirits obtained were tested for their flavour quality using order-of-precedence test. Sensory evaluation was conducted with a panel of 9 consumers whom were asked to evaluate separately the smell and the taste of the spirits. The results were analyzed using the Friedmann statistic test [9].

Experimental set 2

Pear juice preparation

Pear concentrate of 71 ºBrix from Blanquilla variety (donated by Nufri S.A., Mollerussa, Lleida), was diluted with water until a juice of 12.5º Brix was obtained. This juice was characterized by HPLC and by measuring the ºBrix and the pH.

Fermentation process

The pear juice prepared was divided into two fractions. The pH of one of them was adjusted to a value of 3.28 using a mixture of lactic acid (Merk, Darmstadt, Germany) and malic acid (Panreac Química S.A., Barcelona, Spain) in a proportion of 1:25. The other fraction was left at its native pH. Fermentations of the pear juice were carried out in 20 L PVC carafes under semi-anaerobic conditions. A volume of 15 L of juice was put in each carafe and it was inoculated with a dose of 20 g/hL of yeast, according to the instructions provided by the supplier. The yeast used was a commercial strain of *Saccharomyces cerevisiae* (Enoferm BDX, Lallemand, Switzerland). Fermentations were performed in triplicate. After the fermentations finished (total sugars concentration lower than 4 g/L), the carafes were kept closed at room temperature for some days before distilling (in order to enhance the aromatic profile of the ferments).

To monitor the process, samples were collected at different fermentation times. For each one, the pH was monitored with a Crison Basic 20 pH meter and the total sugars content was determined using a GAB kit for sugar analysis (GAB Sistemática Analítica S.L., Spain). Finally, all the samples were subject to HPLC analysis. The final ferments were also subject to GC analysis.

Distillation process

The pear distillates were obtained by double batch distillation of the fermented pear juices in presence of their lees, in a 20 L copper alembic. The operation conditions were the same in all cases: 14 L of the fermented pear juice was distilled using water as the refrigerant and an electric heater as the heat source. The distillations were performed in triplicate. Based on previous experiments (see C**hapter 2** and **Chapter 3**) and literature [10], the fractions collected during the first distillation were: the first 60 mL (corresponding to the head), the following

3.5 L (heart fraction) and the next 600 mL (tail fraction). The heart fraction was second distilled using the same equipment. In this case, the distillation fractions collected were: two of 35 mL each, one of 1.75 L, one of 35 mL and the last one of 210 mL. Samples were put in glass bottles and kept in the freezer until they were analyzed by GC. Based on these results, the head, heart and tail fractions of the second distillation were defined.

HPLC analysis

HPLC analysis was used to characterize the pear juice, the pear fermented juices, and also to monitor the fermentation process. The HPLC equipment was an Agilent 1100 Series with HP Chemstation software (Agilent, Waldbron, Germany) for data acquisition. Sugars, glycerol, and ethanol were measured using a Refractive Index Detector (Agilent, Waldbron, Germany). The column was a Transgenomic ICSepICE COREGEL – 87H3, at an oven temperature of 50 ºC. The injection volume was 20 µL. The mobile phase was a solution of pH = 2.20 prepared with concentrated H_2SO_4 (95-97 %) in Milli-Q water. The flow rate was 0.6 mL/min. All the samples and the mobile phase were filtered before the analysis using cellulose acetate filters (Teknokroma) with a pore size of 0.45 µm. Samples were analyzed in duplicate.

GC analysis

The method used by Cortés et al. for determining volatile compounds in *orujos* was adapted to determine the volatile composition, and the methanol and ethanol content in the samples (distillation fractions and fermented juices) [7]. The equipment used was an Agilent 6890N with a Flame Ionization Detector, automatic injector and HP Chemstation software (Agilent, Waldbron, Germany) for the data analyses. The column was a Teknokroma TR – MetaWax capillary column (polyethyleneglycol stationary phase; 30 m × 0.25 mm × 0.5 µm). The injection volume was 1µL in split 1:5 mode at an injector temperature of 250 ºC. The carrier gas was helium at a column flow rate of 1.1 mL/min. The oven temperature was programmed at 40 ºC for 6 minutes, then increased to 80 ºC at a rate of 1.5 ºC/min. and from 80 to 200 ºC at 3 ºC/min. The detector temperature was 260 ºC, with a H_2 flow rate of 40 mL/min. and an air flow rate of 350 mL/min. Helium was used as the auxiliary gas, at a flow rate of 25 mL/min.

The internal standard was 4-methyl-2-pentanol (Fluka) for all the compounds except ethanol, for which it was acetonitrile (J.T. Baker) [8]. A solution containing these two standards in a concentration of 1.0 g/L and 100 g/L respectively was prepared and mixed at a ratio of 1/10 for each sample. Each sample was injected by duplicate.

Statistical analysis

One-way analysis of variance (ANOVA) was applied to ascertain if there are significant differences (at 5% level) between the *Blanquilla* pear fermented juices. The same analysis was applied to check for significant differences between the hearts of the first distillation process, and also between the pear spirits obtained.

Principal components analysis (PCA) was performed to the pear spirits, to determine the degree of differentiation caused by the variation of pH.

All the statistical analyses were performed by means of SPSS statistical package (version 15.0).

Sensory evaluation

The pear spirits were diluted with demineralized water to an ethanol content of 20% (v/v), according to the guidelines proposed by Frances Jack for the preparation of sensory samples of whisky [9]. Then they were tested for their flavour quality using order-of-precedence tests. Sensory evaluation was conducted with a panel of 24 consumers, whom were asked to evaluate separately the smell and the taste of the spirits. The results were analyzed using the Friedmann statistic test [10].

5.3 Results and discussion

Experimental set 1

Fermentation process

The pear juice prepared from pear concentrated juice had 18 ºBrix and a pH of 4.25. The adjusted pH fraction had a pH of 3.20. During the fermentations at the native pH, the pH slightly increased till a value of 4.45. For the fermentations at the adjusted pH, the pH also increases very slightly, reaching a value of 3.30.

Figure 5.1 shows the fructose and glucose consumption and the ethanol and glycerol formation during the fermentation process at both pH conditions (each value is the mean of two fermentations and two HPLC injections).

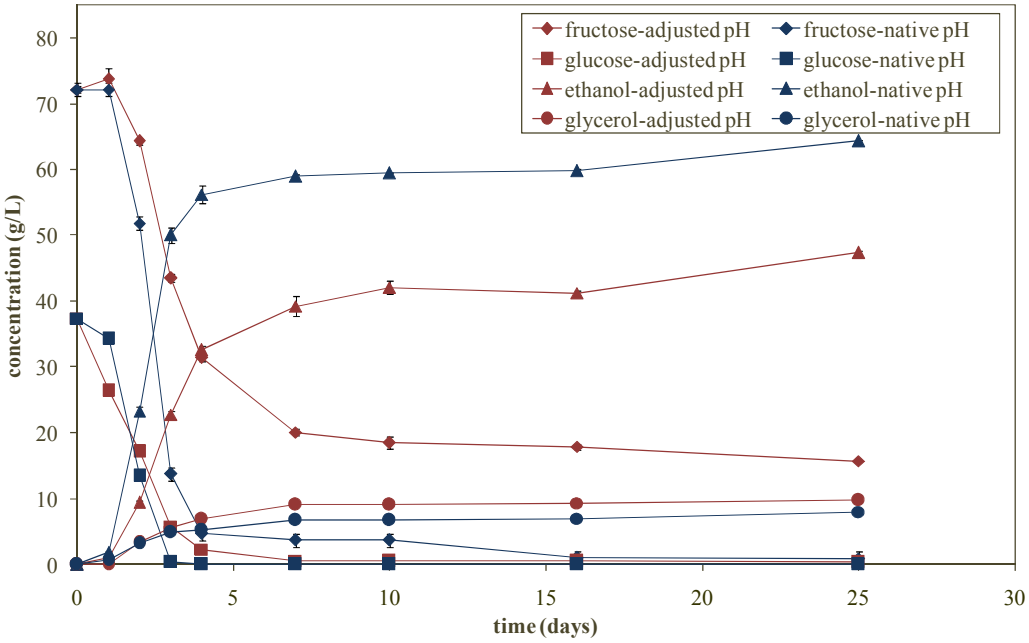

Figure 5.1. Profiles of fructose and glucose consumption, and ethanol and glycerol formation, during the fermentation of *Blanquilla* pear juice at two different pHs (adjusted pH of 3.20 and native pH of 4.25).

As the figure shows, at the adjusted pH of 3.20 a stuck fermentation was observed. During the first five days, the fermentation went more slowly than the one at the native pH; and after the first ten days, the sugars concentration remained almost constant at 16 – 18 g/L. No apparent reason was found for this behaviour. The yeast strain used was proved to achieve successful fermentations at low pHs [12]. On the other hand, the substrate used (pear juice) was completed fermented using the same yeast at the native pH, and using another yeast at low pH (see Experimental set 2). In conclusion, the problem seems to be the combination of the three factors involved: substrate, yeast and low pH.

Distillation process

According to the results of the detaching test for acetaldehyde, the first fraction (20 mL) of the distillations from the juice at the native pH and the first two

fractions (40 mL) of the distillations from the juice at the adjusted pH, were separated. This result suggests a higher production of acetaldehyde in the fermentations performed at the adjusted pH. On the other hand, the organoleptical analyses revealed that the last three fractions (last 70 mL) had fatty acids aroma and only residual smell of pear in all the distillations performed, so these fractions are put together conforming the tail fraction of each distillation. The middle fractions (195 mL for the fermentations at the native pH, and 175 mL for the fermentations at the adjusted pH) correspond to the heart (pear spirit).

Figure 5.2 shows the concentration profiles for the different volatile compounds and the ethanol present in the distillates from pear juice fermented at the native and at the adjusted pH (each value is the mean of two distillations and two GC injections). As it was suspected from the results of the detaching test for acetaldehyde, its concentration was much higher in the distillates from pear juice fermented at the adjusted pH. This was especially observed for the head fraction. Acetaldehyde is mainly produced by yeast during the fermentation process, but it is also considered to be the result of spontaneous or microbial mediated oxidation [13,14]. The slow fermentation observed in the pear juices with adjusted pH could have favoured the growth of some other microorganisms, such as bacteria, which are associated to an increase in the content of acetaldehyde [15]. According to the literature, low fermentation pH might also promote the acetaldehyde production by yeasts [13]. In addition, high concentrations of acetaldehyde may retard or even inhibit yeast ethanol fermentations [13]. Then, the high concentration of acetaldehyde could be the reason for the stucked fermentation.

Acetal is formed from the reaction of acetaldehyde with alcohol [16], so the higher concentration found in the distillates from the adjusted pH pear juices was expected. On the other hand, furfural is a compound formed during distillation by thermal degradation of the sugars [17]. The higher concentration of sugars in the fermented pear juices with adjusted pH (compared to the juices at the native pH), increased the furfural production during the distillation process.

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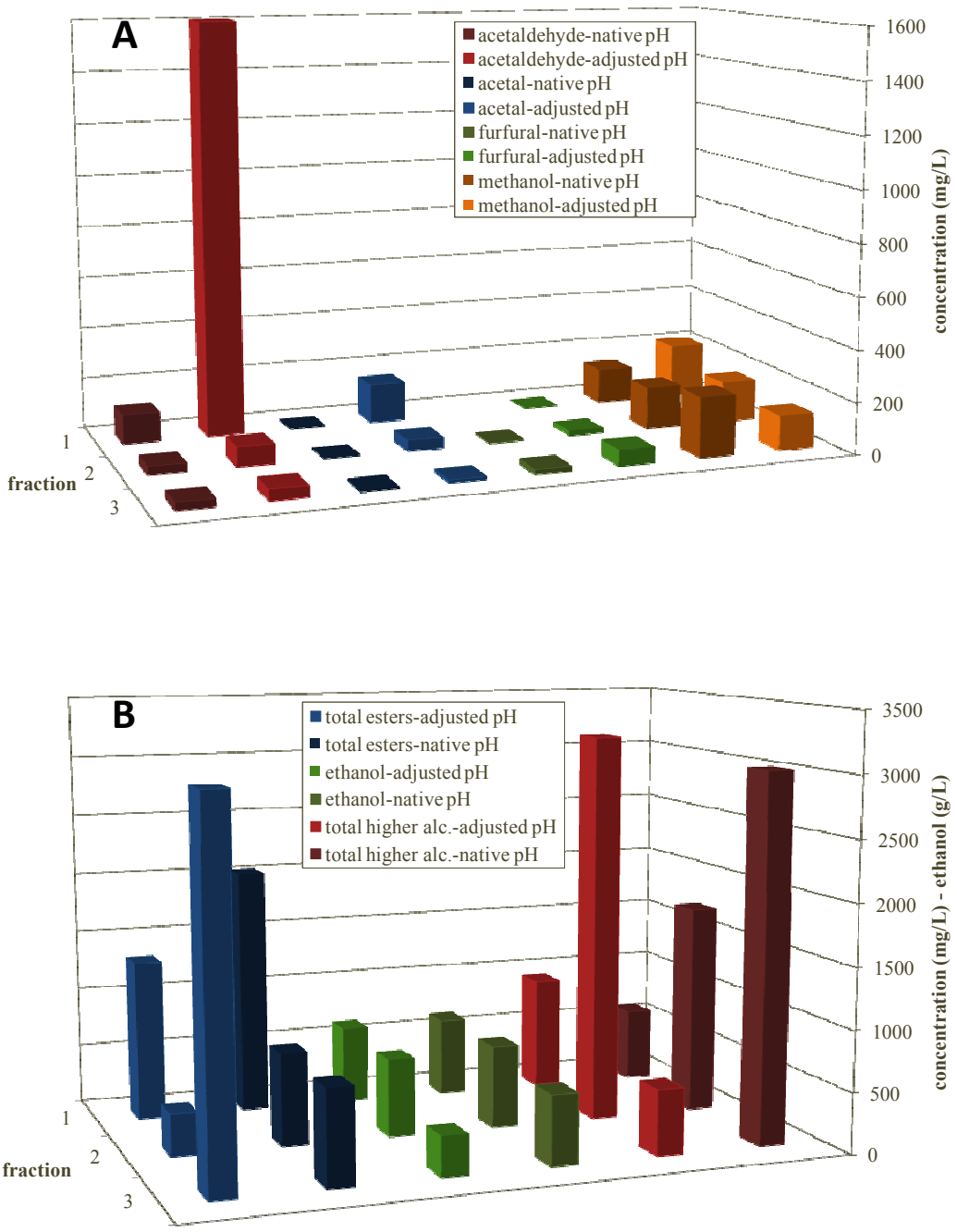

Figure 5.2. Concentration profiles of the different volatile compounds during the distillation of *Blanquilla* pear fermented juice. (A) acetaldehyde, acetal, furfural, methanol; (B) ethanol, total higher alcohols, total esters.
native pH: distillates from the fermentations at the native pH; adjusted pH: distillates from the fermentations at the adjusted pH; total higher alc.: total higher alcohols. Fraction 1: head, 2: heart, 3: tail.

The ethanol concentration in the heads and hearts was quite similar for the two different pHs juices (see **Figure 5.2-B**). On the contrary, for the tail fraction the

concentration was quite lower in the distillates at the adjusted pH. This is because of the lower ethanol production during the fermentation process at this pH. The ethanol concentration at the beginning of the distillations is almost the highest possible (close to the azeotropic point). Once the ethanol concentration in the pot still diminishes, its concentration in the distillate also diminishes. This point was not reached in the distillations of the juices fermented at the native pH, but it was reached in the distillations of the juices fermented at the adjusted pH.

The total esters concentration is higher for the distillates from native pH juice in all the fractions except for the tail. In this case, the total esters concentration in the distillates from adjusted pH increases dramatically due to a very high concentration of ethyl lactate (see **Annex IV**). This compound is produced mainly from bacteria, so its presence is associated to poor storage of the raw material or poor fermentation conditions [18,7]. The high concentration of ethyl lactate in the tails from the juices with adjusted pH, supports in a way the theory of bacterial contamination in the ferments.

The total higher alcohols concentration was higher for the distillates from pear juice with adjusted pH in all the fractions except for the tail. This is probably associated to the much lower ethanol concentration in this fraction. Total higher alcohols are highly soluble in ethanol and less soluble in water. Therefore, their distillation is linked to the ethanol concentration (among other things such as their boiling point, the column plates temperature, and the dephlegmator temperature).

The concentrations of the volatile compounds quantified in the heart fractions of the different distillates are shown in **Table 5.1**, together with the ANOVA test results. Most of the compounds showed significant difference in their concentrations when comparing the distillations from pear juice performed with and without adjusting the pH. However, the concentrations of acetaldehyde, acetal, furfural, phenethyl alcohol and methyl acetate are quite low at both pH conditions. Their values are below the commonly found concentrations in fruit spirits and in most cases below their perception thresholds [3,14,17,18].

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Table 5.1. Mean concentrations (g/hL a.a.) and standard deviations of the main volatile compounds in the heart fractions from Blanquilla pear juice fermented at the native pH and at an adjusted pH of 3.20.

compound	pH- native	pH- adjusted
ethanol (%v/v)	86.2 ± 0.4	81.3 ± 2.8
methanol	18.8 ± 2.9[a]	19.5 ± 4.8[a]
acetaldehyde	3.9 ± 0.9[a]	9.4 ± 2.1[b]
acetal	0.4 ± 0.0[a]	5.0 ± 0.7[b]
furfural	0.1 ± 0.1[a]	2.4 ± 1.9[b]
phenethyl alcohol	0.0 ± 0.0[a]	0.2 ± 0.0[b]
1-hexanol	2.2 ± 1.4[a]	38.6 ± 1.6[b]
1-butanol	0.6 ± 0.2[a]	0.5 ± 0.1[a]
2-butanol	13.9 ± 16.0[a]	0.0 ± 0.0[a]
2-methyl-1-butanol	16.9 ± 4.6[a]	38.2 ± 1.5[b]
3-methyl-1-butanol	96.5 ± 29.3[a]	212.3 ± 10.7[b]
1-propanol	34.9 ± 5.8[a]	30.4 ± 1.4[a]
2-methyl-1-propanol	35.4 ± 7.3[a]	73.6 ± 4.3[b]
total higher alcohols	**200.4 ± 64.6**	**393.8 ± 19.6**
methyl acetate	0.3 ± 0.0[a]	0.0 ± 0.0[b]
ethyl acetate	52.8 ± 4.0[a]	16.6 ± 1.1[b]
ethyl lactate	8.2 ± 2.8[a]	0.0 ± 0.0[b]
ethyl hexanoate	1.6 ± 0.4[a]	0.6 ± 0.1[b]
ethyl decanoate	20.4 ± 8.6[a]	10.4 ± 2.3[a]
ethyl-2-trans-4-cis-decadienoate	5.5 ± 1.6[a]	14.7 ± 6.1[b]
total esters	**88.8 ± 17.4**	**42.3 ± 9.6**

Different superscripts indicate significant differences ($p \leq 0.05$) between parameter values.
pH-native: spirits from *Blanquilla* pear concentrate fermented at the native pH; pH-adjusted: spirits from Blanquilla pear concentrate fermented at the adjusted pH.

In addition, the methanol and total higher alcohols concentrations meet the limits adopted by the European Council regulation for fruit spirits (Nº 1576/89) both for the native and the adjusted pH. These limits are a maximum of 1000 g/hL a.a. for methanol and a minimum of 150 g/hL a.a. for total higher alcohols. Among the higher alcohols, 1-hexanol, the isoamyl alcohols and the 2-methyl-1-propanol had significant differences when comparing the hearts from both fermentation pHs. 1-hexanol has a positive role organoleptically between 0.5 – 10 g/hL a.a., but higher concentrations would supply herbaceous nuances that are negative for the spirit [20]. In our case, the heart from the pear juice fermented at the native pH was within the limits accepted. However, the heart fraction from the adjusted pH fermentation was quite above that limit. The isoamyl alcohols are favourable for the spirit at low concentrations, but they supply disagreeable odours at higher

concentrations [20]. The Regulating Council for the Specific Denomination of Galician Orujo fixes the limits of the sum of 1-butanol, 2-butanol, isoamyl alcohols, 1-propanol and 2-methyl-1-propanol between 225 g/hL a.a. and 600 g/hL a.a. Although pear spirits are obtained from a different raw material, if this value is taken as a reference, the hearts obtained from the native pH pear juice would be slightly below the limit.

In the case of the total esters, the opposite behaviour was observed. The concentration in the heart fractions from the juices fermented at the native pH was more than double of the concentration in the hearts from the juices fermented at the adjusted pH. This is mainly due to the higher concentration of ethyl acetate. Ethyl acetate is a typical head product which supplies nuances of dissolvent or glue to the spirit if present in high amounts [20]. Cortés et al. indicated that it degrades the spirits characteristics if present in concentrations higher than 50 g/hL a.a., so our spirits produced at the native pH would be in the limit. The ethyl lactate also has a negative effect on the spirit when present in high amounts, giving a wet and bakery profile [18]. However, at the concentrations found in our distillates (lower than 10 g/hL a.a.), it stabilizes the aroma and smoothens the firm character of certain substances. Ethylic esters of acids with relatively high molecular weight (C_6-C_{12}) are produced during fermentation, and during the distillation process the heat release significant amounts of them from the yeast cells (where they remain bound) [18]. They have a floral and fruity character, so their presence in the spirits is desired [20]. The distillates from the pear juice fermented at the native pH showed a significant higher concentration of ethyl hexanoate and a significant lower concentration of ethyl-2-*trans*-4-*cis*-decadienoate, typical pear-like aroma compounds [21]. From these results we can observe that both types of spirits have some negative and some positive attributes from the aromatic point of view, so no judgment can be made about their quality.

The PCA analysis divided the compounds in three principal components (PC1, PC2 and PC3). **Table 5.2** shows the compounds that form each component, their loads, and the variance explained by each component. **Figure 5.3** plots the three different components obtained. As it can be seen, the principal component 1 explained almost 70 % of the variance and it differentiated the spirits obtained at the two different fermentation pHs. All of the compounds that conform PC1,

except for the ethyl decanoate, showed a significantly different concentration when comparing the native pH and the adjusted pH spirits using the ANOVA tests. PC2 only separated one of the spirits fermented at the native pH, probably due to its high concentration in 2-butanol compared to the rest (see **Table 5.1**). On the other hand, PC3 mainly separated the spirits at the adjusted pH, probably because of their notoriously different concentration in ethyl-2-*trans*-4-*cis*-decadienoate.

Table 5.2. Principal Component Analysis results for the volatile compounds in the spirits from *Blanquilla* concentrated pear juice fermented at the native pH and at the adjusted pH.

Principal Component	compound	loading	variance explained (%)	total variance (%)
PC1	ethyl lactate	-0.991	69.82	69.82
	furfural	-0.936		
	ethyl decanoate	-0.874		
	ethyl acetate	-0.868		
	acetal	0.820		
	methyl acetate	-0.814		
	phenethyl alcohol	0.769		
	1-hexanol	0.763		
	acetaldehyde	0.723		
PC2	2-butanol	-0.984	19.26	89.08
	1-propanol	-0.965		
	ethyl hexanoate	-0.854		
	1-butanol	-0.823		
	3-methyl-1-butanol	0.788		
	2-methyl-1-butanol	0.761		
	2-methyl-1-propanol	0.743		
PC3	methanol	0.936	7.88	96.96
	ethyl-2-trans-4-cis-decadienoate	0.712		

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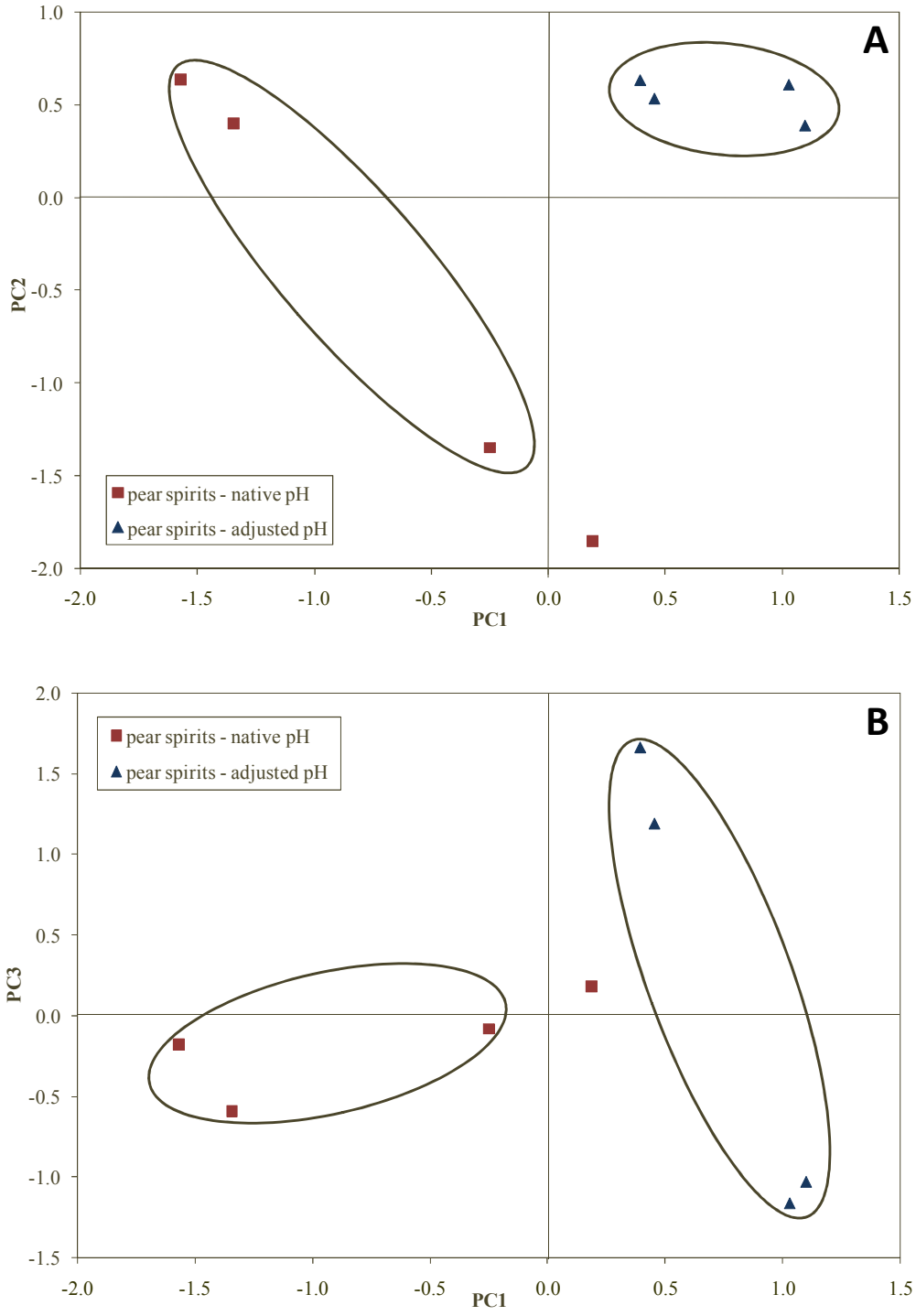

Figure 5.3. Plots of the principal components (PC1, PC2 and PC3) obtained from the Principal Components Analysis of the main volatile compounds present in the spirits from pear juice fermented at the native and at an adjusted pH. (A) PC1 vs. PC2; (B) PC1 vs. PC3.

Sensory evaluation

Finally, the sensorial analysis was performed in order to determine if the chemical differences found can be perceived organoleptically. For the smell parameter, the punctuation of all the spirits was very even and there were no significant differences ($p \leq 0.05$) among them. On the contrary, for the taste parameter, both spirits from the fermentations at the native pH obtained a better punctuation compared to the spirits from the fermentations at the adjusted pH. However, this difference was not significant ($p \leq 0.05$) for any of the distillates tested, both for the whole samples comparison and for the pairs comparison (Friedmann-test).

Experimental set 2

Fermentation process

The pear juice prepared had 12.5 ºBrix and a pH of 4.10. The acidified pear juice had a pH of 3.27. During the fermentation process, the pH of the pear juices at the native pH slightly decreased, reaching a value of 3.90 by the third day of fermentation. After this, it remained constant until the end of the process. The pear juices at the adjusted pH suffered a slight increase in their pH values, reaching a value of 3.49 by the end of the process. The fermentation temperature was between 24 and 29 ºC. The total sugars concentration (measured by the GAB method) went from 103.6 g/L to 3.3 g/L during the fermentation of the pear juice at the native pH, and from 95.3 g/L to 2.8 g/L during the fermentation of the pear juice at the adjusted pH.

Figure 5.4 shows the main sugars, ethanol and glycerol profiles during the fermentation process (each result is the mean of three fermentations and two HPLC injections). The profiles were quite similar for both fermentation pHs, and the sum of the concentrations of the different sugars in each fermentation are in agreement with the total sugars concentration determined by the GAB method. The ethanol concentration reached by the end of the fermentation was around 40 g/L at both pHs.

After 288 hours of fermentation, a microorganism's growth was observed on the surface of the ferments. Therefore, they were taken to a cool room until the moment of distilling.

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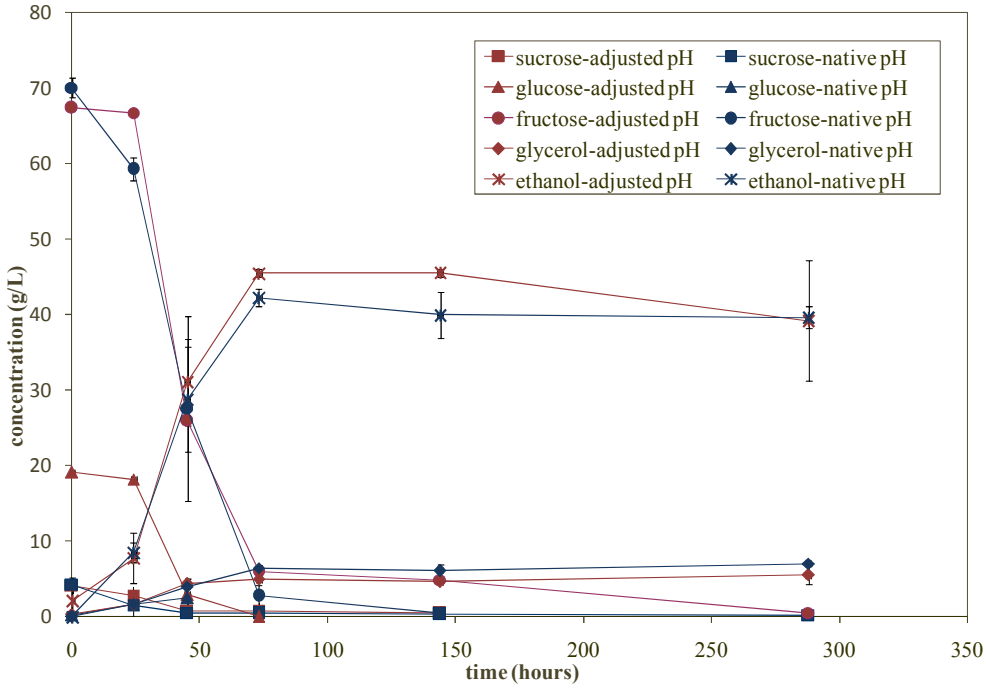

Figure 5.4. Profiles of fructose, glucose and sucrose consumption, and ethanol and glycerol formation, during the fermentation of *Blanquilla* pear juice at two different pHs (adjusted pH of 3.27 and native pH of 4.10).

To determine the volatile composition of the pear fermented juices, they are subject to GC analysis. **Table 5.3** shows the mean concentrations with the standard deviations obtained for each compound, together with the ANOVA test results. These results indicate that the pear juices fermented at the native and at the adjusted pH had significant differences in the concentrations of many of their volatile compounds. This is particularly so for the higher alcohols. The isoamyl alcohols and the 2-methyl-1-propanol presented a significantly higher concentration in the fermented juices with adjusted pH. On the contrary, the 1-butanol, 2-butanol and 1-hexanol presented a significantly higher concentration in the fermented juices at the native pH. Some of the esters also presented a significant difference in their concentrations when comparing the distillates at both pHs. This was the case of the methyl acetate and the ethyl acetate, which also showed a significantly higher concentration in the juices fermented at the native pH. The 1-butanol, 2-butanol, methyl acetate and ethyl acetate are all negative compounds derived from bacterial spoilage, and responsible for bad odours when present at high concentrations [14,17,18,22]. Therefore, these results suggest a possible contamination with bacteria during the fermentation at the natural pH.

This supports the findings of Hernandez-Gómez et al. for melon ferments, which indicated that reducing the fermentation pH controls the bacterial growth during the process [3].

The concentrations of 1-butanol and 2-butanol observed in the experiments performed are very low. Therefore, they would not affect the quality of the beverage. However, methyl acetate and especially ethyl acetate are present in quite high amounts. Apostolopoulou et al. indicated that ethyl acetate, in concentrations higher than 150 – 200 mg/L, can add spoilage notes to the wines [14]. The concentrations observed in the fermented juices at the adjusted pH are around 200 mg/L (400 g/hL a.a.), and in the fermented juices at the natural pH are around 400 mg/L (800 g/hL a.a.), so some negative aromas could be perceived (particularly in the fermented juices at the natural pH). Based on the results obtained it can be concluded that the ferments from pear juices with native pH have a lower quality than the ferments from pear juices with adjusted pH.

Table 5.3. Mean concentrations and standard deviations (g/hL a.a.) of the main volatile compounds present in the *Blanquilla* pear fermented juices at the native and adjusted pH.

compound	pH- native	pH-adjusted
acetaldehyde	33.0 ± 19.1^{a}	27.2 ± 11.2^{a}
furfural	9.1 ± 3.3^{a}	7.5 ± 1.2^{a}
methanol	7.7 ± 2.2^{a}	14.1 ± 10.3^{a}
ethanol (% v/v)	5.1 ± 0.4^{a}	4.8 ± 1.0^{a}
1-butanol	3.2 ± 0.7^{a}	0.0 ± 0.0^{b}
2-butanol	2.6 ± 0.8^{a}	0.0 ± 0.0^{b}
2-methyl-1-butanol	24.3 ± 1.6^{a}	45.8 ± 15.9^{b}
3-methyl-1-butanol	100.7 ± 3.4^{a}	173.4 ± 47.1^{b}
1-propanol	43.9 ± 5.7^{a}	42.5 ± 4.3^{a}
2-methyl-1-propanol	81.0 ± 3.6^{a}	151.2 ± 44.5^{b}
1-hexanol	157.9 ± 70.9^{a}	75.0 ± 22.6^{b}
phenethyl alcohol	43.3 ± 8.8^{a}	42.4 ± 18.1^{a}
methyl acetate	57.6 ± 18.2^{a}	24.7 ± 4.2^{b}
ethyl acetate	799.9 ± 55.5^{a}	425.6 ± 170.7^{b}
ethyl lactate	34.5 ± 8.6^{a}	41.8 ± 27.5^{a}
ethyl-2-trans-4-cis-decadienoate	53.2 ± 24.3^{a}	55.4 ± 26.8^{a}

Different superscripts indicate significant differences ($p \leq 0.05$) between parameter values.

Distillation process

The fermented pear juices obtained at the native and at the adjusted pH were double distilled in a copper alembic in order to obtain the pear spirits. The reason for a double distillation is to reach the ethanol concentration needed for a fruit spirit (37.5º according to the European Union Council (Nº 1576/89)). At the same time, the double distillation helps to better separate the positive from the negative compounds.

During the distillation process of one of the fermented pear juices with native pH, an equipment failure occurred. Consequently, the distillation was not complete and this sample had to be discarded. For this reason, the results presented hereafter are the average of two distilled pear juices fermented at the native pH, and three distilled pear juices fermented at the adjusted pH.

Table 5.4 shows the concentration of the main aromatic compounds in the heart fraction of the first distillation. Most of the compounds in the distillates from the juices at the native pH showed significant concentration differences compared to the distillates from the juices at the adjusted pH. The amounts of acetaldehyde, methyl acetate and ethyl acetate decreased if compared with the pear fermented juices (see **Table 5.3**) because a part of them distilled in the head fractions (see **Annex IV**). These are typical head products, so this behavior was expected [11]. However, the amount of ethyl acetate present in the fermented juices was very high. Therefore, its concentration in the heart fractions of the first distillation is still high. On the other hand, the ethyl 2-trans-4-cis-decadienoate should distill from the beginning to the middle of the distillation [11]. In the present experiments, it was not detected in the heart fraction of any of the distillations performed since it completely distilled in the heads (see **Annex IV**). The ethyl lactate was also present in the fermented juices but it was not detected in the hearts or in the tails. This is probably because it is present in a quite low concentration so it could have remained in the distillation residue.

On the other hand, the methanol concentration increased, meaning that the distillation process concentrates the methanol in the heart fraction. However, its concentration is still very low, far below the maximum limit of 1000 g/hL a.a. fixed by the European Council regulation for fruit spirits (Nº 1576/89).

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Table 5.4. Mean concentrations and standard deviations (g/hL a.a.) of the main volatile compounds present in the hearts of the first distillation of *Blanquilla* pear fermented juices at the native and adjusted pH.

compound	pH- native	pH-adjusted
acetaldehyde	10.2 ± 4.9 [a]	20.1 ± 5.1 [b]
acetal	0.3 ± 0.3 [a]	1.2 ± 0.3 [b]
furfural	6.6 ± 2.8 [a]	6.1 ± 0.1 [a]
methanol	41.9 ± 19.9 [a]	25.6 ± 0.9 [a]
ethanol (% v/v)	18.3 ± 0.1 [a]	21.5 ± 0.7 [b]
1-butanol	1.7 ± 0.2 [a]	1.0 ± 0.1 [b]
2-butanol	0.5 ± 0.4 [a]	0.3 ± 0.0 [a]
2-methyl-1-butanol	24.3 ± 0.7 [a]	34.2 ± 0.7 [b]
3-methyl-1-butanol	104.8 ± 2.1 [a]	138.8 ± 6.3 [b]
1-propanol	41.7 ± 3.8 [a]	26.8 ± 21.0 [a]
2-methyl-1-propanol	84.6 ± 0.7 [a]	123.5 ± 5.7 [b]
1-hexanol	89.3 ± 32.2 [a]	41.4 ± 2.5 [b]
phenethyl alcohol	5.4 ± 0.3 [a]	5.3 ± 0.5 [a]
total higher alcohols	**352.3 ± 40.4**	**371.3 ± 36.8**
methyl acetate	0.0 ± 0.0 [a]	2.3 ± 0.4 [b]
ethyl acetate	463.2 ± 11.0 [a]	181.3 ± 5.9 [b]
ethyl decanoate	0.5 ± 0.1 [a]	0.5 ± 0.2 [a]
total esters	**463.7 ± 11.1**	**184.1 ± 6.5**

Different superscripts indicate significant differences ($p \leq 0.05$) between parameter values

The heart fractions were subject to a second distillation in order to obtain the spirits. The results from the GC analyses of the different fractions obtained during the second distillation are shown in **Figure 5.5**. The acetal was not present or was present in very low concentrations in the heart fractions of the first distillations. However, it was detected in all of the distillates of the second distillation process. This suggests its formation during the second distillation, a fact which was also observed by Rodríguez-Madrera et al. in the production of cider spirits [23]. The concentration of furfural increased from the first to the third fraction, which was expected for its high boiling point and complete solubility in water [11]. Then, its concentration dramatically decreased in the fourth fraction until the end of the distillation. The acetaldehyde distilled mainly in the first fractions, as it was expected for its boiling point and solubility in ethanol. The concentrations of acetaldehyde, methanol and furfural were higher in the distillations from pear juice at the native pH (see **Figure 5.5-A**).

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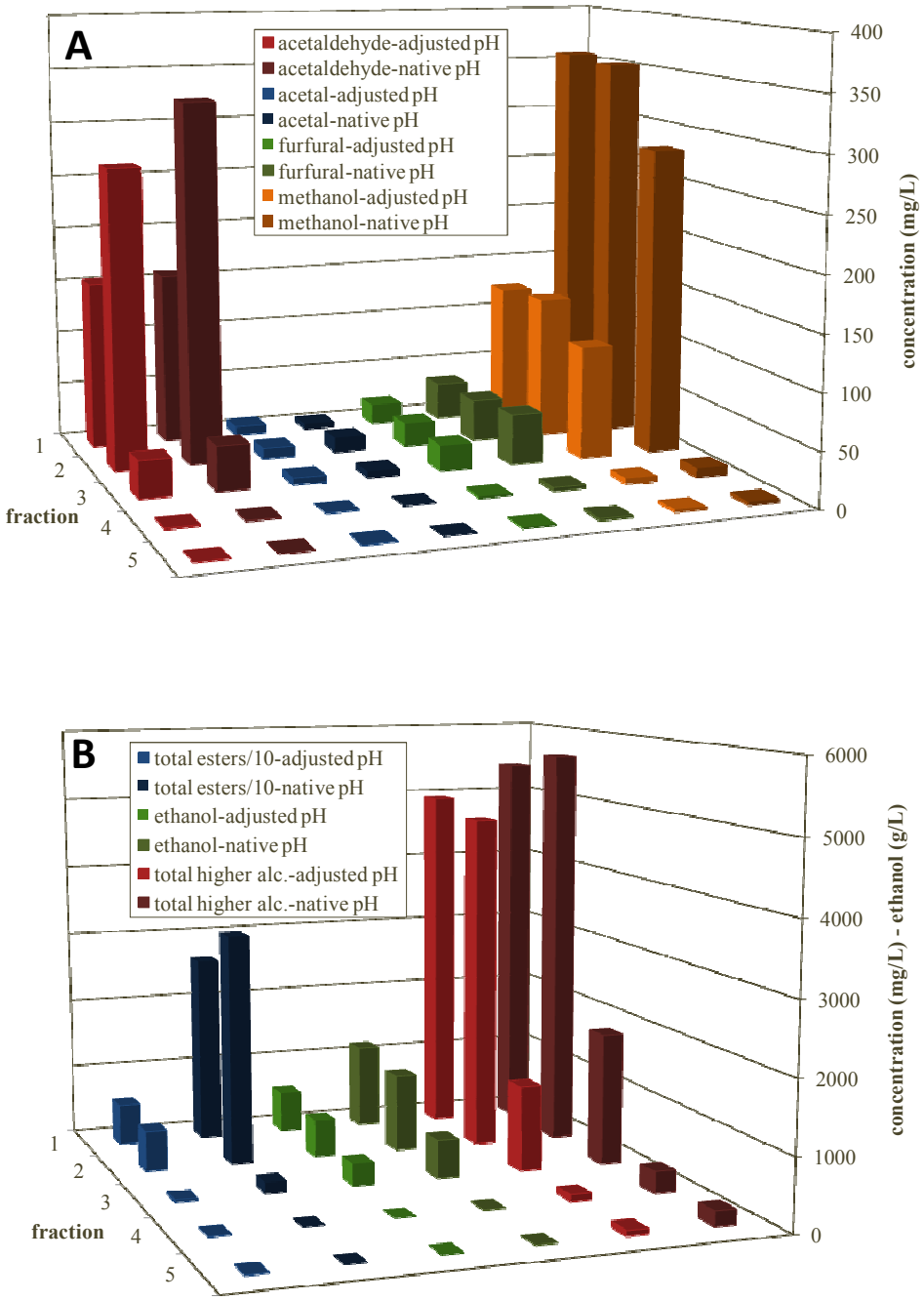

Figure 5.5. Concentration profiles of the different volatile compounds during the second distillation of *Blanquilla* pear fermented juice at two fermentation pHs. (**A**) acetaldehyde, acetal, furfural, methanol; (**B**) ethanol, total higher alcohols, total esters.

Total esters/10: total esters concentration (mg/L)/10, in order to fit the values to the scale of the concentration axis. Native pH: distillates from the fermentations at the native pH; adjusted pH: distillates from the fermentations at the adjusted pH. Fraction 1: 35 mL; 2: 35mL; 3: 1.75 L; 4: 35 mL; 5: 210 mL.

The concentrations of total higher alcohols, total esters and ethanol were also higher in the distillates from pear juices at their native pH (see **Figure 5.5-B**). The large difference between the total esters concentration in the distillates from juices at the adjusted and at the native pH, was mainly because of the much higher ethyl acetate concentration observed in the latest (see **Annex IV**). This difference was already observed in the fermented juices, and it was maintained during the distillations.

From these results it was concluded that the first distillation fraction had to be discarded, mainly due to its high concentration in ethyl acetate. The second fraction also had a high concentration of ethyl acetate, and a quite high concentration of acetaldehyde. However, if this fraction was also removed there would be a very important loss of higher alcohols and fruity aroma esters (see **Annex IV**). As **Figure 5.5-B** shows, the total higher alcohols, ethanol and total esters concentrations dramatically decreased from the second to the third distillation fraction. For this reason, the second fraction was put together with the third one in all of the distillates, forming the pear spirits. The fourth fraction had very low concentrations of all of the compounds analyzed. Therefore, it was mixed with the fifth fraction, forming the tails.

Table 5.5 shows the aromatic composition and ethanol content in the different pear spirits, and also the ANOVA test results. Most of the higher alcohols showed a significant difference between their concentrations in the spirits at the native and at the adjusted pH. The concentrations of 2-methyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol were significantly higher in the spirits at the adjusted pH. The limits fixed by the Regulating Council for the Specific Denomination of Galician Orujo for the sum of 1-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-methyl-1-propanol are 225 g/hL a.a. and 600 g/hL a.a., for the minimum and the maximum respectively. Taking these values as a reference, it could be said that the spirits from pear juice at the native pH were slightly below the limit while the spirits from pear juices at the adjusted pH are within the accepted range. The 1-hexanol has a positive role organoleptically between 0.5 – 10 g/hL a.a. [20]. At higher concentrations it supplies herbaceous nuances which are negative. The spirits at both pHs are far above the upper limit, resulting probably in a loss of quality for the spirits. Finally, the phenethyl alcohol

has floral nuances so its presence in low concentrations, like the ones found in the pear spirits produced, results positive [7].

Table 5.5 Mean concentrations (g/hL a.a.) and standard deviation of the main volatile compounds in the pear spirits from *Blanquilla* variety fermented at the native pH and at an adjusted pH of 3.30.

compound	pH - native	pH - adjusted
acetaldehyde	7.4 ± 3.4^{a}	12.0 ± 3.5^{a}
acetal	1.2 ± 0.5^{a}	2.3 ± 1.3^{a}
furfural	6.8 ± 2.5^{a}	7.5 ± 0.7^{a}
methanol	38.6 ± 18.8^{a}	31.7 ± 6.6^{a}
ethanol (% v/v)	31.8 ± 1.8^{a}	32.7 ± 6.8^{a}
1-butanol	2.1 ± 0.3^{a}	1.4 ± 0.4^{b}
2-methyl-1-butanol	22.4 ± 1.2^{a}	43.0 ± 13.2^{b}
3-methyl-1-butanol	95.1 ± 4.5^{a}	163.8 ± 41.5^{b}
2-methyl-1-propanol	77.0 ± 4.0^{a}	147.3 ± 40.0^{b}
1-hexanol	79.3 ± 32.1^{a}	37.7 ± 4.4^{b}
phenethyl alcohol	3.2 ± 0.2^{a}	5.0 ± 2.5^{a}
total higher alcohols	**279.1 ± 42.3**	**398.2 ± 102.0**
methyl acetate	0.5 ± 0.0^{a}	0.0 ± 0.0^{b}
ethyl acetate	300.9 ± 14.0^{a}	152.2 ± 19.6^{b}
ethyl hexanoate	0.3 ± 0.1^{a}	0.2 ± 0.0^{a}
ethyl 2-*trans*-4-*cis*-decadienoate	0.0 ± 0.0^{a}	0.2 ± 0.3^{a}
total esters	**301.7 ± 14.1**	**152.6 ± 19.9**

Different superscripts indicate significant differences ($p \leq 0.05$) between parameter values.
pH-native: spirits from *Blanquilla* pear concentrate fermented at the native pH; pH-adjusted: spirits from Blanquilla pear concentrate fermented at the adjusted pH.

As it was mentioned before, the ethyl acetate in high concentrations supplies nuances of dissolvent or glue to the spirit [20]. If above 50 g/hL a.a. it degrades its characteristics. In addition, Ferreira et al. found that it was linked to an acidic character in the drinks [24], and concentrations higher than 180 g/hL a.a. result highly negative [18]. The pear spirits obtained in the present experiments have ethyl acetate concentrations that are high above this limit for the spirits at the native pH, and slightly below this limit for the spirits at the adjusted pH. It is interesting to point out that the high ethyl acetate concentrations found initially in the fermented juices diminished during the distillation processes. However, the removal that takes place mainly in the head fractions (see **Annex IV**) was not enough to reach acceptable values in the hearts.

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The concentrations of total higher alcohols observed in the pear spirits at the native and at the adjusted pH were very similar to the ones observed in the experimental set 1. This results quite interesting because a different fermentation yeast was used (which could affect the higher alcohols formation [4]), and also a different distillation system (which could affect the higher alcohols distillation profiles). In particular, the concentrations of 2-methyl-1-butanol, 3-methyl-1-butanol and 2-methyl-1-propanol were significantly higher in the spirits from pear juice at the adjusted pH compared to the spirits at the native pH in both experimental sets. Although more experiments should be performed, these results suggest that the raw material employed (concentrated pear juice from *Blanquilla* variety) does not generate very high amounts of higher alcohols during the fermentation, although the acidification of the fermentation medium increases their production.

On the other hand, the total esters concentrations did not show the same similarity. This is basically because of the much lower ethyl acetate concentration present in the distillates from the experimental set 1. This difference could be explained by the microorganisms growth observed on the surface of the ferments of the experimental set 2, which could be linked to acetic acid and ethyl acetate formation by acetic bacteria [22]. In addition, the spirits of the experimental set 1 presented higher amounts of long chain (C_6 – C_{12}) ethyl esters, which are responsible for the fruity aroma in the spirits [20]. Some of these compounds are already present in the fruit, but their concentrations could increase during the fermentation process [4,25]. As the raw material used is the same for both experimental sets, the total esters difference could be due to the fermentation yeast employed. The distillation system employed could also have had an important role, concentrating these esters in the heart fractions (instead of distilling them in the heads). Related to this point, Claus et al. stated that the distillation in column is preferred to the traditional alembic distillation because it preserves the fruit essences [26].

Finally, it should be noticed that the ethanol concentrations were lower than 37.5º, which is the minimum limit demanded by the European Union Council (Nº 1576/89) for fruit spirits. For this reason, in order to commercialize the beverages, a smaller heart fraction should be collected (to get a higher ethanol content).

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From the results obtained, and based on the compounds analyzed, it could be concluded that the pear spirits obtained from the fermentations at the adjusted pH were of better quality than the ones obtained from the fermentations at the native pH. This statement is based on their higher concentration of total higher alcohols, and lower concentrations of 1-hexanol and ethyl acetate.

The principal component analysis divided the compounds in three different components (PC1, PC2, and PC3). **Table 5.6** shows the compounds present in each principal component, together with their loads and the variance explained. **Figure 5.6** plots these components.

Table 5.6. Principal component analysis results for the volatile compounds in the spirits from *Blanquilla* concentrated pear juice fermented at the native pH and at an adjusted pH of 3.30.

Principal Component	compound	loading	variance explained (%)	total variance (%)
PC1	3-metyl-1-butanol	-0.998	50.74	50.74
	2-methyl-1-propanol	-0.996		
	2-methyl-1-butanol	-0.995		
	ethyl hexanoate	0.927		
	phenethyl alcohol	-0.921		
	1-butanol	0.917		
	methyl acetate	0.731		
	1-hexanol	0.614		
PC2	acetal	0.980	27.38	78.12
	acetaldehyde	0.975		
	ethyl 2-*trans*-4-*cis* decadienoate	0.878		
	ethyl acetate	-0.697		
PC3	methanol	0.939	14.88	93.00
	furfural	0.884		

PC1 and PC2 grouped together the spirits from the pear juices fermented at the native pH. The grouping of the spirits from juices fermented at the adjusted pH

was not so clear. However, it could be observed that the tendency was to separate the spirits from different fermentation pHs in opposite quadrants.

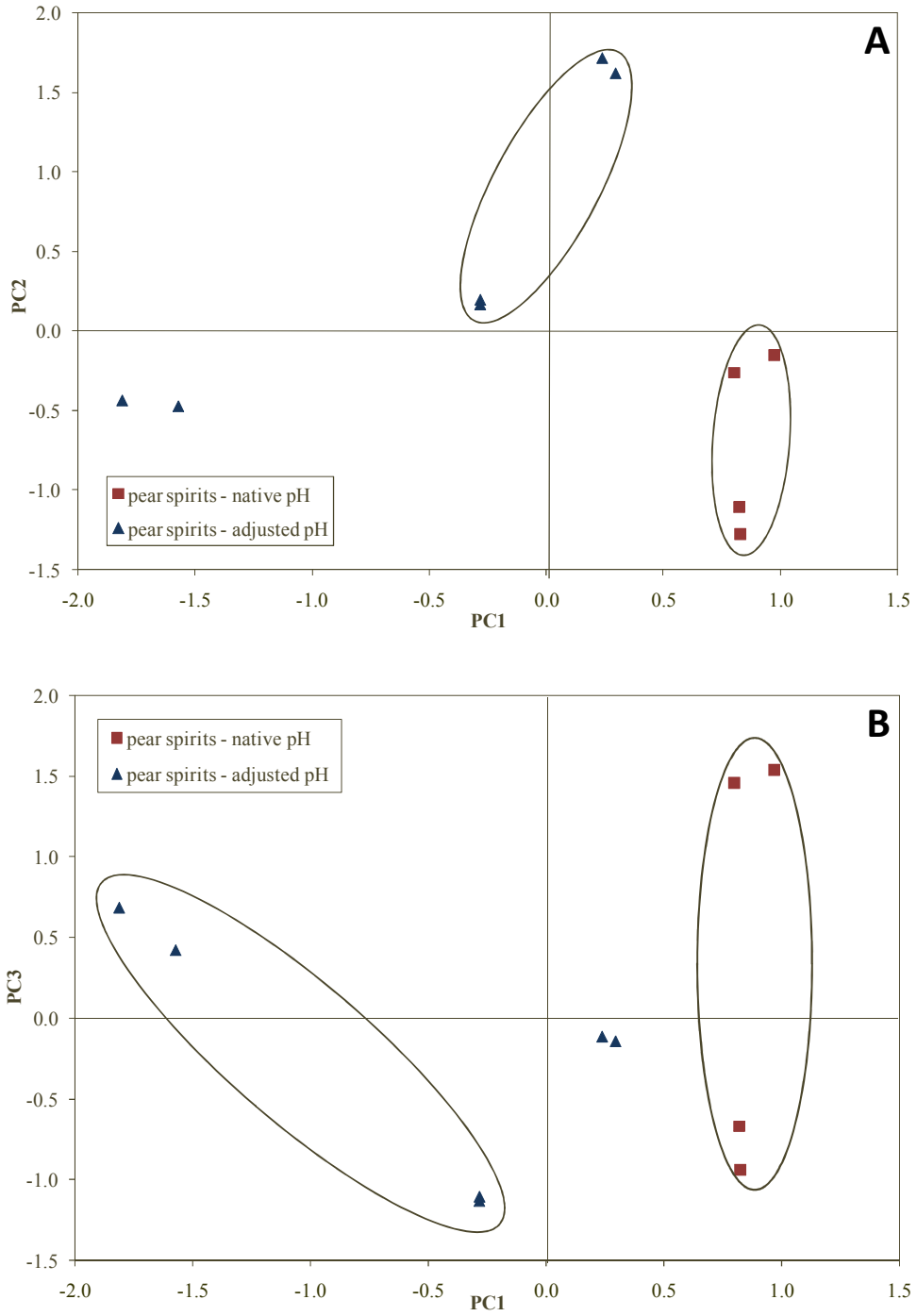

Figure 5.6. Plots of the principal components (PC1, PC2 and PC3) obtained from the principal components analysis of the main volatile compounds present in the spirits from *Blanquilla* pear juice fermented at the native and at an adjusted pH of 3.27. (**A**) PC1 vs PC2; (**B**) PC1 vs PC3.

Therefore, it could be said that spirits from juices at the adjusted pH are differentiated from spirits of juices at the native pH mainly by their concentrations in higher alcohols and esters.

These results are quite in agreement with the ANOVA tests previously performed, which showed a significant difference in all the total higher alcohols concentrations, except for the phenethyl alcohol, and also in the methyl and ethyl acetates.

Comparing these PCA results with the ones obtained in the experimental set 1, some similarities were found. In both experiments the distillates from pear juice concentrate fermented at the native pH could be differentiated from the distillates of pear juice concentrate fermented at the adjusted pH by their higher concentration in ethyl acetate and methyl acetate, and their lower concentration in acetal, phenethyl alcohol and acetaldehyde.

Sensory evaluation

The sensory evaluation was performed in order to corroborate if the chemical differences found by the ANOVA and PCA tests are perceived organoleptically. Samples were diluted to a 20 % (v/v) of ethanol [9]. A lower ethanol degree reduces the saturation of the olfactive cells by ethanol. According to Guichard et al., a taster has six million olfactive cells of which two million are saturated by only one glass of 50 % (v/v) brandy; becoming operational again only after resting for 6 hours [27].

For all the samples tested, no significant differences ($p<0.05$) were found between the spirits fermented at the two different pHs, either for the smell or for the taste. This means that the chemical differences were not as important as to change the sensorial quality of the distillates.

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Chapter 6

INFLUENCE OF RAW MATERIAL

6.1 Introduction

The raw material employed to produce a fruit spirit contribute to its aromatic composition in several different ways. Some compounds (such as ethyl esters of $C_6 – C_{12}$ fatty acids) are present in the fruits, and their characteristic fruity aromas are transferred to the spirits produced from them [1]. In addition, the amount of volatile compounds produced during the fermentation process also depends on the raw material employed (among other factors such as the yeast used and the fermentation conditions) [2].

Arrizon et al. found significant differences in the volatile composition of distilled beverages produced from two different prickly pear varieties [3]. Moreover, Hernández-Gómez et al. also found significant differences in the volatile composition of wines and distillates produced from melon mash, melon juice and melon mash without the fruit skin [4]. One of these differences was attributed to the presence of certain enzymes in the fruit skin, which could contribute to the formation of methanol. In addition, the juices seemed to be a more favorable medium for the development of the native lactic acid bacteria. This generated a higher ethyl lactate concentration in this substrate.

The processing of the fruit to obtain the raw material used for the fermentation could also influence the composition of the spirits. In this sense, Cortés et al. found that the pressing system used to separate the juice from the marc in grapes, strongly influences the methanol concentration present in *orujos* [5].

This chapter is focused on studying the influence of different raw materials from *Blanquilla* pear on the volatile composition of the spirits obtained from them. To this aim, two different experimental sets were performed. In the first one, natural pear juice and pear juice from concentrate were fermented and subsequently

distilled in a 5 L copper alembic. In the second experimental set, natural pear juice and pear juice from concentrate were fermented and subsequently double distilled in a 20 L copper alembic. All the distillates obtained were analyzed by gas chromatography, in order to determine their volatile composition and their ethanol content.

6.2 Materials and Methods

Experimental Set 1

Pear juice preparation

Concentrated juice and natural pear juice from the Blanquilla variety (donated by Nufri, Lleida, Spain) were used. Both were produced on the same day using the same batch of pears to assure comparable results. The concentrated pear juice was obtained by selection and cleaning of the fruit followed by mashing and pressing process. After that, depectinization, clarification, ultrafiltration and concentration to 70°Brix by evaporation were performed. The natural pear juice was obtained after the clarification and it was protected with a dose of 20 mg/L of SO_2. The concentrated pear juice (70°Brix) was diluted with water to 13°Brix so to have a total sugar concentration similar to that of the natural juice. The juices were characterized using high performance liquid chromatography (HPLC) and by measuring the pH, amount of total sugars and density.

Fermentation process

A volume of 3 L of each pear juice was fermented in a 5 L Labfors 3 fermenting reactor (Infors, Switzerland) at 21 ± 3 ºC. The microorganism used was *Saccharomyces cerevisiae* (BDX, ENOFERM, France). The inoculum was prepared in accordance with the instructions provided by the supplier, in a dose of 25 g of yeast/hL of pear juice. When the medium density reached 1040 g/mL, 300 mg/L of di-ammonium hydrogen phosphate (Scharlau) was added as a nitrogen source. Each pear juice was fermented in duplicate.

To monitor the process, samples were collected at different fermentation times. For each one, the temperature was measured and the pH monitored with a Crison

Basic 20 pH meter. Total yeasts were counted using a Neubauer chamber. The density was measured using a Class H Ludwig Schneider densimeter and the total sugar content was determined using a GAB kit for sugar analysis (GAB Sistemática Analítica S.L., Spain). Finally, all the samples were subject to HPLC analysis.

HPLC analysis

HPLC analysis was used to characterize the pear juices and the fermented juices, and also to monitor the fermentation process. The HPLC equipment was an Agilent 1100 Series with HP Chemstation software (Agilent, Waldbron, Germany) for data acquisition. Sugars, glycerol, and ethanol were measured using a Refractive Index Detector (Agilent, Waldbron, Germany). The column was a Transgenomic ICSepICE COREGEL – 87H3, and the oven temperature was 50 ºC. The injection volume was 20 µL. The mobile phase was a solution of pH = 2.20 prepared with concentrated H_2SO_4 (95-97 %) in Milli-Q water. The flow rate was 0.6 mL/min. All the samples and the mobile phase were filtered before the analysis using cellulose acetate filters (Teknokroma) with a pore size of 0.45 µm. Samples were analyzed in duplicate.

Distillation process

The pear distillates were obtained from simple batch distillation of the fermented juices in the presence of its lees in a 3 L copper alembic. The operation conditions were the same for both types of fermented juices: 1 L of the pear fermented juice was distilled at a flow rate of 2 mL/min, using water as the refrigerant and an electric heater as the heat source. The distilled fractions were collected in glass bottles and kept in the freezer until they were analyzed using GC. Based on the literature [6] and on previous results (see **Chapters 2** and **3**), the first fraction (head) was 5 ml, the second fraction (heart) was 295 mL and the third fraction (tail) was 50 mL. The distillations were performed in duplicate.

A second series of distillations was carried out under the same conditions described above, but without the fermentation lees. The fractions collected were also the same. The distillations were performed in duplicate.

GC analysis

Gas chromatography was used to characterize the different samples collected during the distillations. The method used by Cortés et al. for determining volatile compounds in *orujos* was adapted to determine the volatile composition and the methanol and ethanol content in each sample [5]. The equipment used was an Agilent 6890N with a Flame Ionization Detector, an automatic injector and HP Chemstation software (Agilent, Waldbron, Germany) for the data analyses. The column was a Teknokroma TR – MetaWax capillary column (polyethyleneglycol stationary phase; 30 m x 0.25 mm x 0.5 μm). The injection volume was 1μL in split 1:5 mode at an injector temperature of 250 ºC. The carrier gas was helium at a column flow rate of 1.1 mL/min. The oven temperature was programmed at 40 ºC for 6 minutes, then increased to 80 ºC at a rate of 1.5 ºC/min. and from 80 to 200 ºC at 3 ºC/min. The detector temperature was 260 ºC, with a H_2 flow rate of 40 mL/min. and an air flow rate of 350 mL/min. Helium was used as the auxiliary gas, at a flow rate of 25 mL/min.

The internal standard was 4-methyl-2-pentanol (Fluka) for all the compounds except ethanol, for which it was acetonitrile (J.T. Baker) [7]. A solution containing these two standards was prepared and mixed at a ratio of 1/10 for each sample. Each sample was injected in duplicate.

Statistical analysis

One-way analysis of variance (ANOVA) and principal component analysis (PCA) were performed on the volatile compounds present in the heart fraction of the different distillates to determine differences among them. Multiple comparisons of pairs were carried out to isolate the value or values that differed. To this end, the least significant difference (LSD) was used at the 5% significance level. The SPSS statistical package (version 14.0) was used for all the statistical analyses.

Experimental set 2

Pear juice preparation

The natural pear juice and pear juice concentrate from *Blanquilla* variety were produced and donated by Nufri S.A. (Mollerussa, Lleida). They were all obtained from the same production batch, in order to assure comparable results. The

concentrated pear juice was obtained by selection and cleaning of the fruit followed by mashing and pressing. After that, depectinization, clarification, ultrafiltration and concentration to approximately 70°Brix by evaporation were performed. The natural pear juice was obtained by separation after the clarification. The concentrated juice, of 71 ºBrix, was diluted with water until a juice of 12.5º Brix was obtained. This dilution was done when preparing the different fermentation tanks, before the inoculation process. The raw materials were characterized by HPLC and by measuring the total sugars concentration, the ºBrix and the pH.

Fermentation process

The natural pear juice and diluted juice concentrate from *Blanquilla* pear, were separately fermented in 20 L plastic tanks under semi-anaerobic conditions. A volume of 15 L was used for each fermentation. The tanks were inoculated with a dose of 20 g/hL of yeast, according to the instructions provided by the supplier. The yeast used was a commercial strain of *Saccharomyces cerevisiae* (BDX, ENOFERM, France). Fermentations were performed in duplicate. After the fermentations finished (total sugars concentration lower than 4 g/L), the tanks were kept closed at room temperature for some days before distilling (in order to enhance the aromatic profile of the ferments).

To monitor the process, samples were collected at different fermentation times. For each one, the temperature and the pH were measured. Finally, all the samples were subject to HPLC analysis. The final ferments were also subject to GC analysis.

The pH was monitored with a Crison Basic 20 pH meter and the total sugar content was determined using a GAB kit for sugar analysis (GAB Sistemática Analítica S.L., Spain).

Distillation process

The pear distillates were obtained by double batch distillation of the fermented raw materials in presence of their lees, in a 20 L copper alembic. The operation conditions were the same in both cases: 14 L of the fermented raw materials were distilled using water as the refrigerant and an electric heater as the heat source. The distillations were performed in triplicate. Based on previous experiments (see

Chapters 2 and **3**) and literature [6], the fractions collected during the first distillation were: the first 60 mL (corresponding to the head, which was discarded), the following 3.5 L (heart fraction), and finally 600 mL (the tail fraction, which was also discarded). The heart fraction was second distilled using the same equipment. In this case, the distillation fractions collected were: two of 35 mL each, one of 1.75 L, one of 35 mL and the last one of 210 mL. Samples from the heart fraction of the first distillation and the fractions collected from the second distillation were put in glass bottles and kept in the freezer until they were analyzed by GC. Based on these results, the head, heart and tail fractions of the second distillation were defined.

HPLC analysis

HPLC analysis was used to determine the fructose, glucose, sucrose, glycerol and ethanol concentrations in the natural pear juice, the diluted juice concentrate, the pear fermented juices, and the different samples collected during the fermentation. The HPLC equipment was an Agilent 1100 Series with HP Chemstation software (Agilent, Waldbron, Germany) for data acquisition. Sugars, glycerol, and ethanol were measured using a Refractive Index Detector (Agilent, Waldbron, Germany). The column was a Transgenomic ICSepICE COREGEL – 87H3, at an oven temperature of 50 ºC. The injection volume was 20 µL. The mobile phase was a solution of pH = 2.20 prepared with concentrated H_2SO_4 (95-97 %) in Milli-Q water. The flow rate was 0.6 mL/min. All the samples and the mobile phase were filtered before the analysis using cellulose acetate filters (Teknokroma) with a pore size of 0.45 µm. Samples were analyzed in duplicate.

GC analysis

The method used by Cortés et al. for determining volatile compounds in *orujos* was adapted to determine the volatile composition, and the methanol and ethanol content in the samples (distillation fractions and fermented juices) [5]. The equipment used was an Agilent 6890N with a Flame Ionization Detector, automatic injector and HP Chemstation software (Agilent, Waldbron, Germany) for the data analyses. The column was a Teknokroma TR – MetaWax capillary column (polyethyleneglycol stationary phase; 30 m × 0.25 mm × 0.5 µm). The injection volume was 1µL in split 1:5 mode at an injector temperature of 250 ºC. The carrier gas was helium at a column flow rate of 1.1 mL/min. The oven

temperature was programmed at 40 ºC for 6 minutes, then increased to 80 ºC at a rate of 1.5 ºC/min. and from 80 to 200 ºC at 3 ºC/min. The detector temperature was 260 ºC, with a H_2 flow rate of 40 mL/min. and an air flow rate of 350 mL/min. Helium was used as the auxiliary gas, at a flow rate of 25 mL/min.

The internal standard was 4-methyl-2-pentanol (Fluka) for all the compounds except ethanol, for which it was acetonitrile (J.T. Baker) [7]. A solution containing these two standards in a concentration of 1.0 g/L and 100 g/L respectively was prepared and mixed at a ratio of 1/10 for each sample. Each sample was injected by duplicate.

Statistical analysis

One-way analysis of variance (ANOVA) was applied to ascertain if there are significant differences (at 5% level) between the two different *Blanquilla* pear fermented raw materials. The same analysis was applied to check for significant differences between the hearts of the first distillation process, and also between the pear spirits obtained.

Principal components analysis (PCA) was performed to the pear spirits, to determine the degree of differentiation caused by the variation of the raw material used.

All the statistical analyses were performed by means of SPSS statistical package (version 15.0).

Sensory evaluation

The heart fractions of the second distillation (pear spirits) were diluted with demineralized water to an ethanol content of 20% (v/v), according to the guidelines proposed by Frances Jack for the preparation of sensory samples of whisky [8]. Then, they were tested for their flavour quality using order-of-precedence tests. Sensory evaluation was conducted with a panel of 24 consumers, whom were asked to evaluate separately the smell and the taste of the spirits. The results were analyzed using the Friedmann statistic test [9].

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6.3 Results and discussion

Experimental Set 1

Fermentation process

The pear juice obtained from the concentrate had a total sugar content of 79.5 g/L, a pH of 4.3, and a density of 1.052 g/mL. The natural pear juice had a total sugar content of 95.9 g/L, a pH of 4.4, and a density of 1.052 g/mL. **Figure 6.1** shows the total yeast growth, the total sugar consumption and the different concentrations of sugars, ethanol and glycerol (determined by HPLC) for the fermentation process of each pear juice. The results shown are for one fermentation of each juice, the duplicate behaved in a similar way in both cases (see Annex V). Fructose is the main sugar (45.8 g/L in the pear juice from concentrate and 71.6 g/L in the natural pear juice), followed by glucose (22.3 g/L and 21.7 g/L respectively) and sucrose (8.5 g/L and 6.5 g/L respectively). These data are quite in agreement with the results of total sugars found using the GAB kit (79.5 g/L and 95.9 g/L respectively).

The lag phase was longer for the yeasts in the natural juice, showing even a decrease in the yeasts' concentration during the second and third day of fermentation. The juice from concentrate had a very short lag phase (not seen in the figure), so the exponential phase started earlier and was longer than for the natural juice. One reason for this difference may be the addition by the manufacturer of SO_2 as a way to preserve the natural juice. SO_2 inhibits the growth of microorganisms so it probably interfered with the adaptation of yeasts to the medium, and prevented them from growing during the first hours of contact. The concentrated juice has a high sugar content which inhibits microorganisms growth. Therefore, SO_2 is not added during the production process.

For both juices, most of the sugar consumption and the ethanol and glycerol formation occurred during the exponential growth phase. Consequently, the sugars are more rapidly consumed and the ethanol and glycerol more rapidly formed in the pear juice from concentrate. For both juices, the stationary phase was reached after 95 hours of fermentation. During this period some remaining sugars are still consumed, especially the fructose in the natural pear juice.

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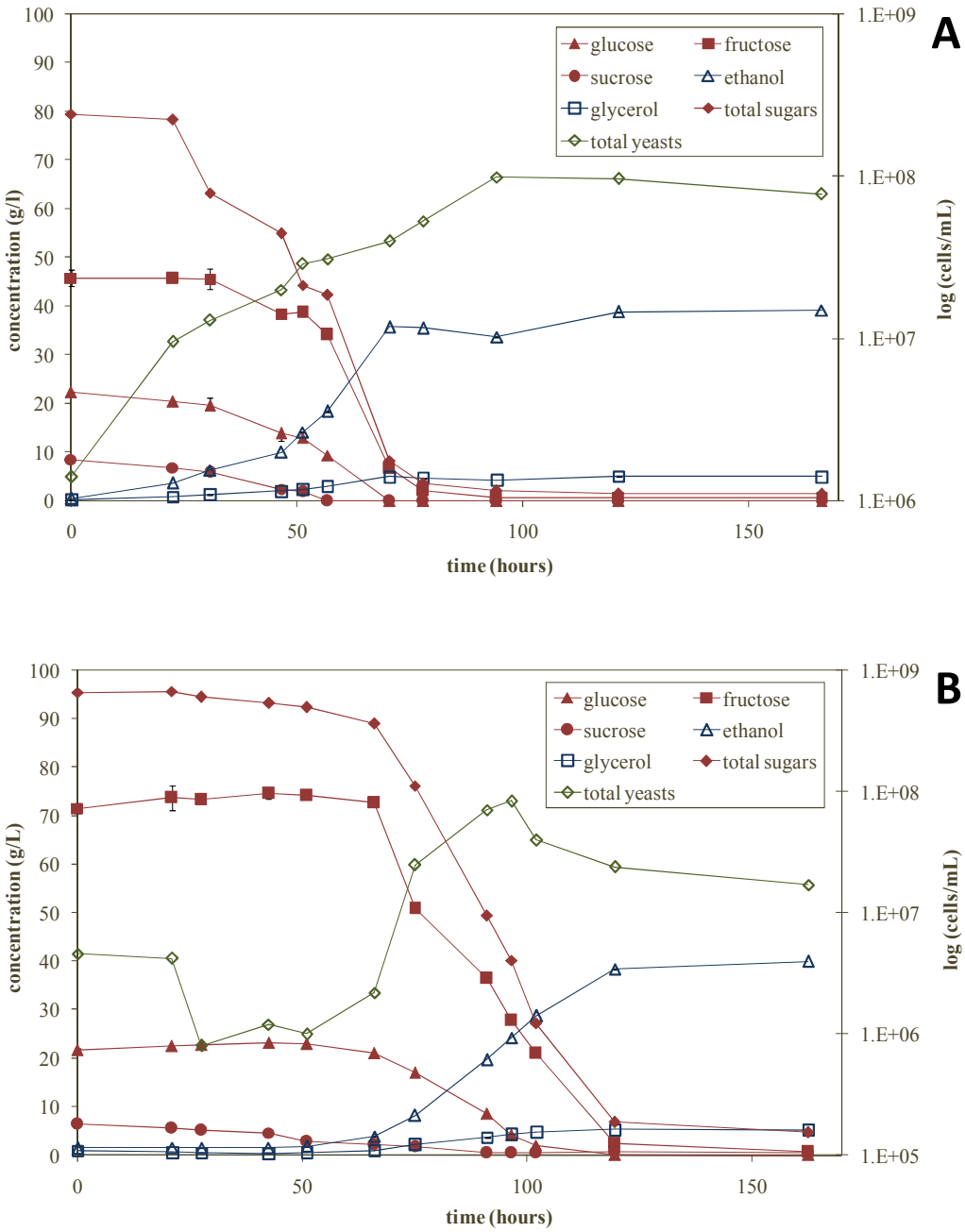

Figure 6.1. Total yeasts, total sugars, glucose, fructose, sucrose, ethanol and glycerol profiles during the fermentation of pear juice. (**A**) concentrated pear juice; (**B**) natural pear juice.

By the end of the fermentation process, the total amount of sugars was 1.5 g/L for both wines from concentrated pear juice and 4.5 g/L for the ones from natural pear juice. The final ethanol and glycerol concentrations were about 40 g/L and 5 g/L respectively for both the fermented juices. The density was 1.008 g/mL for the ferments from concentrated pear juice and 1.011 g/mL for those from the natural

pear juice. The pH also decreased in all cases, reaching a final value of 3.91 and 3.96 for the fermented juices from concentrated pear juice, and 3.51 and 3.43 for fermented juices from natural pear juice.

Both fermentations of the juice from pear concentrate showed the same behavior throughout the process, so the pear fermented juices obtained from them were put together and this mixture was used to perform the distillations. The same procedure was applied to the fermented juices obtained by fermenting the natural pear juice.

Distillation process

The volatile composition of the head fraction (first 5 mL), the heart fraction (following 295 mL) and the tail fraction (the next 50 mL) is determined by GC. **Table 6.1** shows these results for the four types of distillate produced (each result is the mean of two distillations and two GC injections). The ethanol concentration in the head fraction is higher when the distillation is performed with lees. However, in the heart and tail fractions there is no significant difference in the ethanol concentration among the different distillations.

The total concentration of higher alcohols (1-propanol, 2-methyl-1-propanol, 1-butanol, 2-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and 1-hexanol) decreases during the distillation process. This behavior is expected because they have a relatively low boiling point, are soluble in alcohol and partially soluble in water, so they distill at the beginning and in the middle fraction of the distillate [7].

The behavior of the esters was similar to that of the higher alcohols. They can be divided into two groups. On the one hand, there are methyl acetate, ethyl acetate, and ethyl lactate, which are associated with poor quality raw matter or fermentation problems, and which negatively influence the sensory quality of spirits when present in high amounts [5,10,11,12]. On the other hand, there are ethyl hexanoate, ethyl decanoate, and ethyl-2-*trans*-4-*cis*-decadienoate, which are typical of the fruit and impart a pleasant fruity character to the spirits [5,11]. All of them, except for the ethyl lactate, are expected to distill from the beginning towards the middle of the distillation process [7]. This was the behavior observed in all the experiments performed (see Annex V).

Table 6.1. Mean concentrations and standard deviations of ethanol (g/L), methanol (mg/L), and the main volatile compounds (mg/L) in the different fractions of the distillates

sample	compound	head	heart	tail
concentrate with lees	ethanol	676.6 ± 227.3	111.4 ± 19.7	4.2 ± 3.4
	methanol	14.8 ± 2.1	36.9 ± 7.6	28.0 ± 18.1
	acetaldehyde	740.1 ± 31.4	48.3 ± 4.7	0.0 ± 0.0
	furfural	0.0 ± 0.0	4.5 ± 0.4	8.6 ± 0.5
	total higher alcohols	4048.4 ± 106.2	394.6 ± 10.9	0.8 ± 0.5
	total esters	1198.6 ± 170.7	21.3 ± 4.9	0.0 ± 0.0
concentrate without lees	ethanol	365.2 ± 89.6	124.9 ± 17.7	6.8 ± 4.9
	methanol	14.8 ± 2.5	38.4 ± 5.2	21.9 ± 11.4
	acetaldehyde	1051.5 ± 307.0	59.4 ± 11.1	2.4 ± 0.2
	furfural	0.6 ± 0.1	3.9 ± 0.5	7.4 ± 1.0
	total higher alcohols	4141.6 ± 607.0	377.1 ± 18.9	0.1 ± 0.0
	total esters	1073.0 ± 273.8	26.3 ± 2.0	0.0 0.0
natural juice with lees	ethanol	776.6 ± 203.2	121.1 ± 9.1	2.2 ± 0.6
	methanol	2397.1 ± 154.4	1258.5 ± 125.5	59.2 ± 32.1
	acetaldehyde	654.6 ± 97.0		9.7 ± 3.3
	furfural	1.4 ± 0.2	584.7 ± 166.4	16.0 ± 3.0
	total higher alcohols	2987.9 ± 62.4	8.8 ± 2.5 353.8 ± 8.6	2.3 ± 0.3
	total esters	798.2 ± 45.9	23.1 ± 2.8	9.1 ± 2.3
natural juice without lees	ethanol	411.8 ± 46.6	107.8 ± 4.6	8.8 ± 8.6
	methanol	2267.9 ± 50.1	1543.0 ± 163.9 854.8 ± 79.6	63.1 ± 29.3
	acetaldehyde	718.8 ± 213.5		16.6 ± 8.7
	furfural	1.1 ± 0.2		14.8 ± 2.5
	total higher alcohols	3445.2 ± 371.2	11.3 ± 1.1 383.5 ± 16.0	0.0 ± 0.0
	total esters	562.7 ± 309.7	55.5 ± 35.8	15.6 ± 9.0

concentrate = distillate produced from concentrated pear juice; natural juice = distillate produced from natural pear juice; with lees = distilled with the lees; without lees = distilled without the lees.

Table 6.1 also shows the concentration of three of the most negative compounds present in a distillate: methanol, acetaldehyde and furfural. The methanol concentration is much higher in the distillations of pear fermented juices from natural juice (distilled either with or without its lees). This is probably because methanol produced during fermentation is derived from the degradation of pectic substances, which are removed from the concentrated pear juice as part of its production process [8, 13,14]. Therefore, a much higher methanol concentration is

expected in the fermented juices and distillates from natural pear juice than in the ones from concentrate.

Acetaldehyde is formed from the fermented raw materials [12,15]. It can provide the beverage with a fruity character when present in low concentrations, but when present in higher concentrations it provides a sharp smell and taste [16]. Acetaldehyde removal is the main reason for separating the head fraction of a distillation because it has a low boiling point (21ºC) and is soluble in ethanol, so it is expected to distill mainly at the beginning of the process [7]. In the distillates from concentrated pear juice the acetaldehyde removal in the head fraction was almost complete. In the distillates from natural juice, the acetaldehyde amount in the head fractions did not differ much from that in the distillates from concentrated juice. However, the heart fractions had a much higher amount of this compound, indicating that the wine from natural pear juice had a higher concentration of acetaldehyde than that made from concentrated juice. This can be explained by the higher sugars content in the natural pear juice compared to the juice from concentrate; a high sugar content in the juices promotes acetaldehyde formation during the fermentation process [17]. The presence of SO_2 during the fermentation also induces acetaldehyde formation, so another possible cause for its high concentration in fermented juices from natural pear juice could be the addition by the manufacturer of SO_2 to the natural juice as a way to preserve it [10,17]. Furfural behaves in quite the opposite way during the distillation, which is in keeping with the fact that it has a high boiling point (167 ºC) and is also very soluble in water, meaning that its concentration is expected to increase from the middle of the heart to the tails [7]. Its presence in beverages is not desired since it is a toxic compound (reference dose: 3 µg/kg bw/day). Most of it is successfully removed in the tail fraction (see **Table 6.1**), but its concentration is much higher in the distillates from natural pear juice. This may be due to the higher concentration of residual sugars in the wines from natural pear juice compared to the ones made from concentrate. The remaining fermentable sugars (pentoses) can be degraded by heating in acid conditions (i.e. during the distillation process) and/or the Maillard reaction generating furfural [16].

Table 6.2 shows the concentrations of the main volatile compounds in the heart fraction of the distillates, and the results of the ANOVA tests.

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Table 6.2. Mean concentrations (g/hl a.a.) and standard deviations of the main volatile compounds in the heart fraction of the different distillates

compound	conc. w/ lees	conc. w/o lees	natural w/lees	natural w/o lees
ethanol (%v/v)	14.1 ± 2.5 [a]	15.8 ± 2.2 [a]	15.3 ± 1.2 [a]	13.7 ± 0.6 [a]
acetaldehyde	34.3 ± 3.3 [a]	37.6 ± 7.0 [a]	382.2 ± 108.8 [b]	623.9 ± 58.1 [c]
acetal	0.7 ± 0.2 [a]	1.0 ± 0.2 [a]	1.8 ± 0.1 [b]	1.8 ± 0.5 [b]
furfural	3.2 ± 0.3 [a]	2.5 ± 0.3 [a]	5.7 ± 1.6 [b]	8.3 ± 0.8 [c]
methanol	26.2 ± 5.4 [a]	24.3 ± 3.3 [a]	822.5 ± 82.0 [b]	1126.3 ± 119.6 [c]
1-propanol	66.4 ± 2.5 [a]	57.8 ± 3.2 [a]	65.2 ± 1.1 [a]	84.1 ± 3.7 [b]
2-methyl-1-propanol	85.1 ± 2.8 [a]	71.8 ± 1.8 [a]	71.1 ± 1.8 [a]	82.7 ± 1.9 [a]
1-butanol	0.0 ± 0.0 [a]	0.0 ± 0.0 [a]	1.4 ± 0.1 [b]	1.6 ± 0.3 [b]
2-methyl-1-butanol	24.4 ± 0.8 [a]	20.4 ± 0.9 [a,b]	19.8 ± 0.8 [b]	24.2 ± 1.2 [a,b]
3-methyl-1-butanol	102.5 ± 1.5 [a]	87.2 ± 5.8 [a,c]	67.9 ± 1.1 [b]	82.3 ± 4.2 [b,c]
1-hexanol	1.5 ± 0.1 [a]	1.4 ± 0.2 [a]	5.8 ± 0.7 [b]	5.1 ± 0.4 [b]
total higher alcohols	**279.9 ± 7.7**	**238.7 ± 12.0**	**231.2 ± 5.6**	**279.9 ± 11.7**
ethyl lactate	0.0 ± 0.0 [a]	0.0 ± 0.0 [a]	2.3 ± 0.8 [b]	5.3 ± 2.0 [c]
methyl acetate	0.0 ± 0.0 [a]	0.0 ± 0.0 [a]	1.0 ± 0.1 [a]	25.4 ± 21.9 [b]
ethyl acetate	14.8 ± 3.1 [a]	16.6 ± 1.3 [a]	8.5 ± 0.1 [b]	5.8 ± 1.8 [b]
ethyl decanoate	0.3 ± 0.3 [a]	0.0 ± 0.0 [a]	1.4 ± 0.3 [b]	0.7 ± 0.1 [c]
ethyl-2-trans-4-cis-decadienoate	0.0 ± 0.0 [a]	0.0 ± 0.0 [a]	1.9 ± 0.4 [a]	3.3 ± 0.4 [b]
total esters	**15.1 ± 3.5**	**16.6 ± 1.3**	**15.1 ± 1.8**	**40.5 ± 26.1**
phenethyl alcohol	6.0 ± 0.4 [a]	6.3 ± 0.8 [a]	7.6 ± 1.8 [a]	12.4 ± 1.1 [b]

Values with the same letter within the same row, indicate not significant difference ($p \leq 0.05$) among them. Conc = distillate produced from concentrated pear juice; natural = distillate produced from natural pear juice; w/lees = distilled with the lees; w/o lees = distilled without the lees.

As has been already observed in **Table 6.1**, the concentrations of acetaldehyde, methanol and furfural were significantly higher in the distillates from natural pear juice compared to the ones from concentrate. In addition, the distillates from natural pear juice distilled without the lees had significantly higher concentrations of these compounds compared to those from the same juice but distilled with the lees. The concentrations of the higher alcohols did not differ much from one distillate to the other though in some cases there are significant differences. These differences are mainly found in the distillates made from concentrated pear juice and those made from natural pear juice. The higher alcohols in the distillates from

the same type of juice distilled with and without lees showed no significant differences between them, except for 1-propanol in the natural pear juice distillates. Higher alcohols are formed during the fermentation process and make an important contribution to the aroma profile of distillates [10]. For this reason, amounts which are too low (less than 140 g/hL a.a. according to the European Union legislation) or too high (more than 600 g/hL according to the 'Regulating Council for the Specific Denomination of Galician Orujo') have a negative effect on the distillate flavor [5,10]. For our experiments, the total amount of higher alcohols in the heart fraction remained between 230 and 280 g/hL a.a. in all cases.

Table 6.2 also shows that all of the esters, except for ethyl acetate, were present in higher concentrations in the distillates from natural pear juice. In addition, the esters in the distillates from concentrated pear juice showed no significant differences between the distillation with and without lees. On the contrary, the distillates from natural pear juice show a higher ester concentration when distilled in the absence of lees, except for the ethyl acetate (which shows no significant difference) and the ethyl decanoate (the concentration of which decreases when the distillation takes place in the absence of lees). These results suggest that the effect of the lees depends on the raw matter used. Similar results were obtained by Bueno et al. studying the effect of a short contact time with lees on the volatile composition of Airen and Macabeo wines [18].

It should also be noticed that the esters concentrations in all the heart fractions were quite low, the total amount of esters being between 15 and 17 g/hL a.a. in all cases except for in the ferments made from natural pear juice distilled without its lees. In this case, the total concentration of esters was 40.5 g/hL a.a. mainly because of a higher concentration of methyl acetate. The methyl acetate is indicative of aerobiosis in the raw material during the fermentation process or the result of an incorrect separation of the first fraction (head) during the distillation process [5]. In the present experiments, the methyl acetate concentration was very high in the head and heart of one of the distillates from natural pear juice without lees, remaining very low in the other one (this is the reason why the standard deviation of this value is very high). Therefore, a possible aerobiosis during the fermentation process should be suspected.

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In the present experiments no significant difference was observed between the distillations of pear juice concentrate performed with and without lees, while in **Chapter 2** some significant difference was observed for various compounds when distilling under the same conditions. However, these differences were quite small (in spite of being significant). Therefore, the fact of using a pear concentrate that comes from a different production batch could make the difference. In addition, when the results of **Chapter 2** are compared with the ones obtained for the distillates from natural pear juice distilled with and without the fermentation lees in the present experiments, a similar behavior was observed. Consequently, the tendency observed for the distillations with and without the fermentation lees was similar in both sets of experiments.

Table 6.3 shows the main results of the PCA analysis applied to the heart fraction of the distillates. The first two principal components (PC1 and PC2) are plotted in **Figure 6.2**, and they explain 87.5% of the variance.

Table 6.3. Principal Component Analysis (PCA) results for the main volatile compounds in the heart fraction of the different distillates

Principal Component	compound	loading	variance explained (%)	total variance (%)
PC1	methanol	0.986	61.93	61.93
	acetaldehyde	0.985		
	furfural	0.964		
	1-butanol	0.961		
	ethyl lactate	0.918		
	etil-2-*trans*-4-*cis*-decadienoate	0.915		
	ethyl acetate	-0.906		
	1-hexanol	0.890		
	phenethyl alcohol	0.866		
	acetal	0.757		
	methyl acetate	0.654		
	ethyl decanoate	0.636		
PC2	2-methyl-1-butanol	0.963	25.56	87.50
	2-methyl-1-propanol	0.939		
	3-methyl-1-butanol	0.906		
	1-propanol	0.692		

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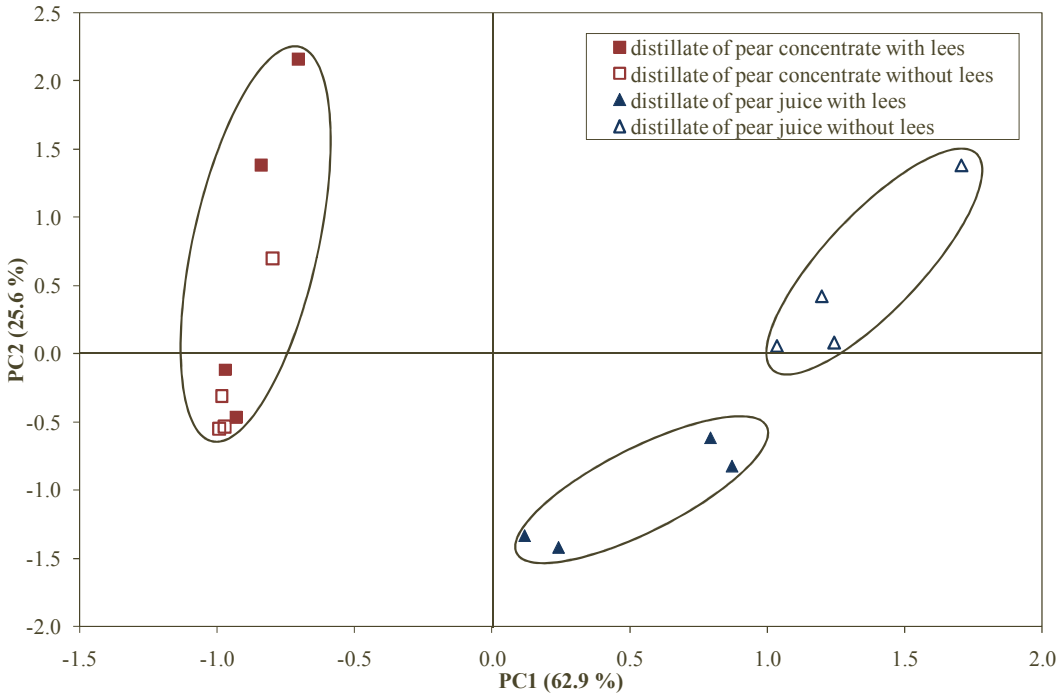

Figure 6.2. Plot of the principal components 1 and 2 (PC1, PC2) for the main volatile compounds in the heart fraction of the different distillates.

From **Figure 6.2**, we can clearly differentiate between distillates from concentrated pear juice, distillates from natural pear juice distilled with lees, and distillates from natural pear juice distilled without lees. PC1 mainly differentiates the natural pear juice distillates from the concentrated pear juice distillates, while PC2 mainly differentiates the distillates from natural pear juice distilled with lees from those distilled without lees. However, PC1 also has a small contribution to this differentiation. These results agree with those obtained from the ANOVA test, confirming that the distillates from concentrated pear juice had no significant difference (for the compounds analyzed) when distilling with or without the lees. At the same time, these distillates can be differentiated from the ones made from natural pear juice because for most of the compounds analyzed there is a significant difference in their concentrations. In most cases, this difference is even larger for the distillates from natural pear juice distilled without the lees.

From these analyses, it can be concluded that the distillates from natural pear juice have a higher amount of volatile compounds. However, this is not necessarily positive. As has been previously shown, some of these compounds have a negative effect on the distillates' quality and some others can affect it positively

or negatively depending on the concentration present in the beverage. In our experiments, most of the compounds whose concentration was higher in the distillates from natural pear juices are negative (furfural, methanol) or become negative in high concentrations (acetaldehyde, ethyl lactate, methyl acetate). In addition, the distillation of the fermented natural pear juice in the absence of lees significantly increased the concentration of some of these compounds compared to the distillation in the presence of lees.

Distilled beverages are a very complex matrix, so quality cannot be accurately predicted by analytical analyses alone. Sensory tests are necessary to complement the results obtained and determine the best quality drink. However, taking all the logistic and economic benefits into account, our results suggest that using concentrated juice from *Blanquilla* pears as the raw material for obtaining distilled beverages may be a viable option.

Experimental Set 2

Fermentation process

The concentrated pear juice had 71.1ºBrix and it was diluted with water to a juice of 12.5ºBrix. The total sugars concentration of this juice was 103.6 g/L, and the pH was 4.10. The natural pear juice had 12.5ºBrix, a total sugars concentration of 83.3 g/L and a pH of 3.96.

The fermentation temperature was between 24 – 29 ºC in all cases. The pH of the fermentations from pear juice concentrate diminished, reaching a value of 3.90 by the end of the process. The fermentations from natural pear juice also showed a slight decrease, reaching a value of 3.87. The total sugars concentration diminished during the process until values of 3.3 g/L and 3.6 g/L for the ferments from concentrated pear juice and natural pear juice respectively.

Twelve days after the fermentation started, most of the tanks presented a superficial microorganisms growth, so they were put in a cool room (4 ºC) until the distillations were performed.

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Figure 6.3 shows the profiles of the sugars, ethanol and glycerol concentrations during the fermentation of the different raw materials used (each result is the mean of two fermentations and two HPLC injections for each).

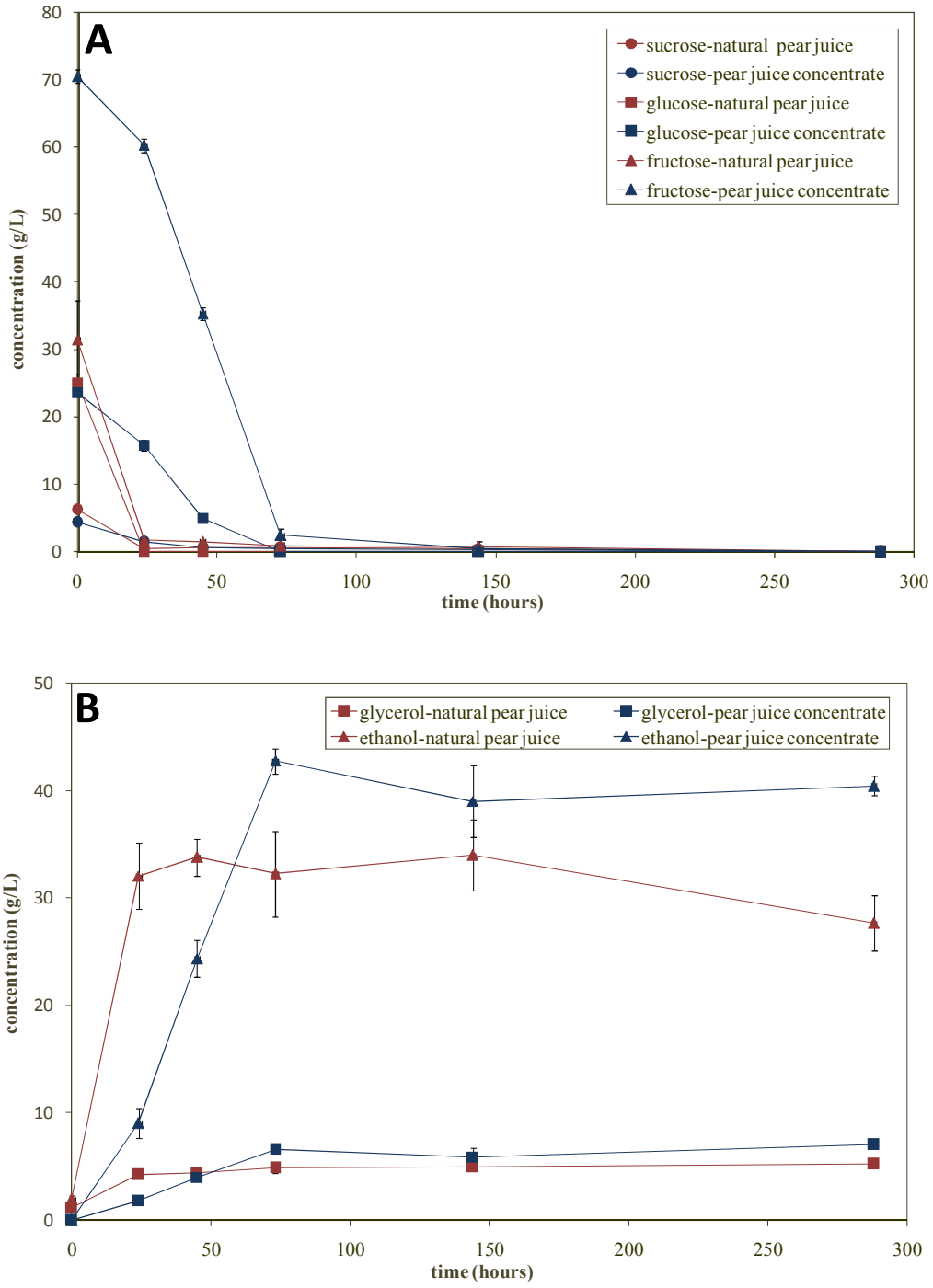

Figure 6.3. Profiles of different main compounds during the fermentation of *Blanquilla* pear natural juice and juice concentrate. (**A**) sucrose, glucose, fructose; (**B**) glycerol, ethanol.

The natural pear juice had the lowest fructose concentration, and a slightly higher glucose and sucrose concentrations (see **Figure 6.3-A**). This fact indicates that the ratio between the main sugars vary depending on the raw material employed. The types of carbohydrate sources in the media have a direct influence on the esters and higher alcohols production during fermentation [19]. Therefore, this fact could be responsible for differences in the aromatic composition between both substrates.

The sugars were almost completely consumed after four days of fermentation in both cases. This confirms that the long lag phase observed for the natural pear juice in the experimental set 1 was probably because of the addition of SO_2.

Figure 6.3-B shows that the ethanol concentration slightly decreased during the last hours of fermentation, especially for the natural pear juice. This was probably due to the microorganisms growth observed on the substrates surface, which could have transformed part of the ethanol present. The microorganisms growth could have been linked to the relatively high temperature at which the fermentations were performed. High temperature and pH during the fermentation process favors a bacterial development (i.e.: acetic and lactic bacteria) [20].

The results of the gas chromatography analyses of the fermented juices obtained are shown in **Table 6.4**. In first place, it was noticed that the concentrations of ethyl acetate and acetaldehyde were high compared to previous experiments (see **Chapter 3**). Ethyl acetate is formed by the reaction of acetic acid with ethanol, and it is usually associated to acetic bacterial spoilage [16,20]. Consequently, a high concentration of this compound could be due to the growing of this microorganism toward the end of the fermentation process.

To confirm this, the acetic acid concentration in the initial and the final fermentation substrates was measured using the same HPLC method and equipment described for the sugars, ethanol and glycerol quantification. The results obtained are shown in **Table 6.5**. As it can be seen, the acetic acid concentration dramatically increased during the fermentation, supporting the theory of acetic bacteria contamination. This results negative for the fermented juices and spirits obtained from them, as ethyl acetate provides a fingernail polish remover and acidic character [16,21]. The high concentration of acetaldehyde

could also be linked to the presence of acetic acid bacteria, since these microorganisms are producers of this compound [22].

Table 6.4. Mean concentrations and standard deviations (g/hL a.a.) of the main volatile compounds present in the *Blanquilla* pear fermented juice concentrate and natural juice.

compound	pear concentrate	pear natural juice
acetaldehyde	22.6 ± 13.5[a]	22.4 ± 11.3[a]
furfural	9.0 ± 4.2[a]	3.5 ± 1.8[a]
methanol	7.0 ± 2.3[a]	733.1 ± 243.2[b]
ethanol (% v/v)	5.3 ± 0.3[a]	3.6 ± 0.3[b]
1-propanol	40.8 ± 3.8[a]	197.6 ± 65.2[b]
2-methyl-1-propanol	80.9 ± 4.2[a]	33.2 ± 8.3[b]
1-butanol	3.2 ± 0.8[a]	6.3 ± 2.8[a]
2-butanol	2.8 ± 0.8[a]	192.0 ± 16.1[b]
2-methyl-1-butanol	23.7 ± 1.6[a]	12.0 ± 2.2[b]
3-methyl-1-butanol	98.7 ± 1.9[a]	65.8 ± 12.4[b]
1-hexanol	183.4 ± 75.9[a]	360.2 ± 98.3[b]
phenethyl alcohol	43.3 ± 11.1[a]	49.9 ± 27.3[a]
total higher alcohols	**476.8 ± 100.1**	**834.6 ± 211.7**
methyl acetate	49.0 ± 14.4[a]	18.8 ± 21.7[a]
ethyl acetate	791.5 ± 69.2[a]	285.8 ± 85.2[b]
ethyl lactate	31.1 ± 8.7[a]	52.9 ± 4.3[b]
ethyl-2-*trans*-4-*cis*-decadienoate	21.9 ± 10.8[a]	130.3 ± 26.2[b]
total esters	**921.5 ± 118.3**	**434.9 ± 133.1**

Values with the same letter within the same row indicate not significant difference ($p \leq 0.05$) among them. Pear concentrate: fermented pear juice concentrate; natural pear juice= fermented natural pear juice.

Table 6.5. Acetic acid concentration (g/L) in the raw materials and final ferments for the fermentation of *Blanquilla* pear juice concentrate and natural juice.

time (hours)	pear juice concentrate	natural pear juice
0	1.0 ± 0.1	1.9 ± 0.5
288	5.8 ± 0.2	4.3 ± 0.8

In addition, several compounds showed significant differences in their concentrations in the two different pear substrates used. Among these compounds, methanol is one of the most remarkable. There is a significant difference in its concentration in the two different substrates used, being the highest the one in the ferment from natural pear juice. Methanol is a highly toxic compound, which can

even produce death when it is present in high concentrations [5]. It is produced by enzymatic degradation of pectic substances present mainly in the skin of the fruits [10]. However, the pressing of the raw material also influences the methanol content by releasing a higher amount of pectolitic enzymes [5,20]. Based on the amount of methanol present in the natural juices, it could be concluded that it was subject to a strong pressing process. As it was previously explained, the concentrated juice is subject to ultrafiltration after pressing and clarification, so the pectolitic substances present are removed. This explains the low concentration of methanol in the ferments produced from this substrate.

The 1-butanol and 2-butanol are associated to bacterial contamination [20]. In spite of being present in all the pear ferments, their concentrations were quite low except for the 2-butanol in the ferments from natural pear juice. The 1-propanol showed a similar behavior to 2-butanol, being its concentration much higher in the ferments from natural pear juice. High concentrations of 1-propanol are also linked to microbial spoilage [16]. The concentration of 1-hexanol was slightly higher in the ferments from natural pear juice, though this difference was not significant. This alcohol comes from the raw material, so its formation is neither linked to fermentation nor to microbial contamination [21]. In kiwi wines its concentration ranges from 0.18 mg/L to 1.63 mg/L, and in grape wines it is usually between 2 mg/L and 3 mg/L [23]. These concentrations are much lower than the ones found in the pear ferments produced in the present experiments, as it can be concluded from **Table 6.4**. In relation to the total higher alcohols, it can be said that they are positive for wines in quantities not higher than 500 – 600 mg/L [22]. According to this, the pear wines obtained from natural juice presented concentrations that are a bit too high.

As it was mentioned before for ethyl acetate, ethyl lactate is also linked to bacterial alteration of wine components such as ethanol and sugars (usually from lactic bacteria) [23]. When present in high concentrations, they both add negative characteristics to the spirits and wines [21,24]. In the pear wines obtained, ethyl lactate was detected, suggesting the presence of lactic bacteria in the medium. However, its concentration was not very high in any case (16.4 mg/L and 19.1 mg/L for the juice concentrate and natural juice respectively), remaining much below the values found for wines which underwent malolactic fermentation (65-

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130 mg/L) [25]. Moreover, its concentration is far below the aroma threshold determined for (S)-ethyl lactate in wines (110 mg/L). The ethyl 2-*trans*-4-*cis*-decadienoate, a typical pear aroma ester, showed a significantly lower concentration in the ferments from pear concentrate [1]. This behavior was expected because it is a very volatile compound, meaning that part of it could have been evaporated during the evaporation process that takes place in the production of pear concentrate.

Distillation process

The fermented raw materials obtained were double distilled in a copper alembic in order to obtain the pear spirits. The results from the GC analyses for the first distillation heart fractions are shown in **Table 6.6**. The concentrations of ethyl acetate were lower than in the fermented juices (especially for the pear juice concentrate). This is because a large part of it was separated in the head fractions (see **Annex V).** Methyl acetate and ethyl-2-trans-4-cis-decadienoate, the same as ethyl acetate, have low boiling points and are highly soluble in ethanol [6]. Consequently, a part of them also distilled in the head fractions (see **Annex V**). On the contrary, phenethyl alcohol has a high boiling point and is soluble in water [7]. Therefore, most of it distilled from the middle of the heart to the tail (see **Annex V**).

The methanol concentration increased in the heart fractions respect to the concentration in the corresponding fermented raw material. The same behavior was observed in previous experiments (see **Chapters 2, 3** and **5**). This is probably because it forms azeotropes, distilling all through the distillation process [26]. Considering this fact and also that the total distilled volume is much lower than the initial volume of fermented juices to be distilled, there is a concentration of methanol in all the distillation fractions. The concentration in the hearts from natural pear juice was particularly high, being close to the limit fixed by the European Council Regulation (Nº 1576/89) of 1000 g/hL a.a. This fact was also observed in the experiments from the experimental set 1. The concentrations of 1-butanol, 2-butanol and 1-hexanol slightly diminished compared to their respective concentrations in the fermented raw materials. However, the concentration of the two last ones is quite high if compared to the results found in the experimental set

1, especially for the natural pear juice. This fact, together with the higher concentration of 1-propanol observed in these distillates, could be linked to microbial spoilage of the raw material before distillation [4,16].

Table 6.6. Mean concentrations and standard deviations (g/hL a.a.) of the main volatile compounds present in the hearts of the first distillation of *Blanquilla* pear ferments from juice concentrate and natural juice.

compound	pear concentrate	pear natural juice
acetaldehyde	10.2 ± 4.9[a]	6.1 ± 0.7[a]
acetal	0.3 ± 0.3[a]	0.0 ± 0.0[a]
furfural	6.6 ± 2.8[a]	4.6 ± 1.5[a]
methanol	41.9 ± 19.9[a]	1045.3 ± 15.5[b]
ethanol (% v/v)	18.3 ± 0.1[a]	13.5 ± 0.1[b]
1-propanol	41.7 ± 3.8[a]	277.1 ± 138.9[b]
2-methyl-1-propanol	84.6 ± 0.7[a]	42.1 ± 3.2[b]
1-butanol	1.7 ± 0.2[a]	3.8 ± 3.8[a]
2-butanol	0.5 ± 0.4[a]	226.6 ± 46.7[b]
2-methyl-1-butanol	24.3 ± 0.7[a]	14.4 ± 1.2[b]
3-methyl-1-butanol	104.8 ± 2.1[a]	77.4 ± 2.3[b]
1-hexanol	89.3 ± 32.2[a]	214.2 ± 41.6[b]
phenethyl alcohol	5.4 ± 0.3[a]	5.8 ± 0.2[a]
total higher alcohols	**352.3 ± 40.4**	**861.4 ± 237.9**
methyl acetate	0.0 ± 0.0[a]	10.9 ± 2.4[b]
ethyl acetate	463.2 ± 11.0[a]	226.8 ± 77.2[b]
ethyl hexanoate	0.0 ± 0.0[a]	0.5 ± 0.6[a]
ethyl decanoate	0.5 ±0.1[a]	0.7 ± 0.1[a]
ethyl-2-*trans*-4-*cis*-decadienoate	0.0 ± 0.0[a]	3.0 ± 1.3[a]
total esters	**463.7 ± 11.1**	**241.9 ± 81.6**

Values with the same letter within the same row indicate not significant difference ($p \leq 0.05$) among them. Pear concentrate: heart fraction from the distillation of fermented juice concentrate; pear mash: heart fraction from the distillation of fermented pear mash; natural pear juice: heart fraction from the distillation of fermented natural pear juice.

The pear spirits are obtained by distillation of the first distillation heart fractions. The concentration profiles of the main volatile compounds in the different fractions collected are presented in **Figure 6.4**. The acetaldehyde and acetal distilled in the first fractions, as expected. Acetal was not detected in the hearts of the first distillation (except for a small amount present in some hearts of the fermented concentrate). In spite of this, it was present in the second distillation first three fractions, in concentrations that are higher for the distillates from pear juice.

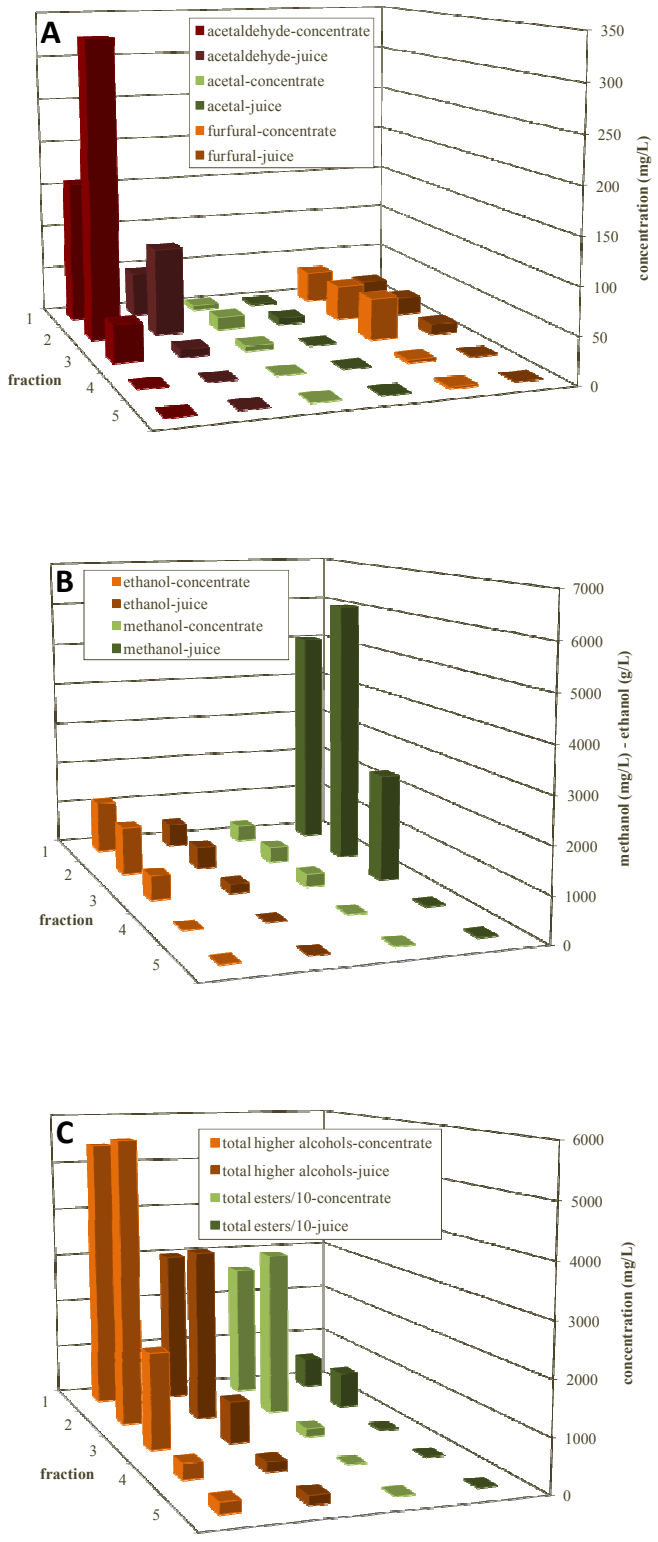

Figure 6.4. Concentration profiles of the different volatile compounds during the second distillation of *Blanquilla* pear fermented juice concentrate and natural juice. (**A**) acetaldehyde, acetal, furfural; (**B**) ethanol, methanol; (**C**) total higher alcohols, total esters.

Total esters/10: total esters concentration (mg/L)/10, in order to fit the values to the scale of the concentration axis. Fraction 1: 35 mL; 2: 35mL; 3: 1.75 L; 4: 35 mL; 5: 210 mL.

This could be due to its formation from acetaldehyde and ethanol during the distillation process [27]. The higher concentration found in the distillates from pear juice concentrate (which presented the higher concentration of ethanol and acetaldehyde) support this theory. On the other hand, furfural's concentration slowly increased during the first distillation fractions and then dramatically decreased for all the distillates.

The ethanol concentration was higher in the distillates from pear concentrate (see **Figure 6.4-B**). This was in agreement with the concentration of ethanol present in the fermented raw materials, which was related at the same time with the initial concentration of fermentable sugars in these substrates. The concentration of methanol was remarkably high in the distillates from pear juice. This was already observed and commented in the fermented pear juice and also in the heart fraction of the first distillation.

The total higher alcohols and total esters distilled mainly in the first distillation fractions (see **Figure 6.4-C**). The highest concentrations of both types of compounds were found in the distillations from pear juice concentrate.

Based on the profiles obtained, it could be concluded that fractions 4 and 5 had negligible concentrations of volatile compounds and ethanol. Therefore, they were put together to form the tail fraction. On the other hand, the first two fractions presented high concentrations of acetaldehyde, methanol, furfural and ethyl acetate (which are negative compounds) (see **Annex V**). However, removing these two fractions would have implied a high loss of higher alcohols, esters and ethanol. Consequently, making a compromise between the positive and negative aspects of the separation, it was decided to separate the first distillation fraction to keep it as the head. Subsequently, the second and the third fractions were joined and kept as the heart (pear spirit). The same separations were performed in all of the distillations.

Table 6.7 shows the volatile composition and ethanol content in the different heart fractions obtained. There is a significant difference between the pear juice concentrate and the natural juice for all the higher alcohols and esters present. The total higher alcohols concentration achieved the minimum required by the European Council Regulation (140 g/hL a.a.) in both cases. The higher

concentrations of 1-hexanol, 2-butanol and 1-propanol found in the hearts of the first distillations from natural juice (compared to the hearts from juice concentrate), were maintained in the hearts of the second distillations. This could result negative from the organoleptic point of view since 1-hexanol in concentrations higher than 10 g/hL a.a. may supply herbaceous nuances to the spirits, and 2-butanol (which is associated to low quality raw materials) can also adversely affect the final aroma of the distillate [4,24].

Table 6.7. Mean concentrations and standard deviations (g/hL a.a.) of the main volatile compounds present in the pear spirits of *Blanquilla* variety produced from juice concentrate and natural juice.

compound	pear juice concentrate	pear natural juice
acetaldehyde	7.4 ± 3.4^{a}	4.7 ± 0.2^{a}
acetal	1.2 ± 0.5^{a}	0.2 ± 0.2^{b}
furfural	6.8 ± 2.5^{a}	4.8 ± 1.7^{a}
methanol	38.6 ± 18.8^{a}	963.4 ± 79.1^{b}
ethanol (% v/v)	31.8 ± 1.8	26.3 ± 3.6
1-propanol	0.0 ± 0.0^{a}	184.5 ± 110.5^{b}
2-methyl-1-propanol	77.0 ± 4.0^{a}	39.1 ± 3.2^{b}
1-butanol	2.1 ± 0.3^{a}	6.5 ± 0.6^{b}
2-butanol	0.0 ± 0.0^{a}	208.9 ± 40.3^{b}
2-methyl-1-butanol	22.4 ± 1.2^{a}	13.6 ± 1.2^{b}
3-methyl-1-butanol	95.1 ± 4.5^{a}	73.0 ± 2.9^{b}
1-hexanol	79.3 ± 32.1^{a}	204.2 ± 41.6^{b}
phenethyl alcohol	3.2 ± 0.2^{a}	4.4 ± 0.4^{b}
total higher alcohols	**279.1 ± 42.3**	**734.2 ± 200.7**
methyl acetate	0.5 ± 0.0^{a}	6.8 ± 1.9^{b}
ethyl acetate	300.9 ± 14.0^{a}	146.5 ± 56.4^{b}
ethyl hexanoate	0.3 ± 0.1^{a}	0.0 ± 0.0^{b}
total esters	**301.7 ± 14.1**	**153.3 ± 58.3**

Values with the same letter within the same row indicate not significant difference ($p \leq 0.05$) among them. Pear concentrate: spirit produced from concentrated pear juice; pear mash: spirit produced from pear mash; pear natural juice= spirit produced from natural pear juice.

In addition, it could be observed that ethyl acetate is present in quite high concentrations, especially in the distillate from pear juice concentrate. Concentrations higher than 180 g/hL a.a. impart an acidic character to the spirit. Therefore, the concentrations found in the spirits from pear juice concentrate could result negative from the organoleptic point of view. It is interesting to point out that the recovery of fruity aroma esters (ethyl hexanoate, ethyl decanoate,

ethyl-2-trans-4-cis-decadienoate) was very low. This should be taken into account for further experiments, when defining the different distillation fractions. Finally, it should be noticed that the ethanol concentration did not reach the minimum required by the European Council Regulation (37.5 % (v/v)). The present experiments were focused mainly on the comparison of the two different raw materials, processed under the same fermentation and distillation conditions. Nevertheless, in future work a smaller heart fraction should be collected in order to fulfill the legislation.

To determine the degree of differentiation of the different raw materials used, PCA was applied. This analysis divided the compounds into two main components (PC1 and PC2). **Table 6.8** shows these results and **Figure 6.5** plots them.

Table 6.8. Principal Component Analysis (PCA) results for the main volatile compounds in the heart fraction of the second distillation of *Blanquilla* pear juice concentrate and natural juice.

Principal Component	compound	loading	variance explained (%)	total variance (%)
PC1	methanol	0.951	83.28	83.28
	2-methyl-1-propanol	-0.934		
	2-methyl-1-butanol	-0.925		
	1-butanol	0.923		
	3-methyl-1-butanol	-0.920		
	methyl acetate	0.915		
	2-butanol	0.890		
	ethyl hexanoate	-0.842		
	ethyl acetate	-0.799		
	phenethyl alcohol	0.788		
	1-hexanol	0.705		
	1-propanol	0.699		
PC2	furfural	0.987	10.28	93.56
	acetaldehyde	0.865		
	acetal	0.798		

As the figure shows, PC1 clearly differentiates the pear juice concentrate distillates from the natural pear juice distillates. From **Table 6.8** it can be seen that the separation is mainly due to the different concentrations of higher alcohols, and the higher concentration in methanol and lower concentration in ethyl acetate of

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the pear natural juice spirits compared to the pear juice concentrate spirits. These differences are quite in agreement with the ANOVA tests results found for the same heart fractions (see **Table 6.7**).

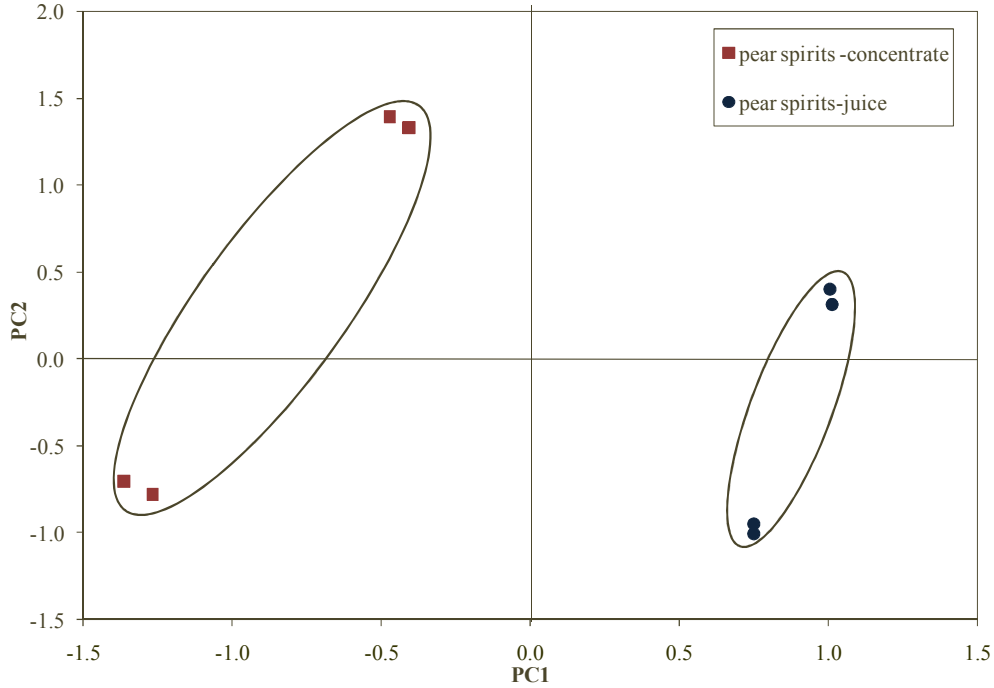

Figure 6.5. Plot of the principal components 1 and 2 (PC1, PC2) for the main volatile compounds in the heart fraction of the second distillation of *Blanquilla* pear juice concentrate and natural juice.

Sensory evaluation

The sensory evaluation of the spirits was performed with a panel of 24 consumers in a sensory evaluation room. In order to reduce the saturation of the panelists' olfactive cells, the distillates were diluted to a 20 % (v/v) of ethanol [28].

Significant differences ($p<0.05$) were found for both taste and aroma between the distillates from natural pear juice and pear juice concentrate. For both senses, the natural pear juice beverages were the preferred ones for their fruity character and more integrated flavors. This result could not be predicted from the chemical analysis. However, it was not surprising. As it was previously mentioned, the manufacturing process of the juice concentrate eliminates part of its volatile composition. Consequently, less aromatic drinks are obtained. For this reason, it would be interesting to add the aroma fraction recovered from the concentration

process to the juice concentrate before the fermentation starts. This would allow maintaining the advantages of using pear juice concentrate, without having the aromatic loss that it implies.

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Chapter 7

CONCLUSIONS

The main conclusion of this thesis is that the *Blanquilla* variety of pear is a suitable substrate for the production of pear spirits.

We also conclude that:

- The fermentation temperature significantly affected the volatile composition of the pear distillates obtained. However, it has been impossible to determine the best fermentation conditions.

- The fermentation pH and the fermentation yeast also significantly affected the volatile composition of the pear distillates obtained. However, the sensory analyses performed on the distillates showed that these differences were not detected by the consumers.

- The volatile profiles obtained indicate that distillations in a copper alembic produced better quality distillates than distillations in a glass device and in a glass device with copper shavings.

- The copper alembic coupled to a copper distillation column seemed to concentrate a higher concentration of fruity aroma esters in the spirits than the simple copper alembic, thus improving the quality of the pear spirits.

- Distilling in the presence of the fermentation lees usually produced distillates of better quality than distilling in the absence of the lees (this conclusion is based on the volatile compounds quantified). However, in

some cases no significant differences were detected between the spirits distilled in the presence or absence of the fermentation lees.

- The raw material employed also played an important role in the quality of the distillates. The composition of the distillates produced from pear juice concentrate was significantly different from that of the distillates produced from natural pear juice. Although the distillates from natural juice had higher concentrations of some negative compounds, the sensory analysis showed that they were preferred to the distillates from pear juice concentrate because of their fruity character and more integrated flavors.

- The use of *Blanquilla* pear juice concentrate for the production of pear spirits is a viable option, although it should be taken into account that there is some aroma loss during the concentration process.

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Future work

- It would be interesting to perform fermentations of pear juice concentrate with the addition of the aroma extract recovered from the concentration process, and compare the distillates obtained with distillates from natural juice.

- More distillations in a copper alembic coupled to a copper distillation column should be performed in order to confirm its benefits.

- Fermentations and distillations should be performed on a larger scale if the process is to be used at the industrial level.

- It would also be interesting to extract the aromatic compounds before they are injected into the GC to detect and better quantify some compounds which are close to the detection/quantification limit (i.e. C_6-C_{12} esters).

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Annex I

Figure Captions

Figure I.1. distillation profiles of higher alcohols during the distillation with lees of *Blanquilla* fermented pear juice in a: glass alembic, copper alembic, glass alembic with copper shavings. (**A**) 1-propanol and 2-methyl-1-propanol; (**B**) 1-butanol, 2-methyl-1-butanol and 3-methyl-1-butanol; (**C**) 1-hexanol, phenethyl alcohol.

Fractions 1-4: 25 mL each; 5-12: 50 mL each.

Figure I.2. distillation profiles of esters during the distillation with lees of *Blanquilla* fermented pear juice in a: glass alembic, copper alembic, glass alembic with copper shavings. (**A**) ethyl acetate and methyl acetate; (**B**) ethyl decanoate and ethyl 2-*trans*-4-*cis*-decadienoate.

Fractions 1-4: 25 mL each; 5-12: 50 mL each.

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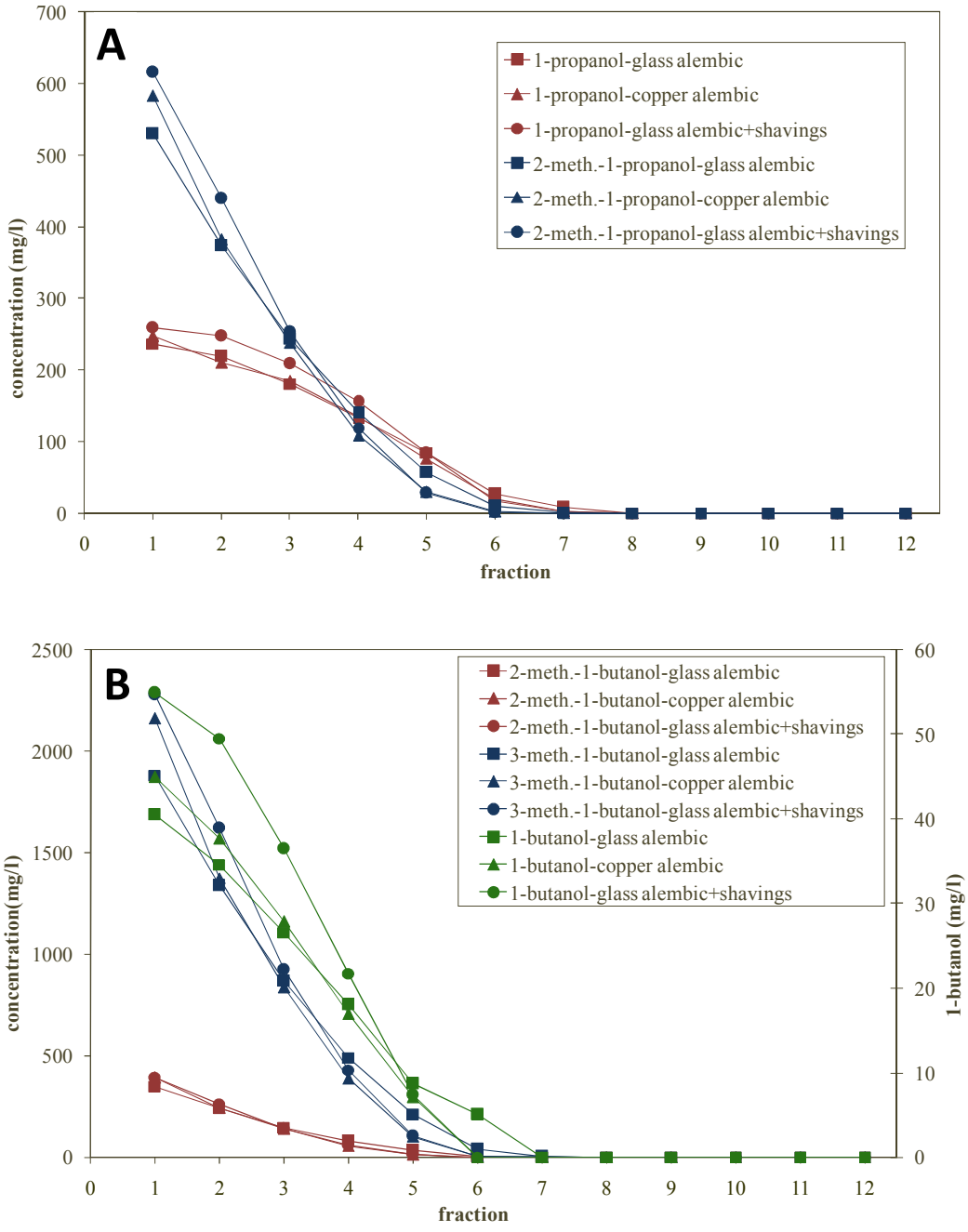

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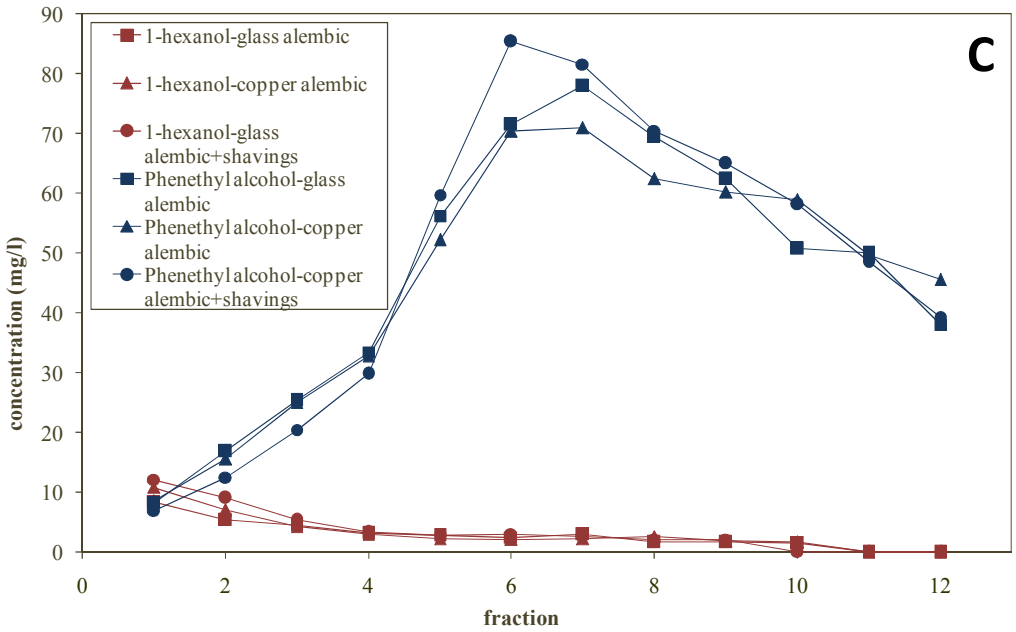

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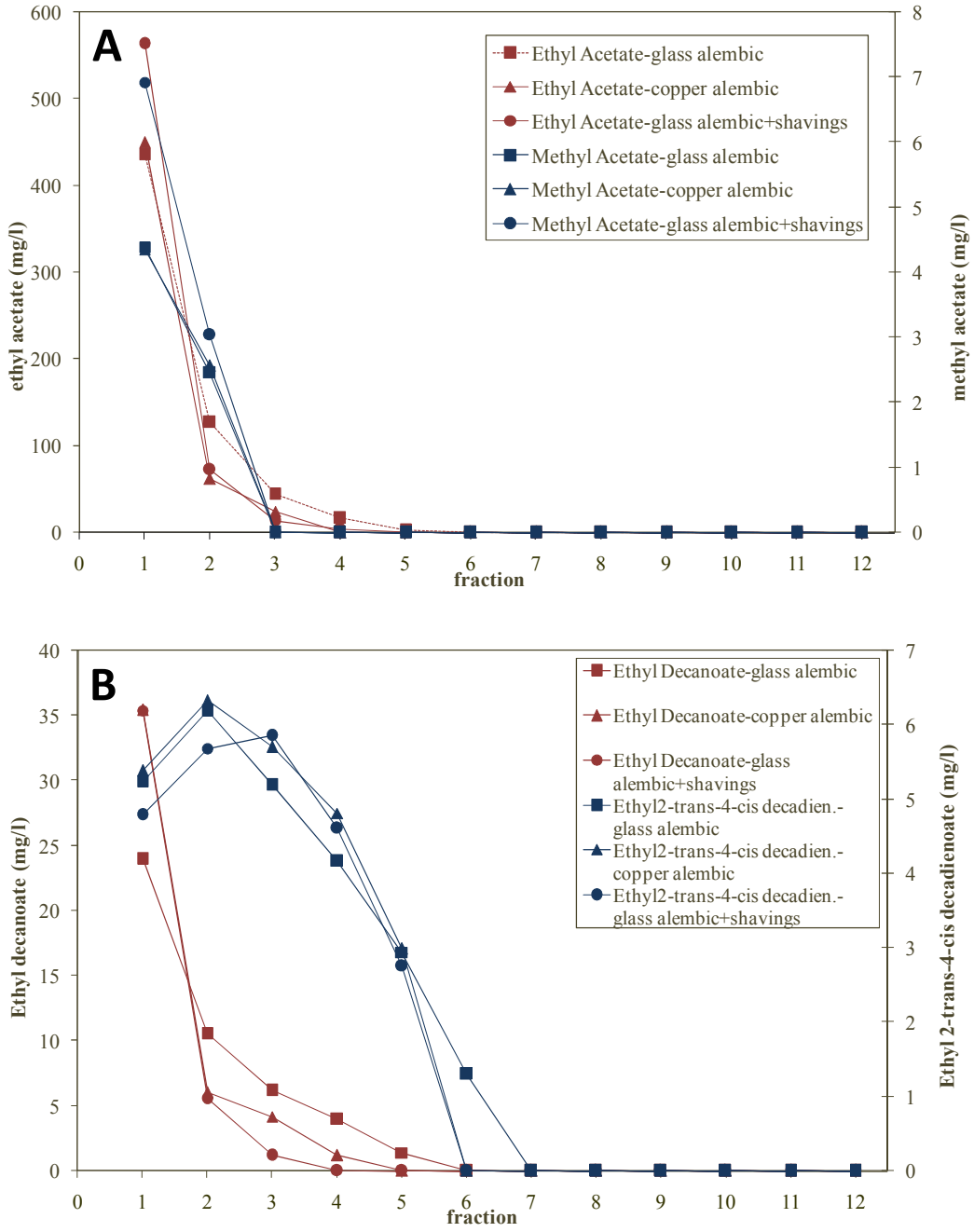
Figure I.2.



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Annex II

Figure Captions

Figure II.1. Concentration profiles during *Blanquilla* pear juice fermentations at 17ºC and 25ºC (duplicate of the fermentations showed in **Chapter 3**). (**A**) sucrose, glucose, fructose and total microorganisms concentrations. (**B**) ethanol, glycerol and total microorganisms concentrations.
m.o. = microorganisms.

Figure II.2. Total sugars concentration profile during *Blanquilla* pear juice fermentations at 17 ºC and 25 ºC. The results shown are for the four fermentations performed (one and a replica at each temperature).

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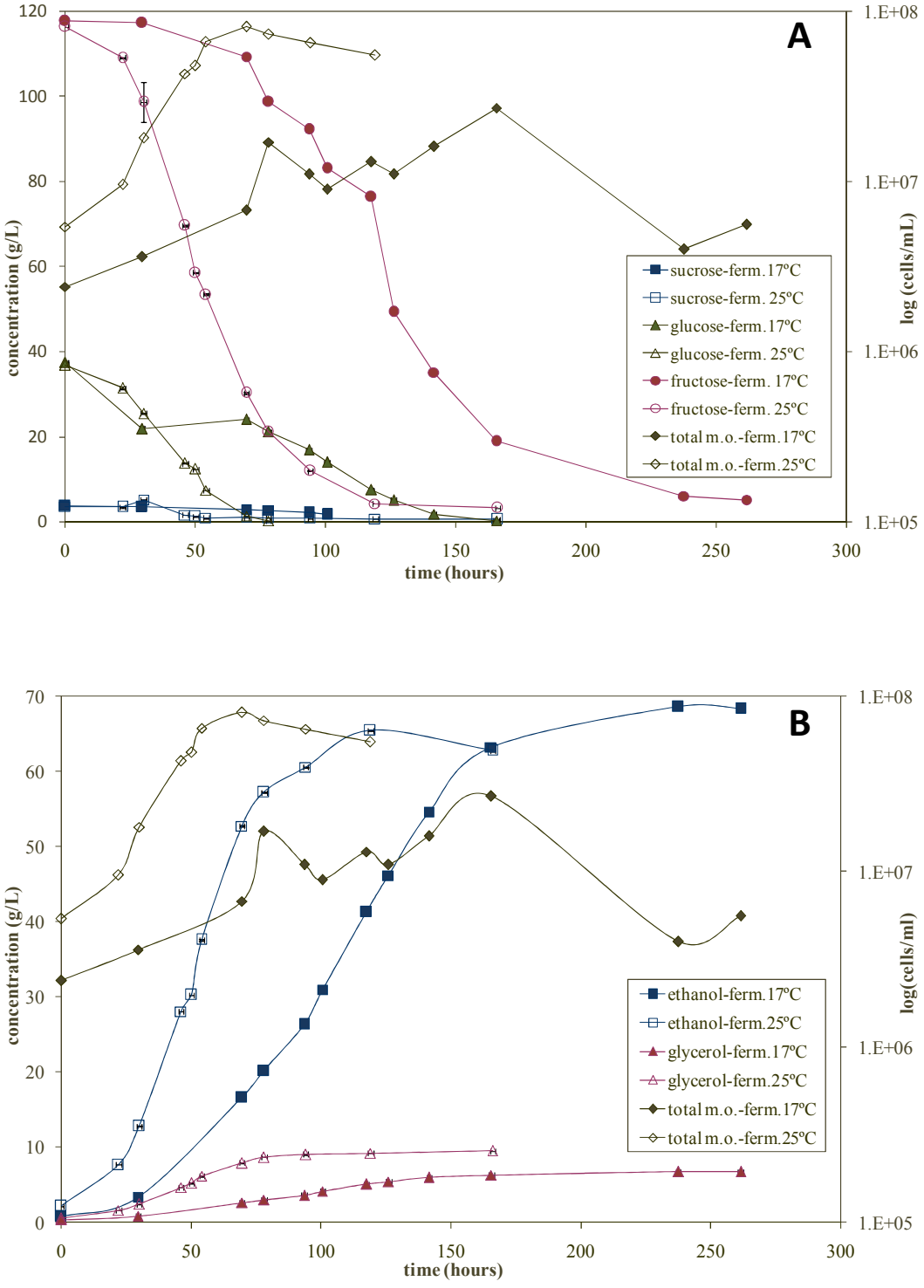

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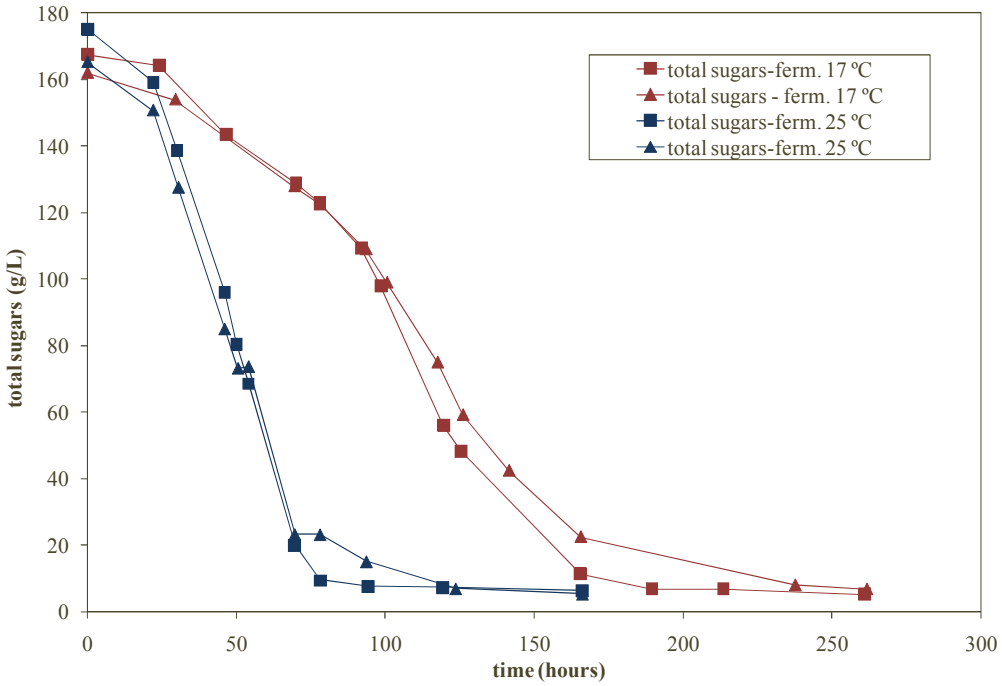

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Annex III

Figure Captions

Figure III.1. distillation profiles of higher alcohols during the distillation of pear juice fermented with *Siha Aktiv6* and *Uvaferm CGC62*. (**A**) 1-propanol and 2-methyl-1-propanol and 1-hexanol; (**B**) 1-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol and phenethyl alcohol.

Siha, fermentations with the *Siha Aktiv6* strain; Uvaferm, fermentations with the *Uvaferm CGC62* strain. Fraction 1: head; 2: heart; 3: tail.

Figure III.2. distillation profiles of esters during the distillation of pear juice fermented with *Siha Aktiv6* and *Uvaferm CGC62*. (**A**) ethyl acetate and methyl acetate; (**B**) ethyl decanoate and ethyl 2-*trans*-4-*cis*-decadienoate.

Siha, fermentations with the *Siha Aktiv6* strain; Uvaferm, fermentations with the *Uvaferm CGC62* strain. Fraction 1: head; 2: heart; 3: tail.

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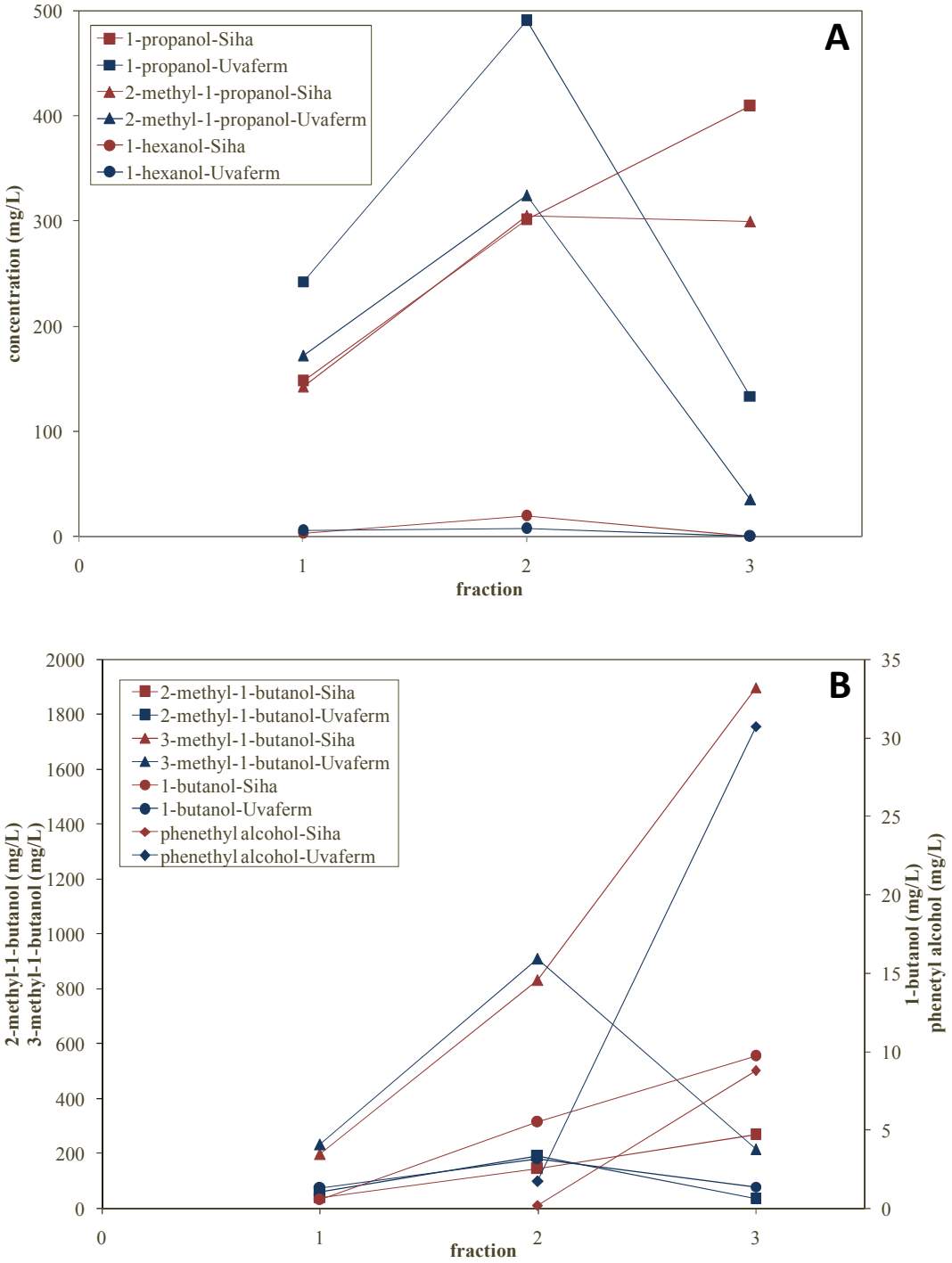

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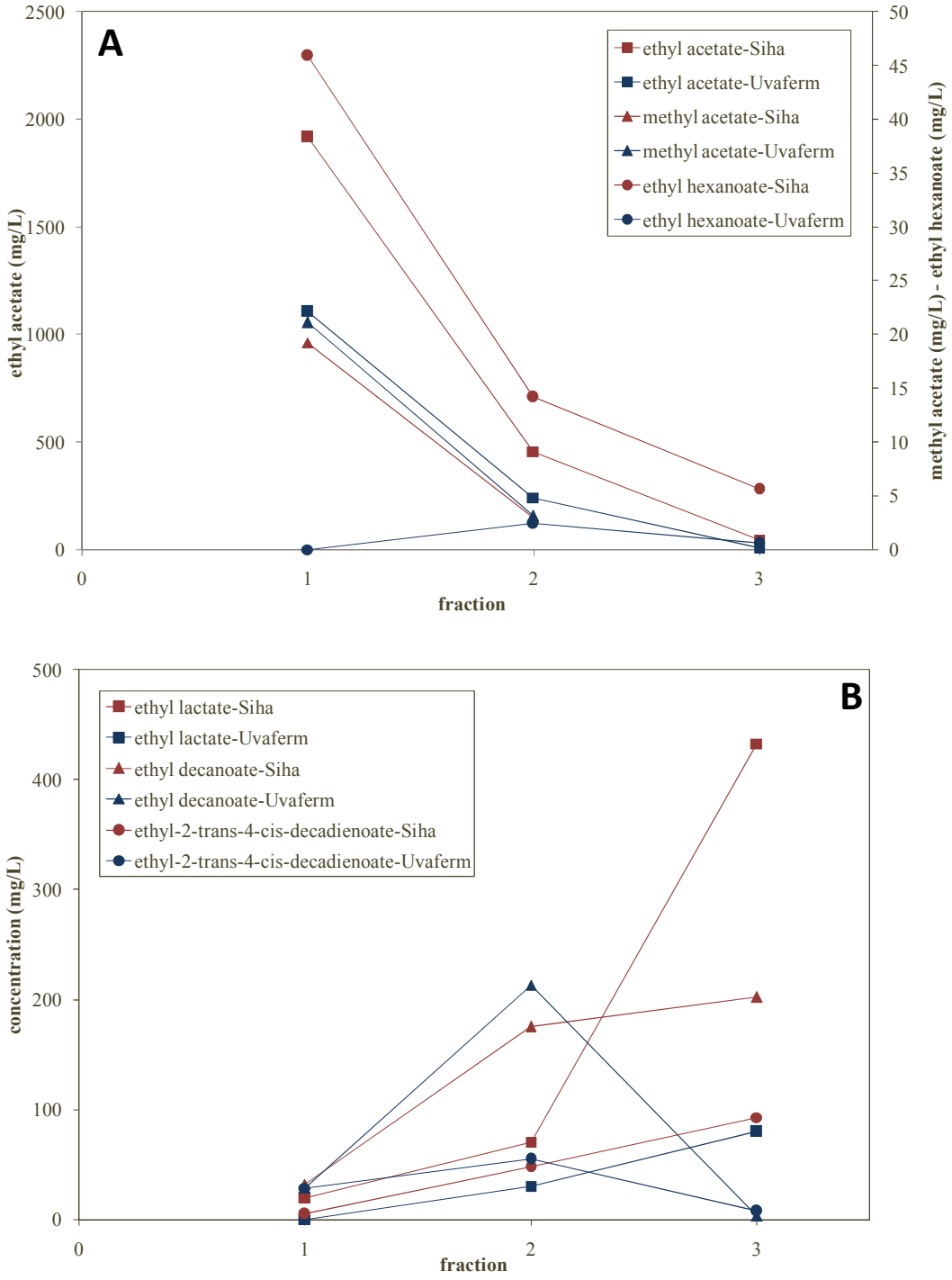

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Annex IV

Figure Captions

Figure IV.1. distillation profiles of **higher alcohols** during the **distillation** of *Blanquilla* pear juice fermented with ***Siha Aktiv6*** at the native pH and at an adjusted pH of 3.20. (**A**) 1-propanol and 2-methyl-1-propanol and 1-hexanol; (**B**) 1-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol and phenethyl alcohol.

Siha, distillates from the fermentations with the *Siha Aktiv6* strain; Siha+AM, distillates from the fermentations with the *Siha Aktiv6* strain with the addition of a malic and lactic acids mixture. Fraction 1:head; 2: heart; 3: tail.

Figure IV.2. distillation profiles of **esters** during the **distillation** of *Blanquilla* pear juice fermented with ***Siha Aktiv6*** at the native pH and at an adjusted pH of 3.20. (**A**) ethyl acetate and methyl acetate; (**B**) ethyl decanoate and ethyl 2-*trans*-4-*cis*-decadienoate.

Siha, distillates from the fermentations with the *Siha Aktiv6* strain; Siha+AM, distillates from the fermentations with the *Siha Aktiv6* strain with the addition of a malic and lactic acids mixture. Fraction 1:head; 2: heart; 3: tail.

Figure IV.3. distillation profiles of volatile compounds during the **first distillation** *Blanquilla* pear juice fermented with a commercial strain of ***Saccharomyces cerevisiae*** at the native pH and at an adjusted pH of 3.30. (**A**) acetal, furfural, acetaldehyde; (**B**) 1-hexanol, 1-propanol, 2-methyl-1-propanol, phenethyl alcohol; (**C**) 1-butanol, 2-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol; (**D**) ethanol, methanol, methyl acetate, ethyl acetate; (**E**) ethyl hexanoate, ethyl decanoate, ethyl-2-trans-4-cis-decadienoate.

Figure IV.4. distillation profiles of higher alcohols during the **second distillation** of *Blanquilla* pear juice fermented with a commercial strain of ***Saccharomyces cerevisiae*** at the native pH and at an adjusted pH of 3.30. (**A**) 1-propanol and 2-methyl-1-propanol and 1-hexanol; (**B**) 1-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol and phenethyl alcohol.

Native pH, distillates from the fermentations at the native pH; adjusted pH, distillates from the fermentations at the adjusted pH of 3.30. Fraction 1: 35 mL; 2: 35mL; 3: 1.75 L; 4: 35 mL; 5: 210 mL.

Figure IV.5. distillation profiles of ethyl acetate, methyl acetate and ethyl 2-*trans*-4-*cis*-decadienoate during the **second distillation** of *Blanquilla* pear juice fermented with a commercial strain of ***Saccharomyces cerevisiae*** at the native pH and at an adjusted pH of 3.30.

Native pH, distillates from the fermentations at the native pH; adjusted pH, distillates from the fermentations at the adjusted pH of 3.30. Fraction 1: 35 mL; 2: 35mL; 3: 1.75 L; 4: 35 mL; 5: 210 mL.

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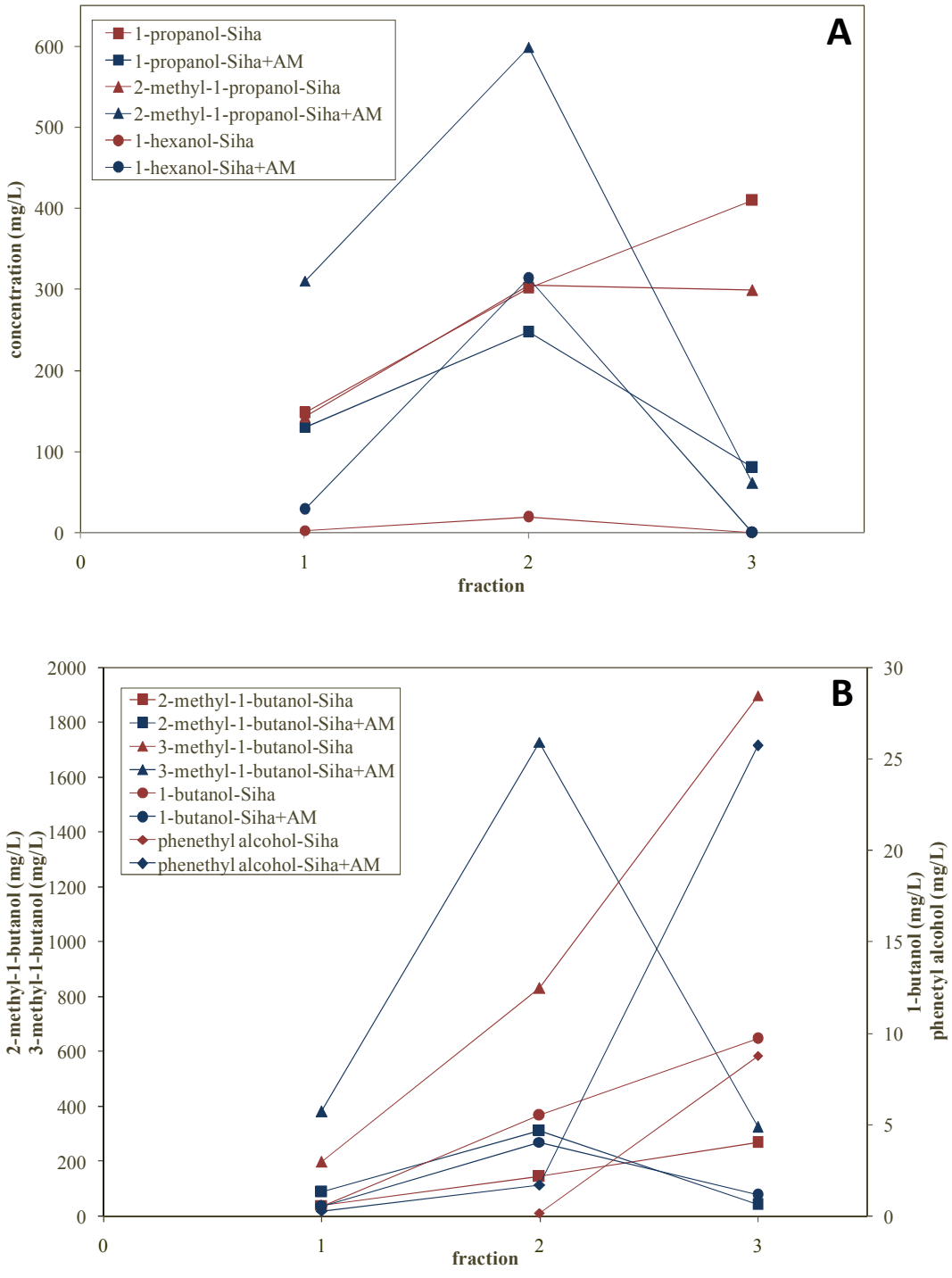

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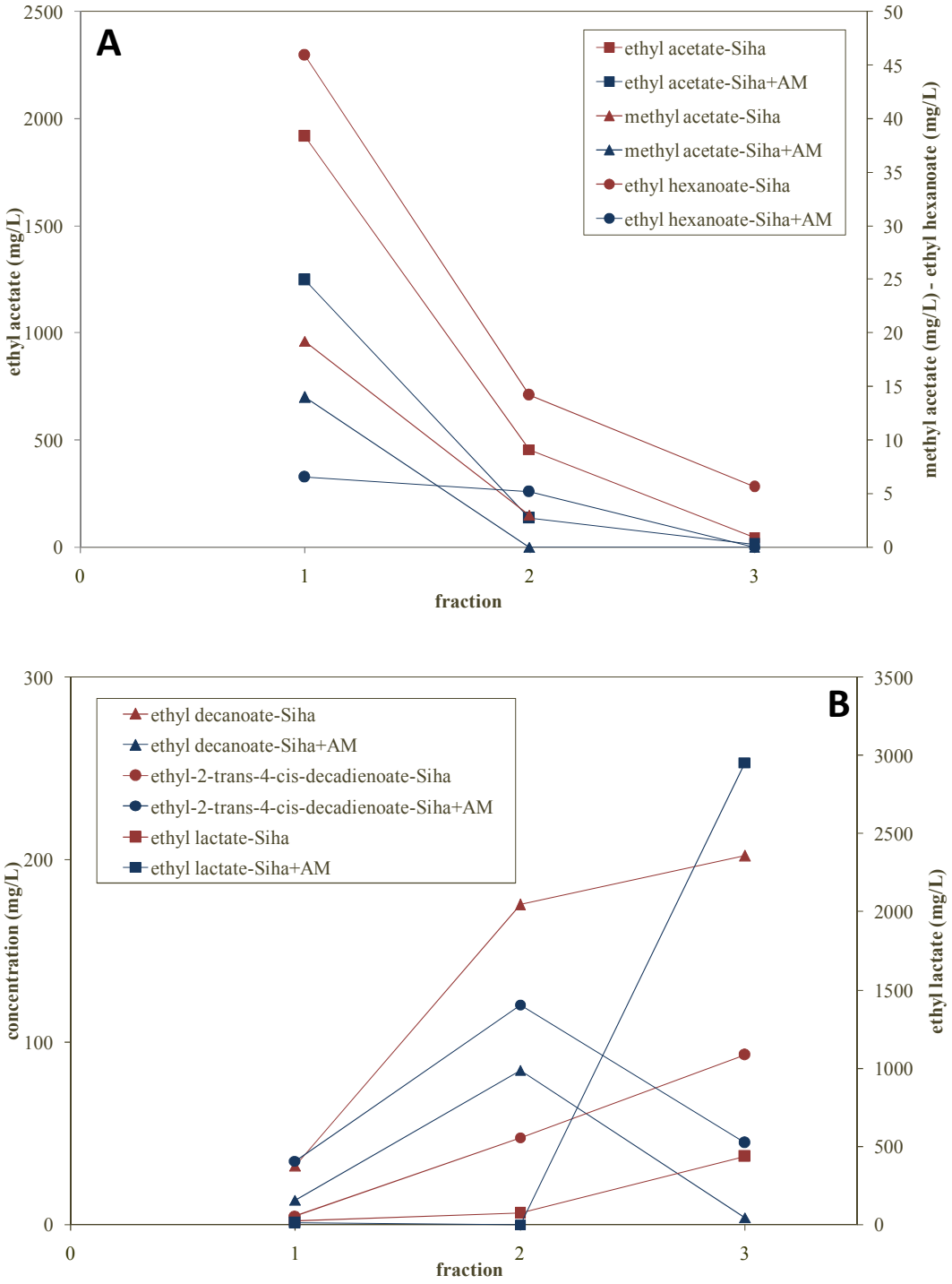

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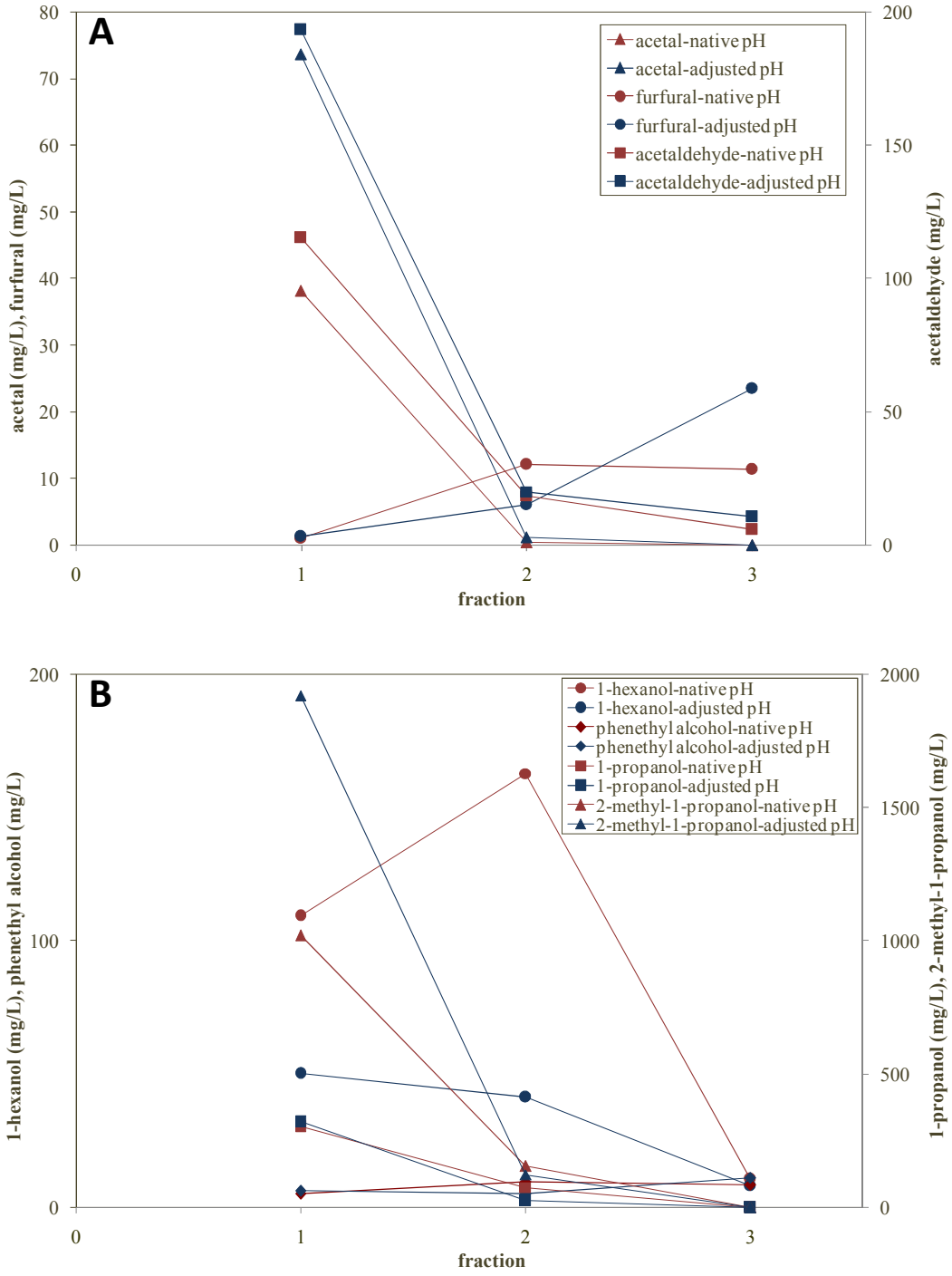

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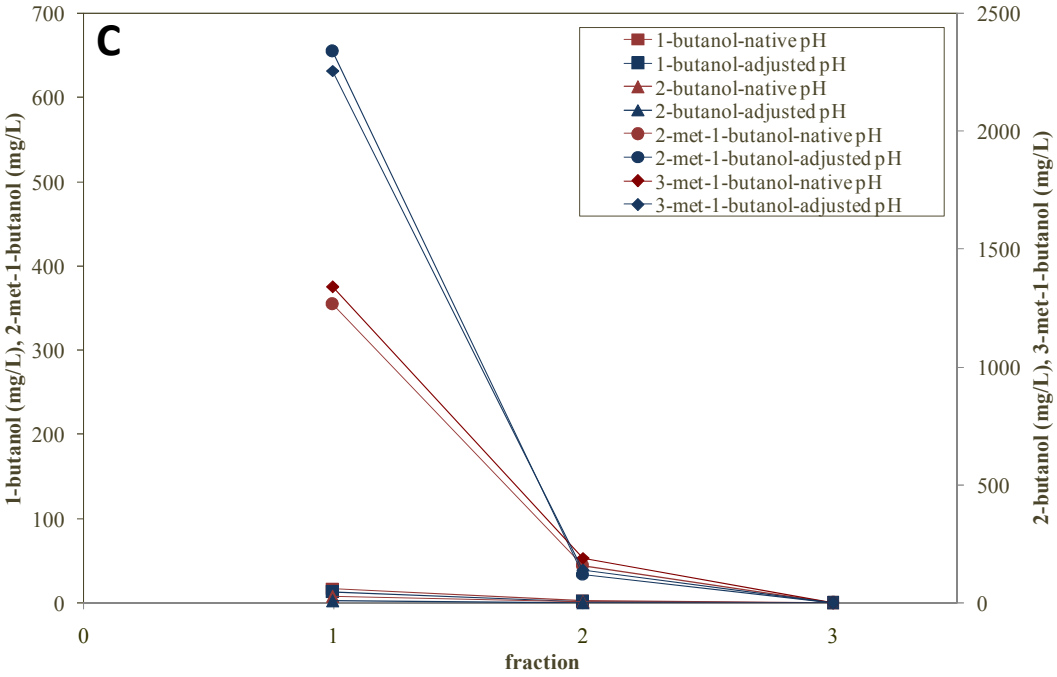

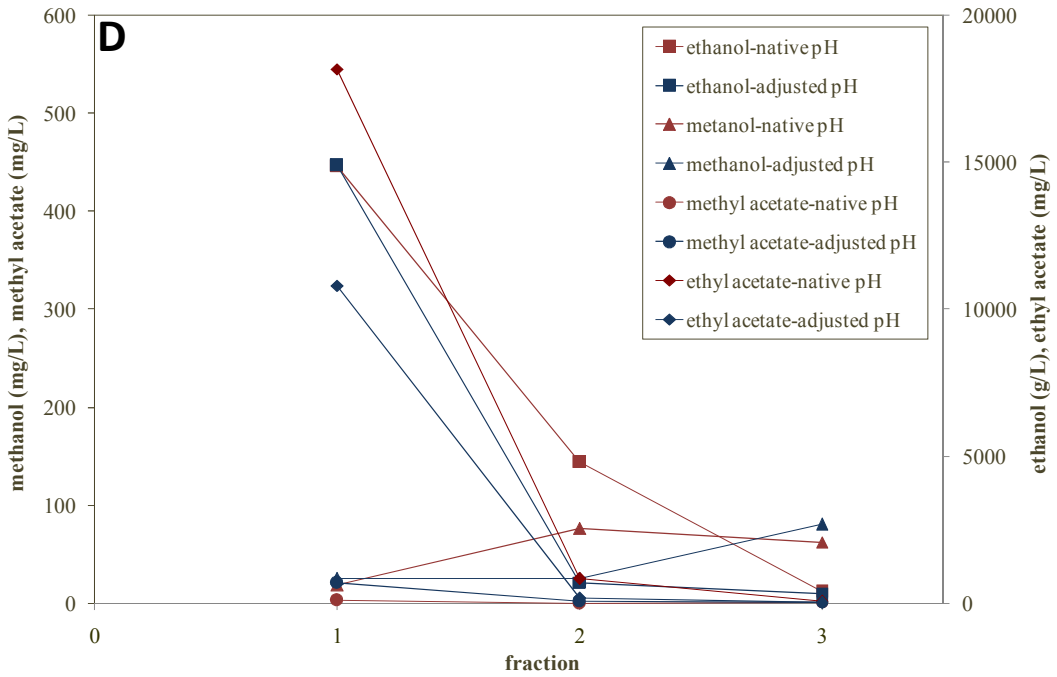

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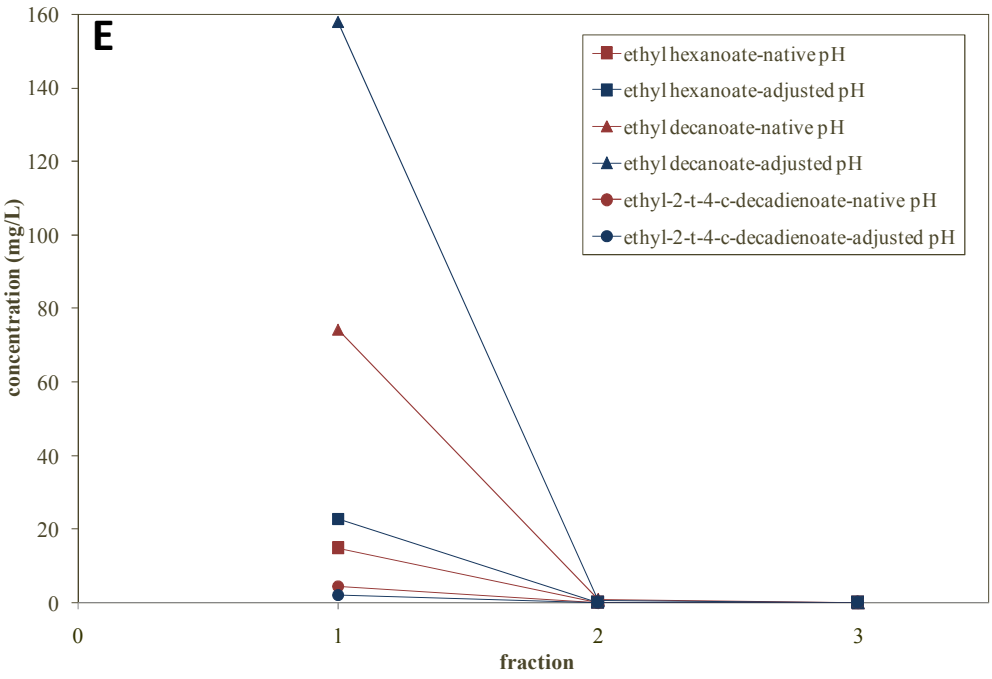

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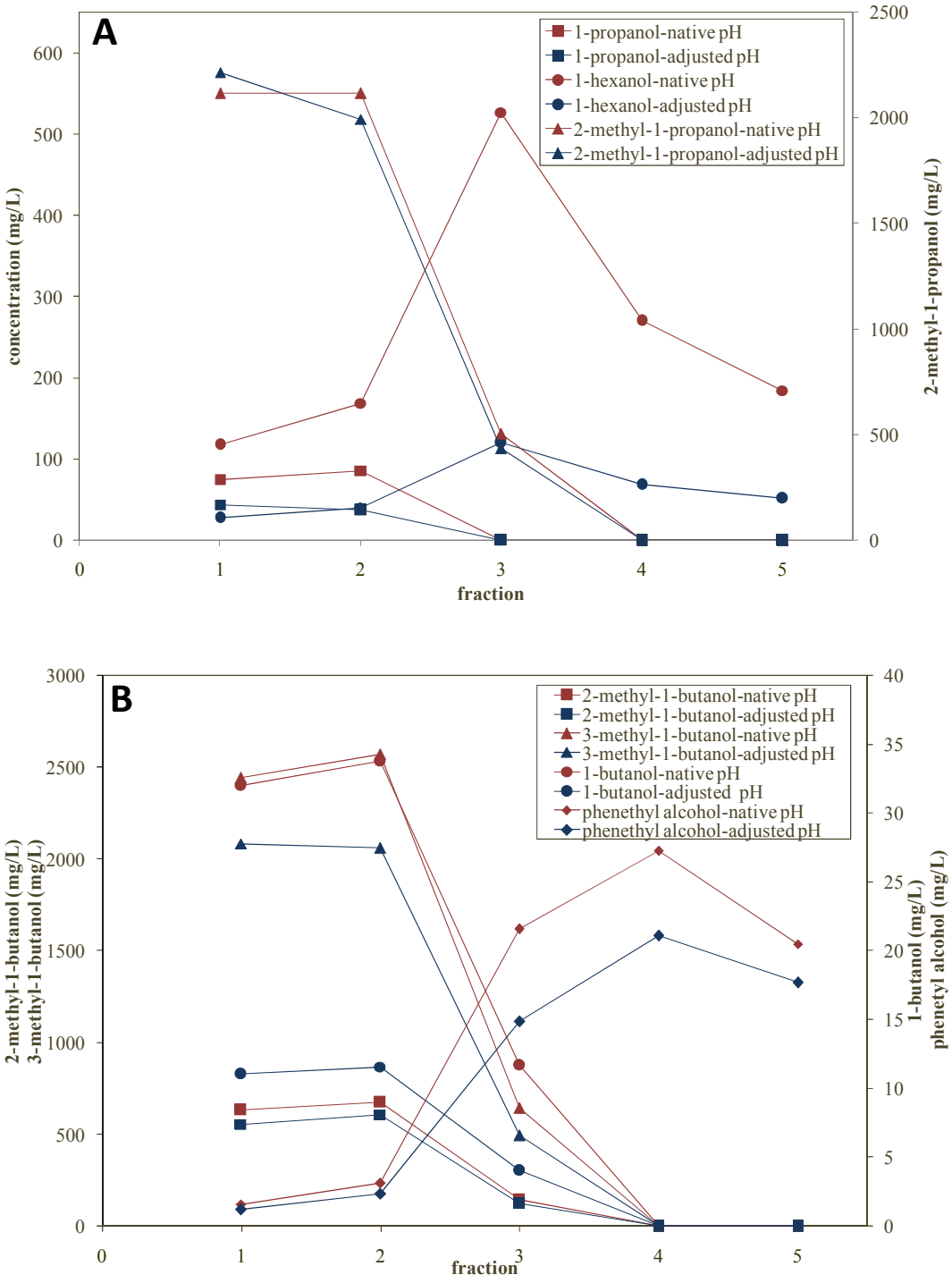

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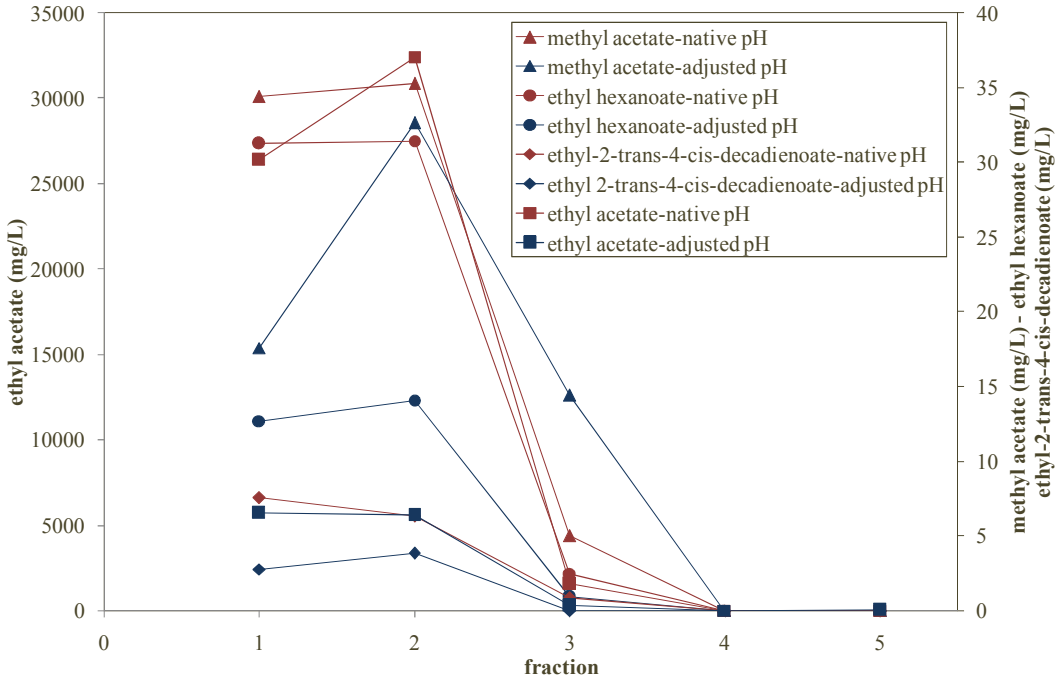
Figure IV.5.



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Annex V

Figure Captions

Figure V.1. total yeasts, total sugars, glucose, fructose, sucrose, ethanol and glycerol profiles during the **fermentations** (duplicate) of pear juice in the **experimental set 1**. (**A**) concentrated pear juice; (**B**) natural pear juice.

Figure V.2. distillation profiles of volatile substances during the distillation of *Blanquilla* **pear juice concentrate** with and without the presence of the wine lees in the **experimental set 1**. (**A**) higher alcohols; (**B**) esters.
w/lees: distillation in presence of the wine lees, w/o lees: distillation in absence of the wine lees.

Figure V.3. distillation profiles of volatile substances during the distillation of *Blanquilla* **pear natural juice** with and without the presence of the wine lees in the **experimental set 1**. (**A**) higher alcohols; (**B**) esters.
w/lees: distillation in presence of the wine lees, w/o lees: distillation in absence of the wine lees.

Figure V.4. distillation profiles of volatile substances during the **first distillation** of *Blanquilla* pear juice concentrate and natural juice in the **experimental set 2**. (**A**) acetal, furfural, acetaldehyde; (**B**) 1-hexanol, 1-propanol, 2-methyl-1-propanol, phenethyl alcohol; (**C**) 1-butanol, 2-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol; (**D**) ethanol, methanol, methyl acetate, ethyl acetate; (**E**) ethyl hexanoate, ethyl decanoate, ethyl-2-trans-4-cis-decadienoate.

Figure V.5. distillation profiles of volatile substances during the **second distillation** of *Blanquilla* pear juice concentrate and natural juice in the **experimental set 2**. (**A**) 1-hexanol, 1-propanol, 2-methyl-1-propanol, phenethyl alcohol; (**B**) 1-butanol, 2-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol; (**C**) methyl acetate, ethyl hexanoate, ethyl-2-trans-4-cis-decadienoate.

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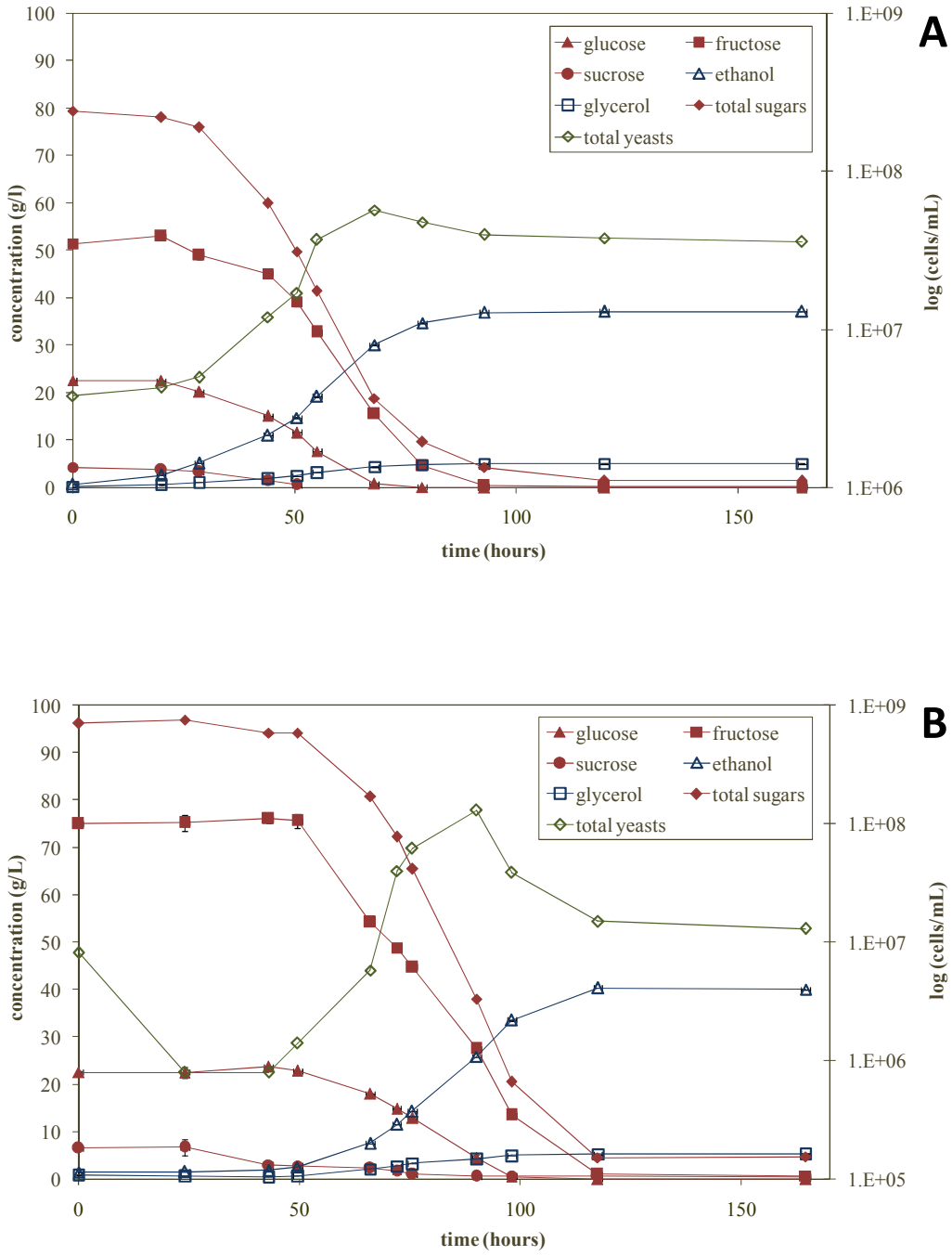

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Figure V.2.

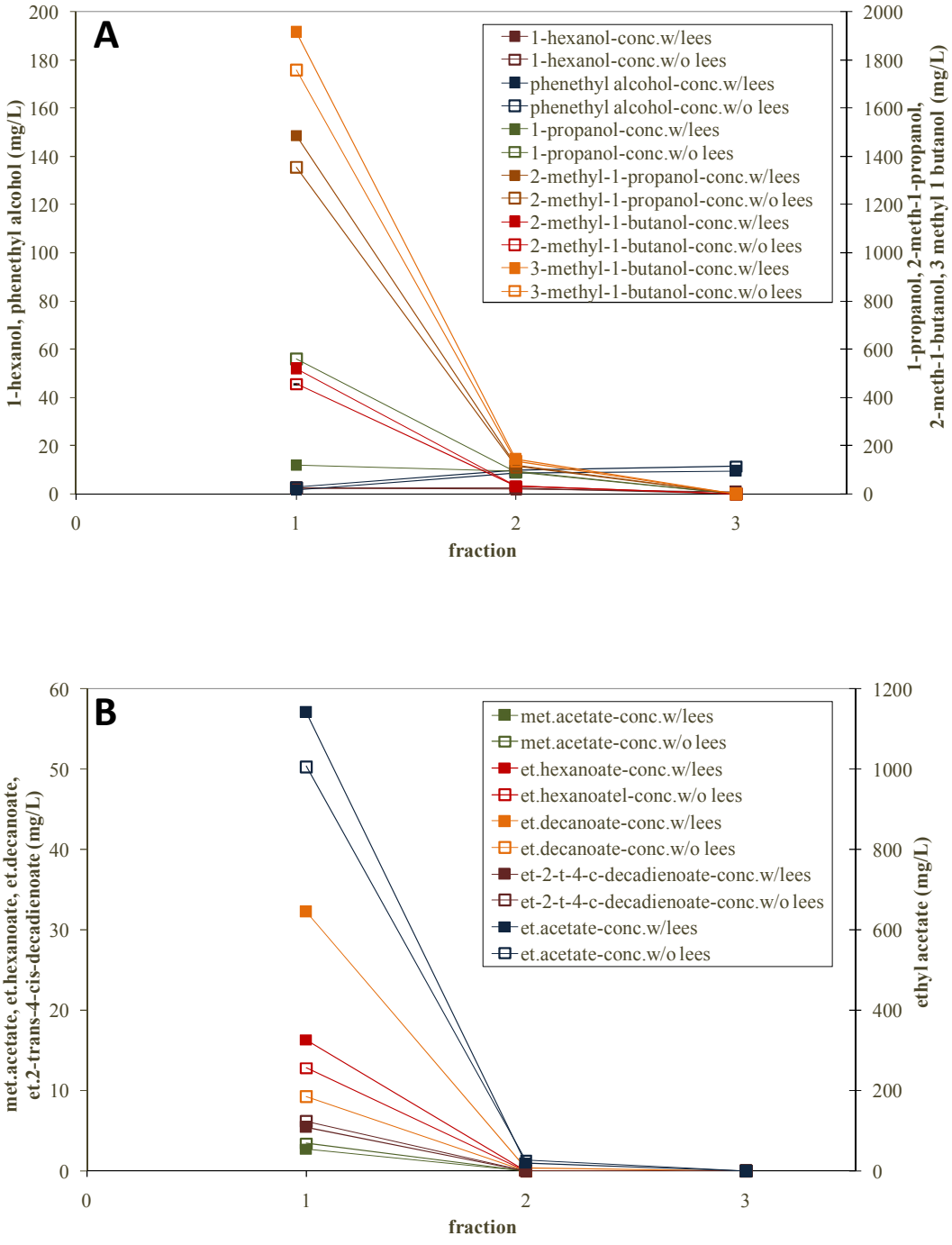

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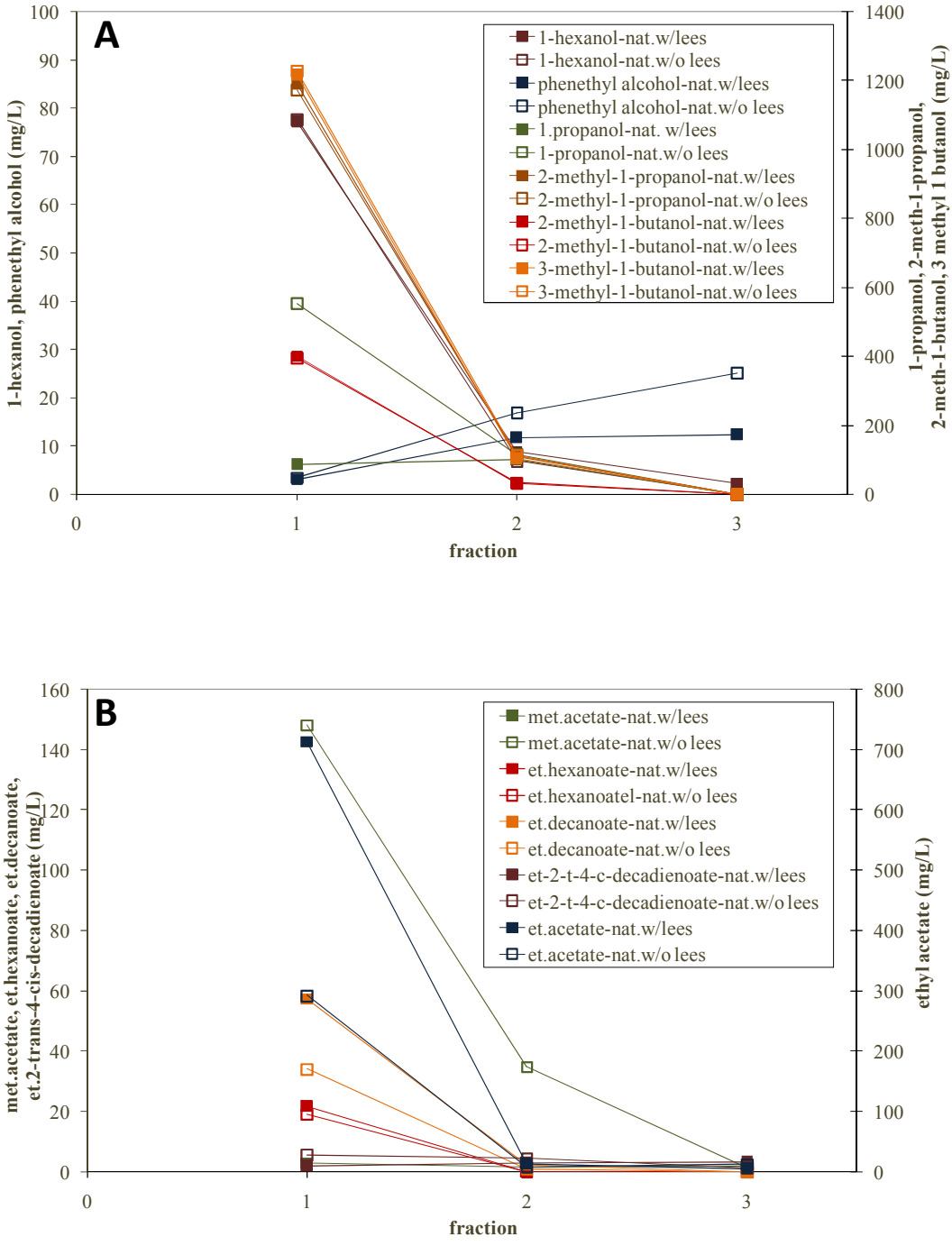
Figure V.3.



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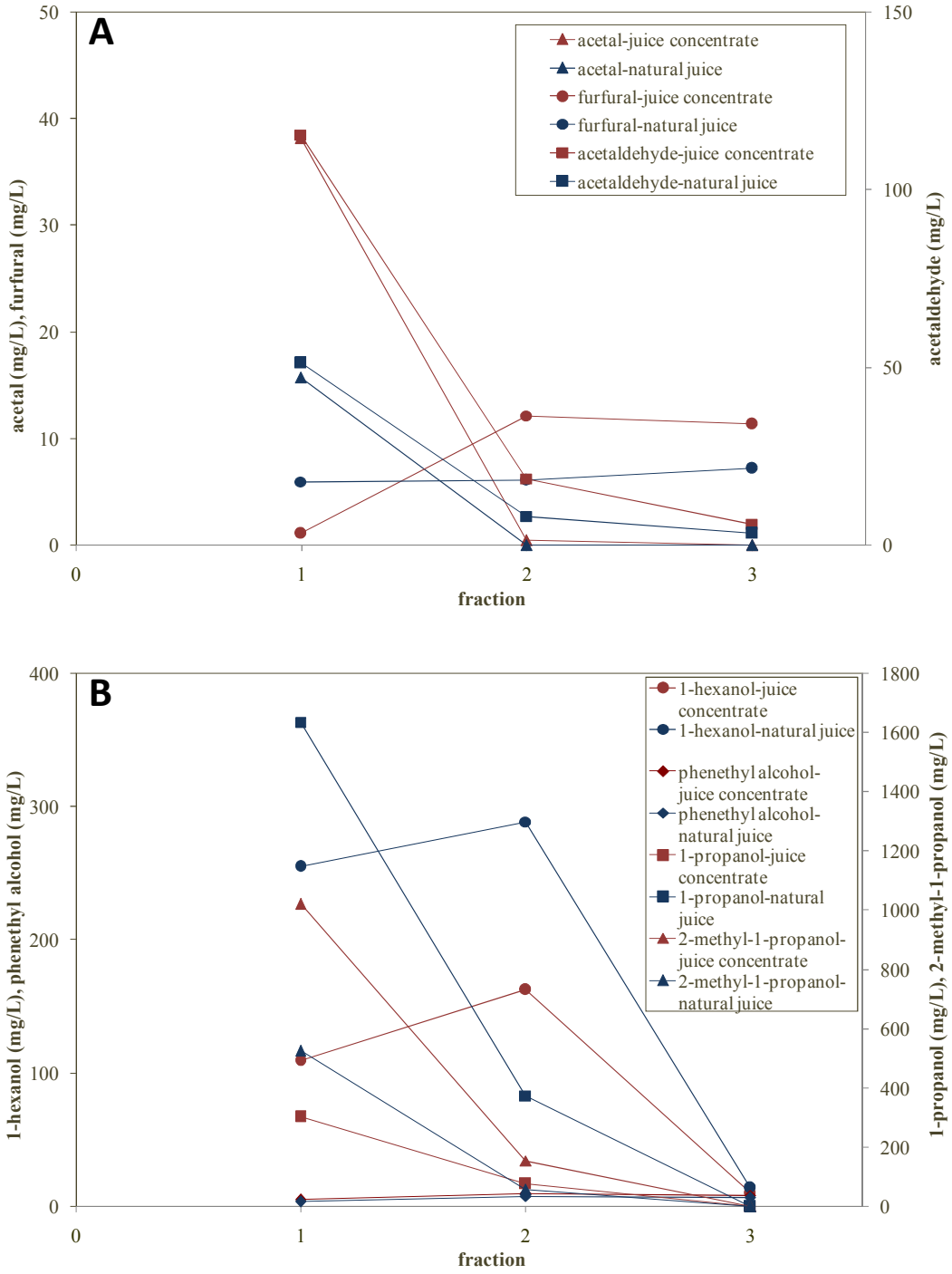

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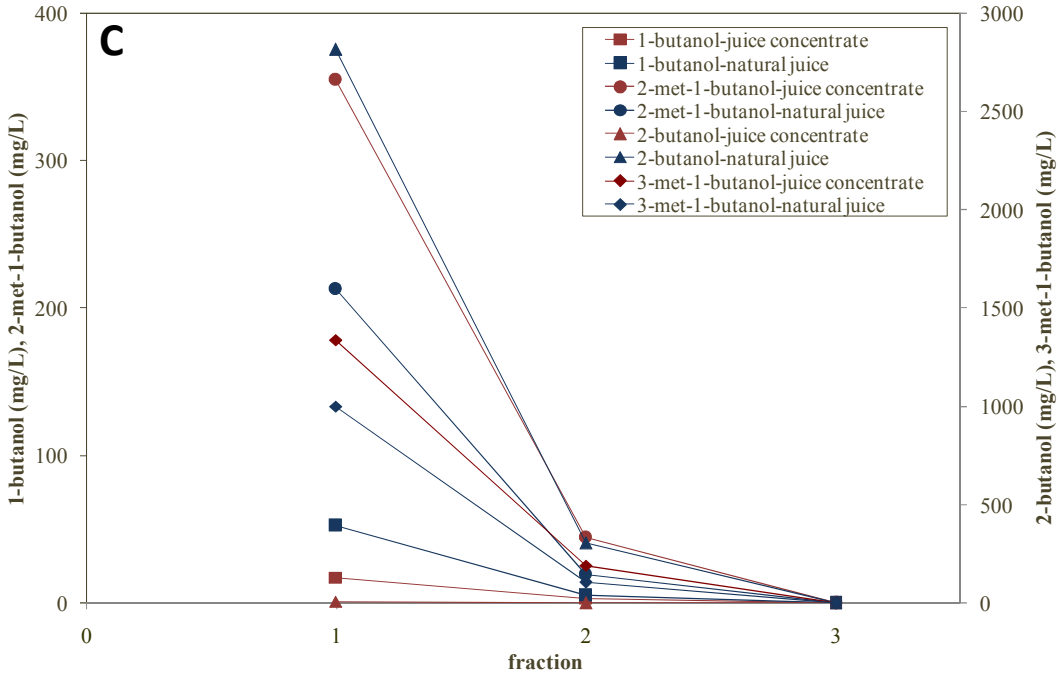

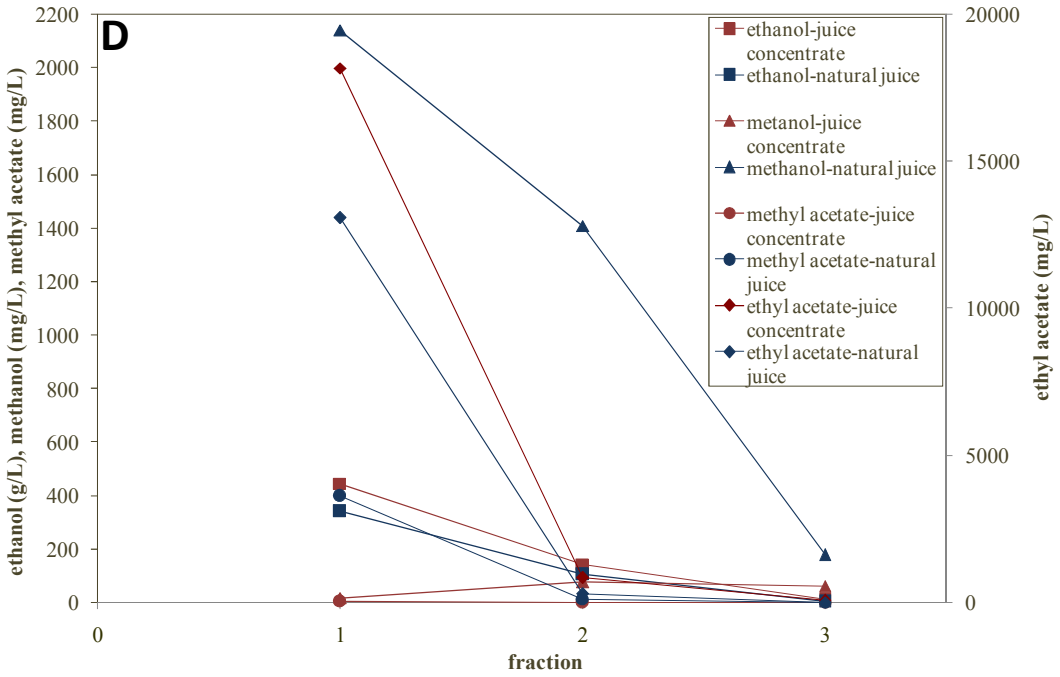

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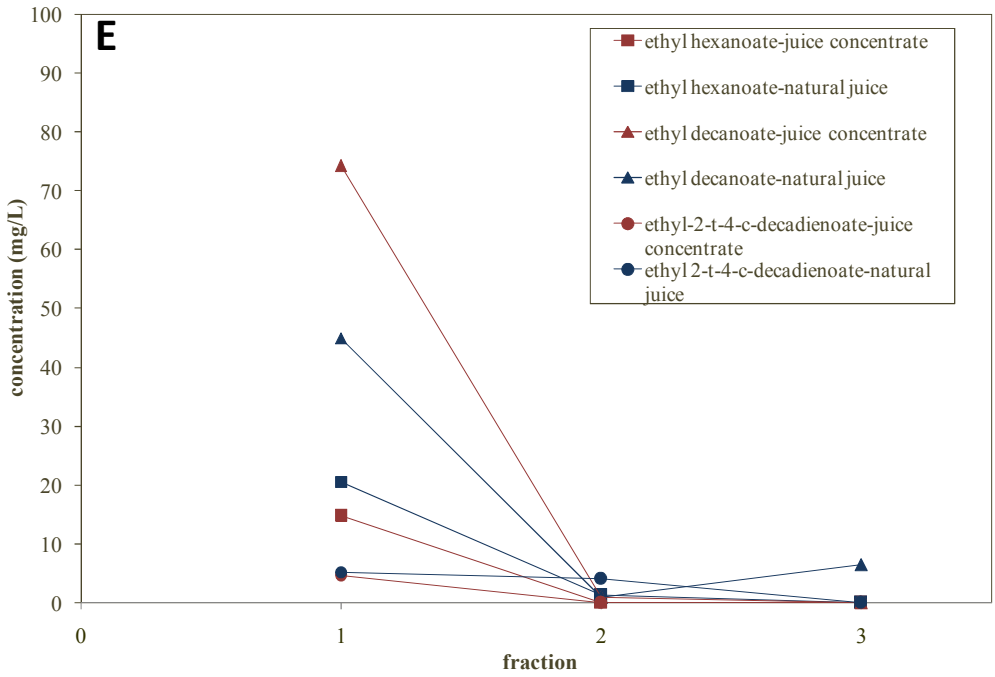

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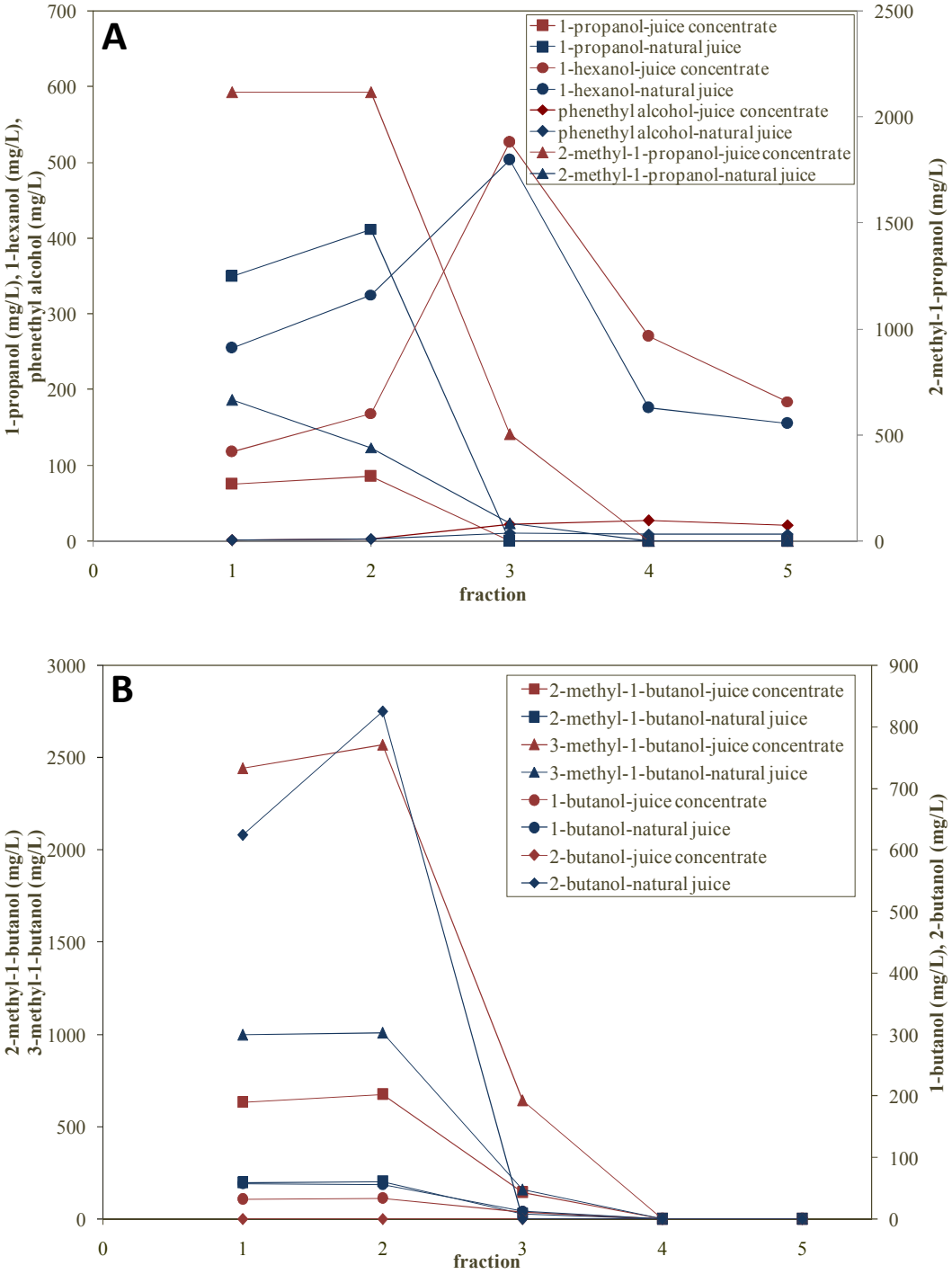

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