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**UNIVERSIDAD AUTÓNOMA METROPOLITANA**  
**Unidad Iztapalapa**

División de Ciencias Biológicas y de la Salud

**Elaborando cerveza con malta de maíz pigmentado: Propiedades químicas, sensoriales y expectativas del consumidor**

**“Brewing with pigmented corn malt: Chemical, sensory properties, and consumer’s expectations”**

T E S I S

QUE PRESENTA

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Para obtener el grado de

**DOCTORA EN BIOTECNOLOGIA**

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*“La utopía está en el horizonte.  
Camino dos pasos, ella se aleja dos pasos.  
Camino diez pasos y el horizonte se corre diez pasos más allá.  
Por mucho que camine nunca la alcanzaré.  
¿Entonces para qué sirve la utopía?  
Para eso, sirve para caminar.”  
-Eduardo Galeano*

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## 2 *Resumen*

El tema de esta tesis está orientado al desarrollo de nuevas cervezas hechas 100% con variedades de maíz pigmentado. El estudio se aproxima desde dos perspectivas: la química y la sensorial. Desde el punto de vista sensorial, esta tesis aborda la conceptualización de la cerveza de maíz, el perfil sensorial de las cervezas de maíz y las expectativas de los consumidores hacia este tipo de cervezas. Desde el punto de vista químico, el estudio se centra principalmente en la caracterización volátil de los granos (maíz azul y rojo), las maltas de maíz y las cervezas de maíz. Además, se realizaron correlaciones entre las propiedades sensoriales y los parámetros químicos para tener una mejor comprensión de la contribución de las maltas de maíz pigmentadas en las características sensoriales de las cervezas.

El objetivo principal de este estudio fue investigar algunos de los aspectos clave que implican el desarrollo de una cerveza elaborada con cereales alternativos como variedades de granos pigmentados y demostrar la viabilidad de utilizar estos granos en la elaboración de cerveza y como una forma de preservar y renovar bebidas tradicionales como Sendecho.

Como resultado del creciente mercado de cerveza artesanal en México, los cerveceros artesanales están en constante búsqueda de cereales e ingredientes alternativos que ayudan a crear estilos de cerveza innovadores y vibrantes para ser añadidos a la oferta de cerveza. Las cervezas artesanales se caracterizan por ofrecer a los consumidores nuevas experiencias sensoriales en términos de sabores y emociones. En la elaboración de cerveza, el maíz ha sido relegado a ser utilizado como una fuente económica de almidón (adjunto). Así, su uso como ingrediente principal apenas se ha explorado y menos aún el uso de variedades pigmentadas de maíz. En este sentido, desarrollamos un proceso para elaborar cervezas 100% con variedades pigmentadas de maíz nativas de México.

Sin embargo, el proceso de creación de un nuevo producto implica muchos pasos que van desde la conceptualización del producto, el desarrollo del producto y las pruebas del consumidor para conocer su aceptabilidad.

Esta tesis se ha centrado en las partes principales que implican el desarrollo de un nuevo producto, en este caso, cervezas hechas al 100% con maíz pigmentado. Esto se abordó en cuatro capítulos, que se describirán brevemente a continuación.

El desarrollo de productos se aborda en los capítulos I y III. Comienza con la caracterización de la composición volátil de los granos (maíz rojo y azul), las maltas y las cervezas finales de maíz, todo en contraste directo con la cebada y las cervezas elaboradas con este cereal. Adicionalmente, investigamos algunos parámetros químicos (compuestos no volátiles) que son indicadores importantes de la calidad de las cervezas.

El capítulo II ha examinado el proceso de conceptualización. Esto implica la creación del concepto de cervezas de maíz y las afirmaciones (emocionales y funcionales) que estas cervezas pueden ofrecer a los consumidores. Esta parte del estudio tenía como objetivo entender si los consumidores aprecian o no la idea de una cerveza hecha 100% con maíz pigmentado. Además, se probaron conceptos en dos países (México y Francia) con el fin de conocer el contraste de la apreciación de los consumidores hacia este tipo de cerveza, así como conocer la viabilidad de ofrecer cervezas hechas con ingredientes locales a la población extranjera.

Finalmente, en el capítulo IV investigamos la aceptación del producto con consumidores de cerveza. Además, determinamos en qué medida las expectativas de los consumidores fueron o no cumplidas.

### 3 *Abstract*

The subject of this thesis is oriented to the development of novel beers made 100% with varieties of pigmented corn. The study approaches from two perspectives: the chemical and the sensory approach. From the sensory point of view, this thesis addresses the conceptualization of a 'corn beer', the sensory profiling of corn beers, and the consumers' expectations towards these types of beers. From the chemical point of view, the study focuses mainly on the volatile characterisation of the grains (blue and red corn), the corn malts, and the corn beers. In addition, correlations between the sensory properties and the chemical parameters were performed to have a better understanding of the contribution of pigmented corn malts in the sensory characteristics of the beers. The main objective of this study was to investigate some of the key aspects that involve the development of a beer made with alternative cereals such as varieties of pigmented corns and to demonstrate the feasibility to use these grains in brewing and as a way to preserve and renew traditional beverages such as Sendecho.

As a result of the increasing craft beer market in Mexico, craft brewers are in constant search of alternative cereals and ingredients that help to create innovative and vibrant beer styles to be added to the beer offering. Craft beers are characterised to offer consumers new sensory experiences in terms of flavours and emotions. In brewing, corn has been relegated to be used as an economical source of starch (adjunct). Thus, its use as the main ingredient has barely been explored, and even less the use of pigmented varieties of corn. In this regard, we developed a process to brew beers made 100% with pigmented varieties of corn native of Mexico. However, the process of creating a novel product involves many steps that go from the conceptualisation of the product, the product development, and the consumer testing to knowing its acceptability.

This thesis has focused on the main parts that involve the development of a new product, in this case, beers made 100% with pigmented corn. This was addressed in four chapters, which will be briefly described below.

Product development is addressed in chapters I and III. It begins with the characterisation of the volatile composition of the grains (red and blue corn), malts, and the final corn beers, all in direct contrast to barley malts and barley beers. Besides, we investigate some chemical parameters (non-volatile compounds) which are important indicators of the quality of the beers.

Chapter II has examined the conceptualisation process. This involves the creation of the concept of "corn beers" and the claims (emotional and functional) that these beers can offer

to consumers. This part of the study was aimed to understand whether consumers appreciate or not the idea of a beer made 100% with pigmented corn. In addition, concepts were tested in two countries (Mexico and France) to know the contrast of consumers' appreciation toward this type of beer as well as to know the feasibility to offer beers made with local ingredients to the foreign population.

Finally, in Chapter IV we investigate the acceptance of the product with beer consumers. In addition, we determined to what extent consumers' expectations were or were not met.

## 4 Thesis accomplishments

### Scientific publications in peer-reviewed journals

**Romero-Medina, A.**, Estarrón-Espinosa, M., Verde-Calvo, J. R., Lelièvre-Desmas, M., Escalona-Buendía, H. B. (2021). "*Pigmented Corn for Brewing Purpose: From Grains to Malt, a Study of Volatile Composition*". Journal of Preservation of Food Processing and Preservation.

Chapter I

**Romero-Medina, A.**, Estarrón-Espinosa, M., Verde-Calvo, J. R., Lelièvre-Desmas, M., Escalona-Buendía, H. B. (2020). "*Renewing Traditions: A Sensory and Chemical Characterisation of Mexican Pigmented Corn Beers*". Foods.

Chapter III

**Romero-Medina, A.**, Verde-Calvo, J. R., Lelièvre-Desmas, M., Escalona-Buendía, H. B. "*Use of native pigmented corn in the brewing process and its influence on consumers expectations*". Journal of the Science of Food and Agriculture or Journal of the Institute of Brewing – (ready for submission)

Chapter IV

### Oral communication in international congresses

**Romero-Medina, A.**, Estarrón-Espinosa, M., Verde-Calvo, J. R., Lelièvre-Desmas, M., Escalona-Buendía, H. B. (2016). "*Influence of corn malt in sensory attributes and volatile aroma compound profiles of Mexican artisan beers*"; 18th IUFoST World Congress of Food Science and Technology, 21st – 25th August, Dublin, Ireland.

### Poster communication in international congresses

**Romero-Medina, A.**, Estarrón-Espinosa, M., Verde-Calvo, J. R., Lelièvre-Desmas, M., Escalona-Buendía, H. B. (2018). "*Influence of product information in consumer's liking of artisan corn beers*"; 8<sup>th</sup> Food Science, Biotechnology & Safety Congress, Latin Food, 14-16<sup>th</sup> November, Puerto Vallarta, Mexico.

**Romero-Medina, A.**, Verde-Calvo, J. R., Lelièvre-Desmas, M., Escalona-Buendía, H. B. (2017). "*Sensory profile of a gluten-free beer made 100% with corn malt*"; 36<sup>th</sup> Congress of the European Brewery Convention", 14-18 May, Ljubljana, Slovenia.

**Romero-Medina, A.**, Estarrón-Espinosa, M., Verde-Calvo, J. R., Lelièvre-Desmas, M., Escalona-Buendía, H. B. (2016). "*Characterization of sensory attributes and volatile aroma compounds in Mexican artisan corn beers*"; EUROSENSE 2016, Seventh European Conference on Sensory and Consumer Research, 11-14 September, Dijon, Francia.

### Lecture presentations

**Romero-Medina, A.**, Verde-Calvo, J.R; Escalona-Buendía, H. B. (2018) "*Corn beer*", FES. Zaragoza-UNAM, October, CDMX, Mexico.

**Romero-Medina, A.**, Estarrón-Espinosa, M., Verde-Calvo, J. R., Lelièvre-Desmas, M., Escalona-Buendía, H. B. (2017). "*Influence of corn malt in sensory attributes and volatile aroma compound profiles of Mexican artisan beers*", CIATEJ- 21st July, Guadalajara, Mexico.



# INTRODUCTION

"La cerveza es un vestigio líquido de la  
prehistoria humana,  
y sus orígenes están estrechamente  
entrelazados  
con los de la propia civilización"  
Tom Standage

## 5 Introduction

As one of the most consumed fermented beverages worldwide, beer has been defined as a multifaceted drink regarding its sensory characteristics. From the consumer's point of view, this beverage is related to a multisensory experience, where a handful of flavours, emotions, and expectations are involved.

Beer is a millenary beverage dating back to Sumerian civilization. Its production process involves natural ingredients such as water, barley, hops, and yeast. The interactions of those ingredients along with all the stages of the brewing process generate a broad range of different chemical and non-chemical compounds, which contribute to the sensory attributes of the product.

The beer market represents a big global industry valued at USD 522 million and dominated by multinational companies. This industry comprises 75% of the alcoholic beverages global market (Beer - worldwide | Statista Market Forecast, n.d.; Poelmans & Swinnen, 2013).

In recent years, this market has experienced a significant change as the number of independent brewers and microbreweries has grown. To gain a place in this competitive market, brewers have adopted a marketing strategy based on products that compete according to their quality, flavours diversity, and consumer's experience, instead of low prices and mass production (like the industrial beer).

Mexico is well known as one of the larger exporters of industrial beer. In the last decade, the craft beer boom movement arrived in the big cities and has resulted in a growing legion of enthusiasts who demand products that are different from the typical lager beer (Calvillo, 2017). Thus, in search of innovation, brewers have let their imagination run away to create beers combining novel ingredients and modern techniques.

The use of native cereals to produce fermented beverages in Mexico dates to Aztec civilization. Aztecs used to produce some sort of beer made from the sprouted kernels of corn (Poelmans & Swinnen, 2013). This tradition has been preserved through some indigenous populations to date. For instance, the Mazahua population settled in the Valley of Mexico and some regions of Michoacán are one of the fewest civilizations which still produce and drink a traditional fermented beverage named Sendeché.

Sendeché is a ceremonial drink of pre-Spanish times produced with sprouted criollo maize (*Zea mays* L) and using techniques that resemble the typical brewing process.

Usually, the beverage is prepared with different varieties of pigmented corn (yellow, red, or blue). This beverage has been passed on through generations and nowadays is a fundamental

part of the heritage, culture, and history of this community, especially for its main ingredient: corn.

In the brewing industry, corn is usually used as an economical source of starch. It is added during the mashing stage as an adjunct (unmalted grain). However, its use as the main ingredient to produce beers is poorly explored.

When it comes to product innovation, it is essential to know the chemical and sensory parameters that determine the quality of the product to understand how the specific changes in ingredients and/or the process affect the overall quality of the beer. In addition, the expectation that a new product brings to the consumers and how they react to a new and unexpected flavour is an important determinant to predict the product acceptance and its potential success in the marketplace.

Innovation in the brewing industry and especially in the craft beer market is essential to have a chance to succeed in this market. Thus, in this constant search of improvement and modernization, we aim to prove that the use of native cereals such as pigmented varieties of corn is a feasible option to produce beers that can compete with the traditional barley beers and meet the expectations of consumers, who become more demanding every day.

As the use of pigmented corn as the main ingredient in brewing is fairly new to the Mexican beer market, the research involves the main aspects of the new product development.

## 6 Literature review

### 6.1 What is beer?

Worldwide known, beer is a beverage that has been defined from the emotional to the technical aspects as well as from the consumer's perspective.

From the emotional point of view, *“Beer is an expression of the human spirit since we use technical sciences as a tool to create it, psychology to market and help sell it, but its essence is, and always will be a form of art. Beer style is the art of combining hundreds of factors to create a consistent combination of beer characters. Beer is complexity and all of the diversity it offers, express the variety of the world's lifestyles”* (Papazian, 2017).

From the technical point of view, *“Beer is an alcoholic beverage that involves the fermentation of malted cereals- most commonly malted barley, although wheat, corn, and rice are widely used. The beverage is made with four essential ingredients: water, malt, hops, and yeast”* (Briggs et al., 2004).

On the other hand, consumers have described the *“Beer as a multisensory experience, as it involves a multisensory perception of a wide range of colours, flavours, and textures, as well as a series of emotions, and feelings”* (Calvillo, 2017; Gómez-Corona, Lelievre-Desmas, et al., 2016).

### 6.2 Its origins: From Mesopotamia to France and Mexico

The origins of beer, as well as bread, date back 6000 BC in Mesopotamia when agriculture first developed in this region. Both, beer and bread, are made in similar ways and with the same ingredients. In general, beer involves two natural processes: germination and fermentation, through which all the ingredients undergo a transformation that ends in the production of beer. There are two theories in the development of the beer. It is said that beer was discovered accidentally through bread-making by using grains that were fermented. Others say that beer was developed intentionally as an intoxicant, after bread (Meussdoerffer, 2009; World History Encyclopedia, n.d.).

In Mesopotamia, people used to drink beer as a daily dietary staple to avoid infectious diseases. In the Sumerian civilization, beer was regarded as a gift from the gods to promote human happiness and wellbeing.

Also, in ancient Greece and Rome, this beverage was considered a food, as it contains vitamins and minerals. The Gauls, who named the beer “Cervisia” in honour of Cérès (the Goddess of the harvest), invented the barrel which made it possible to better control the

fermentation and storage of the beer. Thus, beer played an integral role in early societies (Meussdoerffer, 2009).

However, beer as it is recognized nowadays was developed in Europe, specifically in Germany. It was during the Middle Ages period, when the 'knowledge of brewing' spread along with European countries. In Germany and France, the monasteries were the first places where this drink was brewed, firstly for self-consumption, and then in larger and commercial quantities. Thus, the French and Germans perfected the original manufacturing technique, using similar methods as today.

It was in 1489, with a new law in Paris for regulating breweries, when the term "beer" became popular. By this time, only master brewers could brew beer and only from 3 ingredients: grain, water, and hops. The introduction of hops in brewing happened in the 9th century. This ingredient was incorporated due to its antiseptic properties. Yeast (unknown at that time) was added later to the main ingredients to make beer.

In the middle of the 19th Century, the brewing industry grew rapidly both in Europe and in North America. The Industrial Revolution accelerated technological advancements, and with that, massive industrialization started. However, in France, this movement had a negative impact on the local beer production as many companies merged between them, leading to a loss of a long tradition of local brewing. By this time, the most popular beer was the "Pils" or most commonly called "Lager". This golden and clear beer brewed between 7 to 12°C (bottom-fermented beer), was quickly established itself among consumers thanks to its refreshing and clear nature (Meussdoerffer, 2009).

On the other side of the ocean, in the United States, the spreading of breweries was thanks to millions of immigrants that came from Germany and Ireland. The consumption of beer in the "New World" was introduced by Europeans mostly because they believed that water was polluted and carried diseases. Europeans found that in some regions of North America and Latin America there already exists some sort of "beer" made from sprouted kernels of corn (Poelmans & Swinnen, 2013)

Back in 1542 in Mexico, the first Mexican brewery named "El Portal" was established in Amecameca, State of Mexico. This drink was not as popular as today, and it was only consumed by the rich people of the country. By 1882 "Toluca Lager" was established as the first Mexican Lager beer (Rivera Mena et al., 2016).

It was in 1890 when the beer boom took off in Mexico. In the mid-19th Century, the national production of beer was industrialized and in the 20th century, the great Mexican breweries began to emerge. In the year of 1995, the craft beer movement (inspired by the American craft

beers) resurged, and the first ale beer (top fermentation) was created in Mexico City (Secretaría de Agricultura y Desarrollo Rural, 2019).

Nowadays, Mexican beer has a presence in more than 180 countries, since 2018 this sector generates more than 120 million hectolitres, of which around 40 million are exported, making our country the largest exporter. This allowed Mexico to position itself as the first beer exporter and the 4th largest beer producer worldwide (Secretaría de Agricultura y Desarrollo Rural, 2019).

In contrast, due to the II World War, many breweries in France were destroyed leading to a decrease in beer production as well as in the diversity of types of beer. Between 1919 and 1933, the beer market was extremely transformed due to the American prohibition, dropping the number of breweries to a few dozen. Those who survived eventually expanded their influence. This led to the first wave of consolidation of the dominance of the US beer market by Lager beer.

In France, the revival of craft beer comes straight from the United States. It was until 1978 that craft brewing was allowed again. By that time, Americans rediscovered the passion for brewing, especially for new styles such as IPAs. In the 1990s, the number of breweries grew quickly, and this phenomenon was spread to Europe and France. Today, France is the first European country in number of breweries and micro-breweries (named “brasseries”) and is the 8th largest beer producer country in Europe. Nowadays, French breweries represent more than 10,000 different beers (*Brasseurs de France*, 2021).

Beer is a beverage in constant evolution that has changed according to the culture and the technology available. This drink has coevolved along with us, and it has gone from being considered a “food” and part of our diet to an “aspirational” luxury (Forde, 2017).

### **6.3 What is craft beer?**

According to the Brewer’s Association (BA) defining craft beer has been a ‘difficult task as it can be very subjective and a personal experience’. However, this association remarks three points to be considered as “craft beer”: small, independent, and traditional (Papzian, 2015).

The “Asociación de Cerveceros Artesanales” de México (ACERMEX), a not-for-profit organisation that represents small and independent breweries in Mexico, has taken the same parameters as the Brewers’ Association to define a craft beer: 1) to be small: having an annual production up to 1% of the national beer market or less; 2) to be independent: no more than 25% of the business is owned by another “beverage alcohol industry member”; 3) to be traditional: the beer has to be made always with the four ingredients (malted cereal, hops,

yeast and water) and adjuncts could be added to enhance the flavour and experience not to reduce cost. (ACERMEX, 2020).

Many attempts have been done to define 'craft beer' around the world. However, there are no universally accepted definitions of what craft beer is. Nevertheless, some concepts related to craft beer have emerged (ACERMEX, 2020; Brewers Association, n.d.; Morgan et al., 2020):

- Is not mass-produced: it has not to be associated with multinational brewing and organisations.
- The hallmark of craft beer is innovation, in terms of flavour, but maybe either traditional or modern.
- Is made using traditional brewing processes and traditional ingredients, interesting and sometimes non-traditional ingredients are often added for distinctiveness.
- Craft beer uses the best quality raw ingredients with adjuncts to encourage innovation.
- Craft beer is perceived as 'honest', as 'luxury item', as 'a high-quality beer made with care.

Now, the term craft beer has become more mainstream due to the rapid growth of microbreweries around the world (Murray & O'Neill, 2012). The creativity of the brewers has pushed the creation of more flavourful beers, where the use of alternative and local ingredients has been the hallmark of this industry.

#### *6.4 Craft beer movement in Mexico and France*

The development of beer in Mexico and France has taken different ways through history. However, both countries have shown a similar evolution in terms of the craft movement and the development of different styles of beer. In both countries, the boom of microbreweries has been seen as an urban and social phenomenon that has led to the consolidation of an emerging craft industry in continued growth (ACERMEX, 2020; *Brasseurs de France*, 2021).

Before the craft beer movement, the market of beers was dominated by lagers, pilsners, and Vienna-style light. Thus, both Mexicans and French consumers were used to the golden, crispy, and light-flavour beers. Now, microbrewers have leapt into that creative void, creating ambitious styles, many made using local and indigenous ingredients like corn, agave, hibiscus flower in Mexico (Martinez, 2018), and buckwheat and mirabelle plum in France (Greenacre, 2021), to reflect their identity.

Thus, both countries are experiencing a change in beer conception, changing their perspective as well as their consumption habits and likings. Craft beer consumers are more open to

exploring new products and experiencing new flavours. Currently, in Mexico, there is a lack of regulation in terms of definition for craft beers. Thus, Mexico follows the German Purity Law.

#### *6.4.1 A snapshot of the craft beer in Mexico*

The craft beer movement began in the 1960s in the U.S and since then, it has been extended around the world. In Mexico, the first craft brewery has been born in the mid-90s with the creation of the first ale beer inspired by American craft beers (Calvillo, 2017). From 2011 to date, the craft beer market in Mexico has experienced a noticeable growth, with an average annual growth rate of 53% (*BeerectorioMX: Estadísticas*, n.d.).

In concert with this increase in the production and consumption of craft beer, there has also been a marked increase in the number of innovative microbreweries operating in Mexico. According to ACERMEX, Mexico has approximately 940 independent craft brewers along with the country, where Jalisco is the main producer with 34% of the national production, followed by Nuevo León with 15% and Baja California with 8%. Some of the most popular microbrewers in Mexico are Allende, Beer Factory, Calavera, Cinco de Mayo, Ceiba, Concordia, Colima, Casa Cervecera Cru Cru, Fortuna, La Silla, La Patrona, Minerva, and Nevado. These brands are popular because they re-create European beer styles but with some Mexican variations (*BeerectorioMX: Estadísticas*, n.d.).

In seek of innovation and a twist of new flavours, brewers have been experimenting with the use of unconventional ingredients in their beers. Many craft beer brewers are incorporating flavours of Mexican gastronomy (spicy peppers, chocolate, coffee, agave, and even corn) into their beers for interesting results.

#### *6.4.2 A snapshot of the craft beer in France*

As mentioned before, France suffered a decline in beer production and consumption during the Industrial Revolution. During the last three decades, beer was considered a standardised product made for men (“a man’s drink”). It was until the beginning of the 21<sup>st</sup> century that the craft beer market has been in full swing. The number of microbreweries has grown, from having 442 active breweries in 2011 to 2300 in 2021, making France the country with the largest number of breweries in Europe, and the 1<sup>st</sup> in number of independent breweries. Nowadays, France is considered the European leader in the craft beer market (Greenacre, 2021).

Just like in Mexico, there is an organisation that represents the craft and independent breweries in France: the “Syndicat National des Brasseurs Indépendants” (SNBI). According to the SNBI, there are certain criteria to qualify an independent brewery:

- Its production volume must not exceed 200,000 hl per year.
- The brewing must be made by the craftsman, in no case, it can be subcontracted.
- The bottles must mention their name and address.

This beer sector is passing by a grand and fast development, opening on average 5 microbreweries per week. Also, beer (especially craft beer) has become the second preferred drink after wine (Mascr, 2019).

According to Jean-François DROUIN, President du SNBI “French craft beer is booming, as consumers are moving away from standardised industries and are turning to craft, qualitative and local beer, promoting naturalness, transparency and inventiveness” (machopinette.com, 2020).

As well as in Mexico, this industry is reinventing the concept of craft beer by offering a range of beers with new flavours and made with local raw materials such as stale bread and coffee grounds (Fitzpatrick, 2020).

### *6.5 Some sorts of “beers” made with corn*

Along with the development of barley beer in Europe, some types of fermented alcoholic beverages made with corn were developed in South America among ancient indigenous populations during the pre-colonization era (Rivera Mena et al., 2016).

The cultivation of corn began in Mexico about 5,000 years BC. Corn was the basis of the further development of the classical civilizations of Mesoamerica and the Andes (Cassá, 2003). Due to its centrality, this crop was the main important cereal in the social life of the American cultures, taking place the connotation of the plant of religious meaning. Thus, it was associated with rites and myths concerning the Mesoamerican civilizations and divinities (Barkin & David, 2002).

Due to the nutritional properties and versatility of corn, this cereal has been processed into a variety of food and beverages, taking place in uncountable traditions. In Mexico, corn has been consumed in its fermented form for hundreds of years. Since pre-Hispanic times, a great number of traditional fermented and non-fermented beverages have been made and maintained by some populations despite the passage of time. These beverages were mainly consumed during religious ceremonies.

Although the liking of Mexicans towards these beverages has unchanged, the consumption of many of them has declined due to urbanization, the change in food habits over the last twenty years and the decrease and almost disappearance of landraces of corn due to abandonment of the field.

In many of the corn-based beverages, the use of other ingredients such as cocoa, chilli and cinnamon in combination with corn is typical to enhance flavours of the traditional Mexican gastronomy. A list of the most consumed fermented beverages made with corn is presented in Table 1.

Table 1. Fermented corn beverages consumed in Mexico.

Beverage	Description	State
Tejate	Fermented beverage made with cooked corn grains in ash water and grinding them into smooth 'masa' or dough. Other ingredients are added such as cocoa, cinnamon, piloncillo and some flowers.	Oaxaca
Pozol	Non-alcoholic acidic beverage based on maize liquor. Balls prepared from fermented dough are enveloped in banana leaves, dried chilis, honey and cocoa are added.	Southeast of Mexico
Tepache	Alcoholic beverage fermented from corn grains, brown sugar, and water.	Southeast of Mexico
Tejuino or Tesgüino	Alcoholic beverage produced from germinated corn, both ground and cooked with fragments of plants that serve as enzyme sources.	Jalisco
Sendechó	Alcoholic beverage fermented from germinated corn and red chili. Corn dough is resuspended in water, boiled, bestowed, cooled, and inoculated with Pulque	Mexico State

## 6.6 *Sendechó*

Also known as 'Sende', 'Sendechyecho' or 'Senditha', this beverage has been passed on through generations. The Mazahua and Otomi indigenous populations have been responsible to prepare and preserve this traditional drink. It is widespread in some regions of the State of Mexico and the Otomi and Mazahua regions of Michoacán. Sendechó is made with the sprouted corn of the Chalqueño race. Its elaboration process consists of three main steps: a germination process, cooking, and fermentation steps.

- Germination: the corn is left to sprout for about 8-10 days in a hole dug in the ground, covered with soil and 'ocote' leaves. Once the sprouts reach 2-3 cm, they are sun-dried for a few days.
- Cooking: The dried grains are cooked for 8-10h in hot water, where some chilis are added and mixed.
- Fermentation: After the cooking, the drink is cooled and then some 'Pulque' which serves as inoculum is added to it.

Sendechó can be prepared with blue or red pigmented corn varieties. Its taste is always sweet and sour; it is delicate when it's been freshly made and becomes more intense after a few days (*Sendecho*, n.d.). This beverage is a key element of the culture and heritage of many

indigenous communities. It is still used for ceremonial rituals, like weddings or the Otomi carnival. It can only be found in the local Mazahua and Otomi communities, and as young people are no longer interested in such an ancient and labour-intensive product, it is at risk of disappearing (Bernal-Gil et al., 2020).

### 6.6.1 Corn

According to the Food and Agriculture Organization of the United Nations (FAO), corn (*Zea mays* L) is one of the most important cereals both for human and animal consumption and is grown for grain and forage (FAO, 2000). Mexico has been known as the birthplace of this cereal. Corn was domesticated from teosinte (the wild ancestor of corn) in Mexico some 7,000 to 10,000 years ago and quickly spread through the Americas (Pruitt, 2016). Present world production is about 1,404 million tons of grain from about 235 million ha (Food and Agriculture Organization of the United Nations, 2018). Mexico occupies the 7<sup>th</sup> world's place in the production of this crop, with 27.2 million tons. Of this, 86.7% belongs to white corn variety, 12.9% to yellow corn and only 0.4% to other varieties of corn (Food and Agriculture Organization of the United Nations, 2018; Inforural, 2019).

Mexico is home to more than 300 varieties derived from 64 races of native corn. This diversity gives rise to a wide variety of grain colours that range from white to black, yellow, purple, blue, and red (Chaudhary et al., 2014; Herrera Cabrera et al., 2004). The classification of the corn races depends mainly on the geographical zone of cultivation and the food use for which they are intended.

In Mexico, corn represents the main basis for feeding for all Mexicans from the urban to the rural areas. The great importance of this crop in Mexico lays in its implications on the economic, social, and cultural sectors. For most Mexicans, corn is not only a grain but a deep cultural symbol intrinsic to daily life. Since pre-Hispanic times, people have included corn in every possible context like religion, literature and even art. This cereal has helped shape Mexican culture at large. Corn is endemic to the region and is still deeply embedded in indigenous populations where plays an important role in supporting rural livelihoods (Sweeney et al., 2013).

Even though corn takes place in the daily life of Mexican gastronomy, its production has been declining over the last decade due to the internationally market trades, such as NAFTA (The North American Free Trade Agreement). This has led Mexico to increase dependence on imports, especially from the U.S (Barkin & David, 2002; Sweeney et al., 2013).

In this scenario, farmers try to rescue and preserve the diversity of native varieties of corn grains maintaining its production in rural zones. For instance, in some parts of the State of

Mexico, the smallholder farmers grow pigmented varieties of native corn (blue, red, purple, and black) along with the white corn crop (Salinas Moreno et al., 2012).

### 6.6.2 Pigmented corn: Chalqueño race

Mexico has been the centre of the domestication and diversification of corn. According to Sanchez et al. (2000), Mexico possesses 64 races out of the 220-300 existing corn races in all the American continent. The classification of these races is based on their morphology, genetic (isoenzymes) and their final use (tortillas, popcorn, biscuits, snacks, soups, flour and beverages) (CONABIO, 2020; Fernández Suárez et al., 2013). Among them, the Chalqueño race stands out as being the more productive crop. It is used in the preparation of different beverages (Sendechó, atoles, pinoles), soups, biscuits (burritos) and tortillas (Fernández Suárez et al., 2013).

The growth of the Chalqueño race contributes to the conservation and generation of *in situ* genetic diversity of this crop. Also, it preserves traditions as it is reproduced from one generation to another. Chalqueño race belongs to the corn varieties group named 'Cónico', which includes other varieties such as Negrito, Palomero, Mixteco, Elotes cónicos, Arrocillo, among others. Due to their characteristics, the races of this group can grow at high altitudes (> 1800 m) in volcanic soils, as well as in soils with high humidity. The Cónico group is also characterised by its pyramidal-shaped ears and coloured tooth-shaped grains. One of the most prominent characteristics of this group is the high content of anthocyanins found in those pigmented varieties.

The Chalqueño race can be distinguished from the other racial groups as it presents blue grain, although there are some red grained populations, long grain and greater diameter of the ear, and a floury endosperm (Herrera Cabrera et al., 2004). This is one of the most popular races in Mexico because of its high yield. Chalqueño grows mainly in the high central valleys of Mexico in almost all the localities of the Valley of Chalco-Amecameca in the State of Mexico and surrounding regions. It is also widespread all over the states of Michoacán, Durango, Zacatecas and in the area known as Mixteca Oaxaqueña (Herrera Cabrera et al., 2004; Mijangos-Cortés et al., 2007).

As mentioned before, the Chalqueño breed comes mainly in blue and red colours, but there are many varieties of this breed that differs in colours and textures. These varieties are used in different ways. For instance, the yellow or white kernels -known as 'cream'- are used to make tortillas and tamales, and the blue and red -this one also known as 'colorado'- are better used to make beverages and small dishes named 'antojitos'.

The grains of the blue varieties of the Chalqueño race takes colours in the hue range of dark blue to purple, while the red grains go from pink to dark red. These grains owe their colour to

anthocyanins, which predominate in these varieties of corn (Salinas-Moreno et al., 2012). The colour is mainly related to the anthocyanin type and content and the size of the grains. For instance, the predominant anthocyanins in the red and blue varieties of corn are pelargonidin-3-glucoside and cyanidin-3-glucoside, respectively. These compounds have been associated with a broad variety of biological activities such as antioxidant, antimicrobial, antimutagenic and anticancer capacity (Abdel-Aal et al., 2006; Ortíz et al., 2011). The use of these varieties of pigmented corn is a matter of high interest due to all the benefits that these compounds bring to health. Additionally, the use of coloured grains has a direct impact on the colour of food and beverages, making these grains of particular interest to the food industry.

### *6.7 Corn in brewing*

As mentioned before, beer is made with malted cereal, hops, yeast and water. The malted cereal preferred par excellence is barley, but some countries opt for the use of alternative and indigenous cereals such as rice, wheat, oat, sorghum and corn as they help to support local agriculture as well as reduce the cost of raw materials. Additionally, beers made with these cereals can be considered gluten-free, pointing out to the increasing gluten-free market developed for celiac disease patients and consumers that want to avoid gluten-containing foods (Bogdan & Kordialik-Bogacka, 2017; Rubio-Flores & Serna-Saldivar, 2016).

Corn grains and their products have traditionally been the adjunct (any unmalted grain used as a complement to the malt base of the beer) of choice among brewers due to their low-cost vs malted barley. Generally, it is added to the brewing process in its unmalted form in conjunction with barley malt to increase the number of fermentable sugars available in wort (Briggs et al., 2004). The use of corn as raw material in the malting and brewing process has recently caught the attention, especially in the craft beer industry.

The knowledge in the use of corn as the main ingredient to develop alcoholic beverages is not new. As corn is deeply ingrained in the Latin American character, it makes sense that it would make its way into beer. Historically, all populations have made use of native cereals available in their region to convert them into beverages. Many indigenous communities of Latin America and African countries have used this cereal to produce traditional corn beer-like beverages. To name just a few: Chicha (Peru), Tesgüino, Sendeché and Ostoche (Mexico), Busaa, Ogi and Pito (Nigeria), Abati (Paraguay and Argentina) (Sangwan et al., 2014).

The use of corn in brewing has three main purposes: to cut costs (as an adjunct, corn grains are cheaper sources of fermentable sugar than malted barley), to improve foam and head retention (corn brings protein into the wort), and to lighten the body and flavour of a beer, which increase drinkability that in some cases could be favourable (Bogdan & Kordialik-

Bogacka, 2017). Additionally, corn has been used in countries where the quality of the barley is poor and expensive. Also, the particular laws that take place in each country about beer taxation have forced brewers to develop alternative brewing procedures to utilise local crops such as corn, rice, wheat and sorghum (Goode & Arendt, 2006). Thus, the use of adjuncts, and especially the use of corn as an adjunct differs among countries. For instance, the United States and countries of Africa use to replace between 40 to 75% of barley malt with unmalted grains, and corn is the preferred cereal to use (Bogdan & Kordialik-Bogacka, 2017).

## 6.8 Corn beers

As mentioned before, brewers and in particular, the craft beer industry is facing a more competitive beer market. Many craft brewers are taking ingredients of their gastronomic culture (like chocolate, cinnamon, vanilla, and corn) and adapting them to the brewing process.

Nowadays in the beer market, it can only be found a few brands (Table 2) that use corn as the main ingredient, but two important differences can be observed: 1) the corn is used in the unmalted form and 2) the beers are made with yellow varieties of corn. These beers are promoted as gluten-free alcoholic beverages. Table 2 shows a list of the beers that can be found in the market and that declared corn as an ingredient.

Table 2. Beers in the market made with corn.

Brand	Beer name	Characteristics	Country
El Bolsón	Celiac's beer rubia	A lager style, made with yellow corn.	Argentina
World Top	Against the grain	Made from lager malt, maize, hops, and yeast.	Britain
San Miguel	San Miguel gluten-free	Made with water, barley malt, corn and hops.	Spain
La Brū	La Brū maíz azul	A cream ale style made with native blue corn, hops and water	Mexico
Teufel	Babalao	Made with purple corn of Oaxaca	Mexico

In the last decade, the research group of the laboratories of Enology and Sensory Evaluation of UAM- Iztapalapa have carried out some studies in the malting technology of pigmented varieties of corn and its use as raw material in the development of beers made from 100% with malted corn. Previous studies have focused on the adequation of the malting procedure of the corn, with the development of a Patent (Verde Calvo et al., 2019). Additionally, other studies addressed the sensory and chemical characterisation of beers made with 100% red and blue corn (Romero-Medina, 2013) and the development of 6 beer styles of blue corn with different types of corn malt (Flores-Calderón et al., 2017).

These beers incorporate the main ingredients of the Sendecho beverage into the brewing process. As Sendecho follows almost the same steps as beer to be produced, the main ingredients (pigmented corn grains and chilli) were transferred to the brewing process. This aims to maintain its pre-Hispanic character, adding ingredients of Mexican cuisine (corn and chilli), in a more popular and consumed beverage: beer.

## **6.9 Product development in scientific research**

To innovate products, it is necessary to pass through a process of product development, which is defined as: *“The sequence of steps or activities which an enterprise employs to conceive, design and commercialize a product”* (Ayağ, 2016). This process involves the research, design, creation, marketing and commercialization of new products or existing products with new features (Ayağ, 2016). The product development process by Ulrich and Eppinger (2018) shows the steps in the creation of new products (Figure ) (Ulrich & Eppinger, 2018).

Inside this process, there is a step named “Research & Development” (R&D), which serves to conceptualize new products and test the viability of the potential product(s). Product development is a continuously expanding list of research actions, involving issues connected with the core product itself and issues concerning quality assurance, technical aspects of production, nutritional value, concepts, labelling, raw materials, technology and marketing (Halagarda, 2008). This process is one of the critical stages as is the linkage between the business organization and its market.

It is well known that Universities are an important resource of science and technology, and the scientific knowledge produced by them can contribute to product innovation in many commercial areas. However, the transfer of scientific research to market has not been successful and only a few research manages to reach commercial product applications. Thus, Mesa et al.(2019) have explored the idea that the product development process could help bridge the gap (known as “the valley of death”) between the laboratory and commercial applications and propose that this process should be integrated early in a scientific research project by building the concept, demonstrating potential and developing the product (Mesa et al., 2019).

In general, to bridge the gap between research and market, it is important to understand and recognise how design and science are related and how they differ. While science is a source of knowledge and is focused on discovering, the design claims innovation and novelty (Mesa et al., 2019).

Creating concepts is one of the main steps in the product development process and is in that phase where the research made by science has more implications. These concepts serve as

an intermediary between science and market and can translate different needs and interests into a shared language.

According to Mesa et al; (2019), science can share some responsibility of each phase of the product development process, especially in the early stages, to be able to transfer the knowledge (product, technology) to the market. Figure 1 describes the phases of the product development process proposed by Ulrich and Eppinger (2018) and shows the role that science takes in each one when a new product is conceived.

The goal of incorporating the product development process in this academic research is to create prototypes of new style(s) of beer that have solid bases of research in terms of functionality, viability, desirability, and feasibility of the product. Though, this project was focused only on the phases of the product development process (Figure 1) that were possible to be carried out in our laboratory and pilot plant. Also, as this doctoral thesis is part of a big project in the development of corn beers, previous research has already demonstrated the feasibility of the use of varieties of pigmented corn in brewing. Thus, this investigation will be focused on some specific parts that have not been taken into account in previous research.



Figure 1. Product development process and science role in each phase.

### 6.10 Sensory evaluation for new product development

Nowadays sensory evaluation has been widely used in industry especially for quality inspection, product design and marketing (Zeng et al., 2008). However, it has been difficult for this discipline to get its voice heard in food research and development. But what does sensory evaluation mean?

The classical definition of sensory evaluation is: *“Sensory evaluation is a scientific discipline used to evoke, measure, analyse, and interpret reactions to those characteristics of products or material as they are perceived by the sense of sight, smell, taste, touch and hearing”* (Dijksterhuis, 1996; Stone & Sidel, 2004).

This discipline was developed to study the reactions of consumers to certain characteristics of food products. But sensory evaluation can be used in other situations. For instance:

- It may be used for preliminary analysis to measure the sensory characteristics of a product (colours, odours, flavours and textures) and to provide clues for later chemical or physical analysis,
- To measure changes in sensory characteristics due to a change in ingredients and processing of the food,
- To measure consumers acceptability and expectations to find out whether they like or not a specific product,
- To know how a group of subjects uses their senses to interact with the environment, products and other persons.
- To launch or modify a product based on the sensory characteristics and the consumer’s responses (hedonic liking, perceptions, attitudes and emotions)

In brewing science, sensory evaluation has been used to know the sensory properties of the beverage, as well as the sensory changes during the beer process, fermentation and storage (Vanderhaegen et al., 2006). Additionally, some studies have explore the consumer’s hedonic response, preferences, acceptability (G. Donadini et al., 2014; Guinard et al., 2001), the cultural representation and emotional response of beer (Chaya et al., 2010; Gómez-Corona, Escalona-Buendía, et al., 2016; Gómez-Corona, Lelievre-Desmas, et al., 2016).

Descriptive analysis is used in product development to:

- Characterise prototypes
- Determine the effect of ingredients substitution and/or process changes
- Provide insights into consumers’ evaluations
- Benchmarking against existing products and in-market competitors

### ***6.11 Challenges in the development of beers corn-based***

The process of developing a new product always face challenges from both the technological and the marketing points of view. When it comes to food development the process begins with

a concept and ends with either the entry of the product in the market or the maintenance of the product in the marketplace (Rudolph, 1993). In this case, the development of beers made with pigmented corn malts involves many steps of the development process:

1. The creation of a 'corn beer' concept that best reflects the properties of the product and that appeals to the emotional response of the target consumers.
2. The development of the beer prototypes, which will begin with a micro-scale production and eventually it will scale up. This step also encompasses the malting and the brewing process, which must be adapted to the use of pigmented corn.
3. The product analysis will be focused on both the chemical and the sensory aspects.
4. The product testing, which will consist in determining the consumer's liking and expectations of the new products.

From the technological point of view, the use of corn to produce malt has not been as explored as barley. Much less has been the research of pigmented corn grains in the malting process, and to our knowledge, there are no studies on this field. The main challenges come with the chemical composition of the corn that will directly affect the malting process and the chemical and sensory properties of the beers. Also, it is well known that even small changes in the ingredients and/or the process produce changes in many properties of the product. Thus, it is expected that the replacement of barley malt with pigmented corn malt will influence the chemical product quality and its sensory properties such as colour, flavour, taste. All of these factors along with the tradition and customs will influence the consumer's expectations of the beers (Goode & Arendt, 2006).

From the marketing point of view, the development of 'new' styles of beer is always challenging in the craft beer industry. Innovation has become the insignia of the craft beer movement, and brewers are always looking for innovative and novel ingredients and procedures that make their beers unique, making brewing a matter of trial and error. Additionally, beer is a familiar product category in which consumers often have clear expectations about its flavours and tastes. Thus, when it comes to a new product with novel sensory properties it could be difficult to meet consumers' expectations.

Using pigmented corn as the main ingredient in brewing could be a striking idea for Mexicans, as we are used to consuming corn in our daily life. In addition, craft beer consumption is continuously increasing in Mexico and around the world. Considering this, it is reasonable to assume that this kind of beer could be more appreciated by Mexican beer consumers than foreign consumers. Thus, a good question to address is if corn beers could have a potential

market in countries where are used to drinking craft beers but are not used to eating corn or drink beverages made with corn, such as France.

Taking this into consideration, determining the liking and expectations of the concept of “corn beer” with Mexicans and with foreign beer consumers (i.e., French consumers) could help us to explore the viability of this product with potential consumers early on, and improve its development from their feedback.

Considering that Mexicans have a culture based on corn consumption it is reasonable to assume that the concept of ‘corn beer’ may be accepted more favourably by frequent users of corn. However, in the last years, the popularity of craft beers has increased in France and its beer market is increasing its consumer base for innovative and flavoured craft beers. Beer is now considered a trendy beverage in France and French consumers have a great appreciation for beers made with new ingredients. Thus, a beer made from an intangible cultural ingredient such as pigmented corn could also be attractive for the French beer market.

## 7 *Justification*

Given the previous theoretical background, there is a need to deepen the study of the development of a beer made with native cereals (i.e., pigmented varieties of corn). The use of alternative cereals in the brewing process allows us to create beers with new flavours, but also it gives an added value to the final product. However, as a new ingredient, it must be tested along all the stages of the product development, from the concept to the consumer's acceptability of the final beer.

Testing the concept of a new product is one of the main steps to assure you will launch a product that consumers want to buy. As product developers, testing the feasibility of the concept is a powerful tool to learn more about the needs and desires of our potential consumers, so that potentially successful new products can be identified early on. In addition, the information recollected through consumers serves to optimize the product by listening to their opinions and by focusing on the product concepts with the greatest probability of acceptance. For these reasons, in the early stages of product development, it is important to test as many ideas as possible and explore with different target consumers (i.e., beer consumers from countries with different beer cultures) to know which concept works best and helps you match the product with an ideal market. In this way, we will improve the development of the product by knowing three important aspects of each idea: its acceptability, weaknesses and strengths.

The cultural differences in beer knowledge and choices are noticeable between Mexico and France. However, both countries are showing a growing interest in craft beers, especially those made with authentic ingredients.

When a product is developed it is always necessary to know about all the aspects that involve the product such as its chemical composition and sensory characteristics for full details on product features. This will provide us with the tools to optimize the product to be able to offer the best one to the market.

Finally, when the product is finished, testing the final product with the target consumers will let us know if the beer likes or not and if the product met the expectations of the consumers.

## 8 Objectives

### General objective

The purpose of this thesis is to contribute to a more complete understanding of the use of pigmented corn varieties in the brewing of their chemical and sensory aspects to provide the craft brewing industry with the knowledge of the feasible use of native cereals as an alternative to the production of innovative beers which in turn, could help to revalue traditional drinks like Sendecho.

This was addressed by studying corn beers from two perspectives: the chemical and the sensory perspectives.

### **Chemical perspective:**

- By examining the volatile composition of the raw ingredients (pigmented corn grains and malts).
- By examining the chemical composition (volatile and non-volatile compounds) of the final beers.

### **Sensory perspective**

- By examining the influence of the use of pigmented corn in the sensory properties of the beer.
- By investigating the consumers' perception of the concept of "corn beer".
- By examining the consumers' liking and expectations towards beers made with pigmented corn.

To this end, the specific objectives and sub-objectives were:

**Objective 1:** to have a thorough knowledge of the volatile composition of two varieties of pigmented corn (red and blue Chalqueño varieties) used for brewing and compare it to usual barley malts.

- *Objective 1.1:* To identify the volatile compounds generated during the malting process of the two varieties of pigmented corn grains (red and blue) and the volatiles that comes from the grains and remains in the corn malts.
- *Objective 1.2:* To identify and compare the volatile compounds in the two pigmented corn malts and two commercial brewing barley malts.

- *Objective 1.3:* To determine the key volatile compounds that can serve as markers of pigmented corn malts.

**Objective 2:** Evaluate the perception of potential consumers towards the concept of corn beer in order to know the weaknesses and strengths of the product to produce a beer(s) that meet the expectations of the consumer.

- Objective 2.1: Develop two types of concepts and determine their perception and relevance among two potential beer consumers market (Mexican and French)
- Objective 2.2: Obtain the sensory characteristics that the consumer expects to find in the corn beer with the end of being used in the formulation stage of the product.

**Objective 3:** To build the experimental prototypes of corn beers based on the developed concepts by varying the main ingredient (malt).

**Objective 4:** To understand how the use of pigmented corn malt influences the chemical composition and sensory characteristics of beers

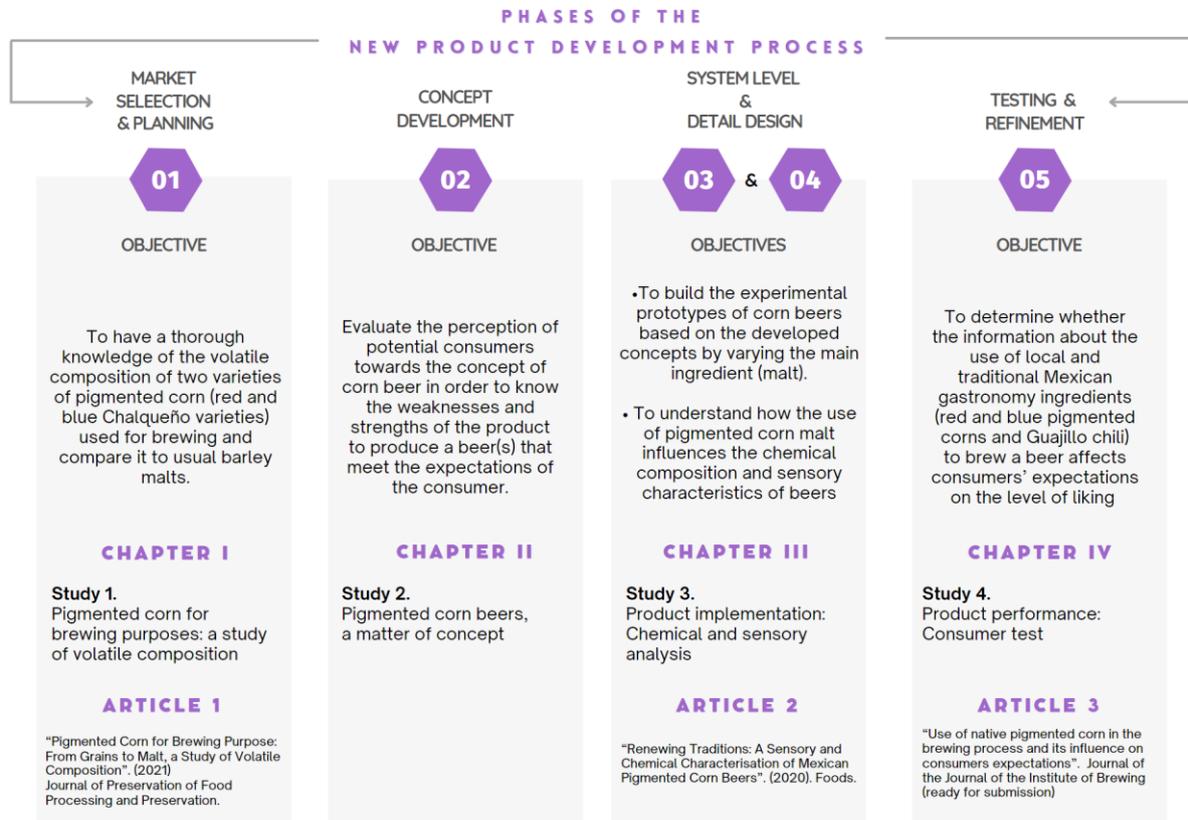
- Objective 4.1: Characterise the sensory properties of beers made with pigmented corn malt.
- Objective 4.2: Characterise the volatile composition and non-volatile parameters of the beers.
- Objective 4.3: Identify sensory attributes that could be influenced by the volatiles and non-volatiles parameters.
- Objective 4.4: identify components (sensory, volatiles and non-volatiles) that can be used as indicators of the use of pigmented corn malt.

**Objective 5:** To determine whether the information about the use of local and traditional Mexican gastronomy ingredients (red and blue pigmented corns and Guajillo chili) to brew a beer affects consumers' expectations on the level of liking

- Objective 5.1: Determine the effect of the information of the beers in three different conditions (blind, expected and informed) on the liking of consumers.

## 9 Thesis structure

A schematic overview of the research objectives and related studies is given below:





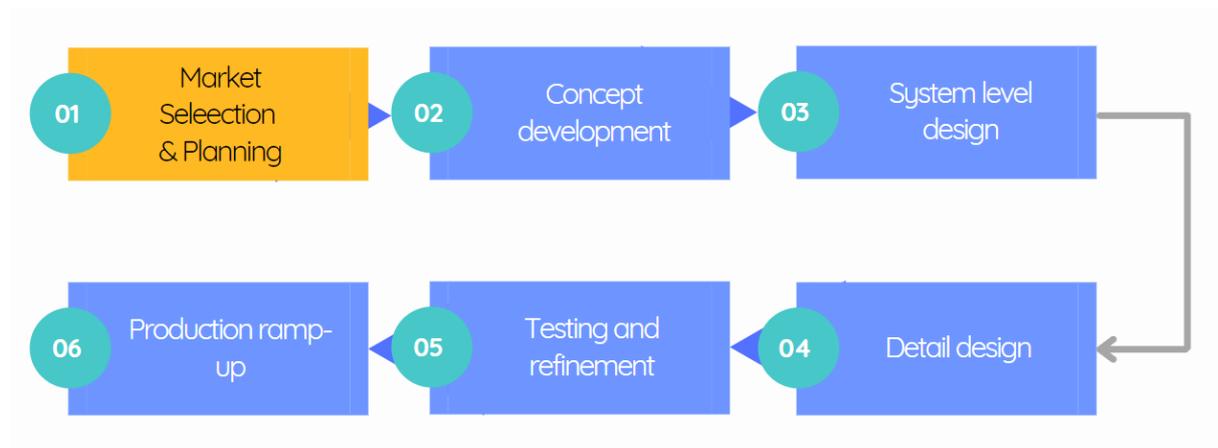
# CHAPTER I

## **Pigmented corn for brewing purposes**

*“Craft is about brewing before marketing,  
about flavour before packaging, about  
integrity and honesty before segmentation  
and exploitation.”*

Pete Brown (beer writer)

## 10 Chapter I – Pigmented corn for brewing purposes



Study 1 comprises Phase 1 of the “New Product Development Process” (Figure 1). This phase is focused on the research to demonstrate the feasibility of the proposed ideas/technology. In this phase, science has a major and active role. Usually, is in this phase where universities spend more resources (money and time), and on the contrary, industry spends little.

### 10.1 Introduction

Beer is a fermented beverage made from four basic ingredients: water, malt, hops and yeast. Each one of these ingredients plays a large and very important role in creating the wide spectrum of aromas, flavours and overall impression in beer. The beer flavour is the result of complex combinations of the raw material along with the malting and brewing process (Bettenhausen et al., 2018). Thus, the understanding of the composition of the main ingredients and their interactions is crucial for optimising the brewing process to develop a beer with a specific flavour character.

Malt is one of the primary ingredients in brewing. It provides the fermentable sugars that are crucial for fermentation, but it also provides volatile and non-volatile compounds that impart colour, flavour, and body to the final beers. There are many types of malt, and its classification is based on the temperatures and duration of the kilning and roasting process. The most common malts are base, caramel, and dark roasted. Each type of malt has its flavour profile, which is largely developed through the malting process (Guido et al., 2007).

Malting is a process consisting of three steps: induce the artificial germination of the cereal (barley, corn, wheat, oats, rye, etc.) to allow the development of enzymes that will convert the grain starches into fermentable sugars. Then the spouting is stopped by drying the grains, preserving the starch for brewing. Finally, different length of temperature and time is applied to the grains in a kiln to obtain the malt. It is the final step of malting that has the biggest impact

on the finished beer as the combination of time/temperature determines the colour, flavour and fermentable sugars that each malt will have and that eventually will contribute to the brew.

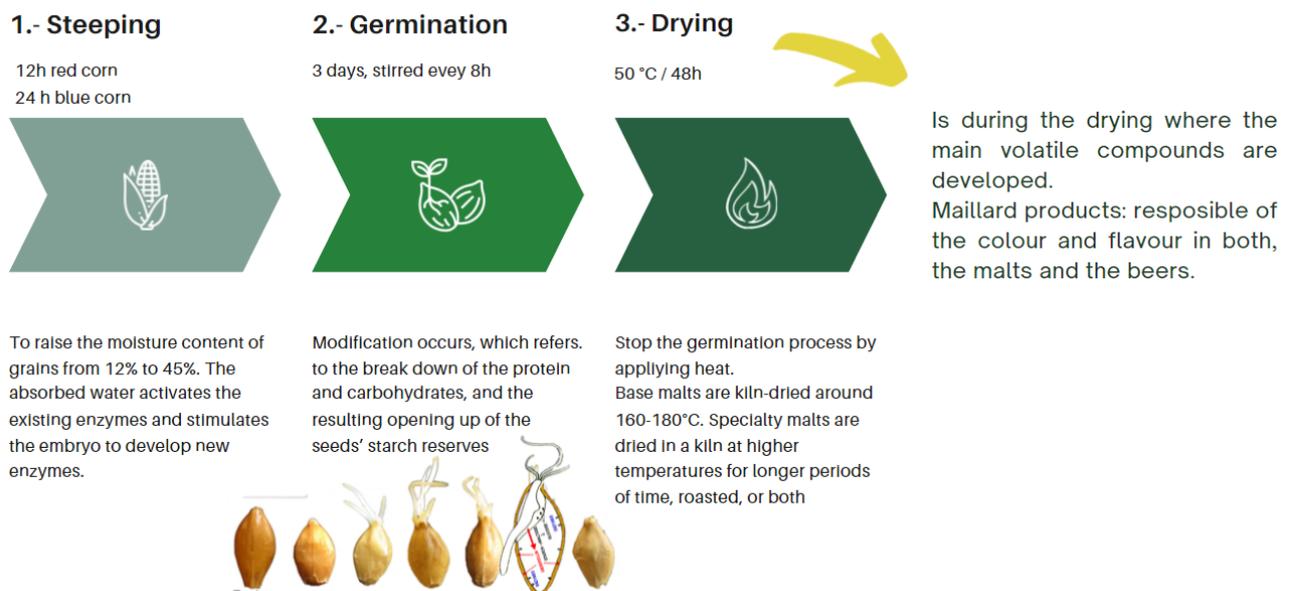


Figure 2. Malting process

Malt is mainly grouped in two categories based on the drying process summited and the quantity of fermentable sugar it contains base malts (low temperatures, high quantity of fermentable sugars and enzymes), speciality malts (high temperatures, low quantity of fermentable sugars and enzymes).

It has been demonstrated that malt flavour is influenced by the type of cereal, its genetic, environmental factors, and their interactions. Also, some metabolites of the malt have a direct influence on beer flavour and mouthfeel sensations (Bettenhausen et al., 2018). However, there is still a poor understanding of the origins of malt flavour, whether developed during malting or kilning or comes from the cereal itself. Although, the composition of the grains could influence the development of certain compounds as the grains are a source of proteins, enzymes and volatile compounds precursors (Bettenhausen et al., 2018).

To this end, this study was performed to investigate the volatile composition of the grains (pigmented red and blue corn) and malts (two varieties of pigmented corn malt and two varieties of barley malt). The specific objectives for this study were:

**Objective 1:** To demonstrate the feasibility of the use of pigmented corn in the brewing process in terms of the volatile composition of the raw material (malt).

- *Objective 1.1:* To identify the volatile compounds generated during the malting process of the two varieties of pigmented corn grains (red and blue) and those volatiles that come from the grains and remain in the corn malts.
- *Objective 1.2:* To identify and compare the volatile compounds in the two pigmented corn malts and two commercial brewing barley malts.
- *Objective 1.3:* To determine the key volatile compounds that can serve as markers of pigmented corn malts.

**Hypothesis:**

There are volatile compounds that comes from the pigmented corn grains that influence the volatile profile of the beers and serve as a fingerprint of the use of pigmented corn malt

**Outcomes:**

- Article 1 - Pigmented Corn for Brewing Purpose: From Grains to Malt, a Study of Volatile Composition

## 10.2 Article 1- Pigmented Corn for Brewing Purpose: From Grains to Malt, a Study of Volatile Composition

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# Pigmented corn for brewing purpose: From grains to malt, a study of volatile composition

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#### Abstract

Malt is one of the most important contributors to beer flavor, this research aimed to characterize the volatile profiles of two varieties of pigmented corn in their unmalted and malted form and determine the differences and similarities between pigmented corn malts and barley malts. A total of 173 volatiles were identified, where phenols and terpenes such as 2-methoxy-4-vinylphenol, phenol, 4-ethyl-phenol, limonene, and geranyl acetone are the main compounds that characterize pigmented corn malts. Volatiles such as 4-ethyl-phenol, 4-ethyl-2-methoxy-phenol, limonene, geranyl acetone, and  $\alpha$ -terpineol might be considered as flavor markers of the red and blue corn grains and corn malts. The results demonstrated there is a group of volatiles specific to corn malts and others that have in common to barley malt. These volatiles can be used as specific key markers for pigmented corn malts. The similarities between barley and corn malts open the possibility to use pigmented corn for brewing purposes.

#### Practical applications

The similarities between corn and barley malts in their volatile profiles make pigmented corn a good alternative to produce beers using native ingredients from Mexico.

## 1 | INTRODUCTION

Beer is a popular fermented beverage prepared from malted cereals, hops, yeast, and water (Briggs et al., 2004). Malt is made from grains, which undergo a germinated process under controlled conditions. Even though any cereal could be converted to malt, barley is the most used and preferred cereal in brewing (Meussdoerffer & Zarnkow, 2009). Sometimes unmalted grains are mixed with ground malt to improve the starchy materials in the mashing process (Briggs et al., 2004). Due to the recent boom of the craft brewing industry in Mexico and around the world, brewers have faced the challenge to create new and original styles of beers. The use of alternative cereals such as wheat, rice, oat, sorghum, and corn, has caught the attention of brewers who have used them in brewing as mash tun adjuncts or as a substitute for barley malt (Flores-Calderón et al., 2017; Rubio-Flores & Serna-Saldivar, 2016).

Presently in brewing, corn is used as unmalted grains (as an adjunct) along with some malted barley to reduce the cost of beer production (Bogdan & Kordialik-Bogacka, 2017; Meussdoerffer & Zarnkow, 2009). Corn (*Zea mays* L.) is the most domesticated and cultivated crop in Latin America. In some regions of Mexico, other varieties named "criollo" are distributed among indigenous communities to preserve the high diversity of pigmented grains. In the State of Mexico, the Chalqueño type is the predominant variety. It comes in different colors that range from blue, red to yellow and white (Herrera-Cabrera et al., 2004). These varieties are mainly destined to prepare traditional dishes and to produce non-alcoholic and fermented beverages (e.g., Atole, Pox, Pozol, Sendecho, Tazcalate, Tejate) (Sangwan et al., 2014).

Recent studies have demonstrated the feasibility of using malted pigmented corn (red and blue grains) to produce beers (Flores-Calderón et al., 2017; Romero-Medina et al., 2020). This option

offers several benefits. First, corn is an economical source of starch for brewing. The use of local varieties of pigmented corn seems an option to limit the need to base beer production on expensively imported barley malt, thus reducing the cost of raw materials while supporting local agriculture (Rubio-Flores & Serna-Saldivar, 2016). Second, pigmented varieties of corn provide a nutritional added value as they are rich in anthocyanins and carotenoids that have antioxidant and anticarcinogenic effects, respectively. Third, at a time when the craft beer market is booming and when consumers are looking for beers with more and more original flavors (Donadini & Porretta, 2017), the use of pigmented corn as raw material seems quite relevant from a sensory point of view.

The flavor is the main parameter for brewers and consumers to judge the quality of a beer. The volatile compounds that come from the raw materials and those generated through the brewing process contribute to the beer flavor. Thus, beer flavor depends largely on the type of malt used (Dong et al., 2015). Previous studies have been carried out focusing on the volatile composition of brewing barley cultivars and the volatiles generated during the malting process (Dong et al., 2015; Gupta et al., 2010; Scholtes et al., 2014). However, to our knowledge, no studies on the volatile composition of pigmented varieties such as red and blue corn in their unmalted and/or malted form have been published yet. The evaluation of grains and malts as raw ingredients is useful not only for quality control but also to improve the understanding of how they contribute to the final sensory characteristics of the beer. As the replacement of barley with alternative cereals such as pigmented corn has been recently explored (Flores-Calderón et al., 2017; Romero-Medina et al., 2020), it is relevant to know which volatiles come from the cereal itself and which are formed during different stages of the process such as the malting process.

The main objective of this study was to have a thorough knowledge of the volatile composition of two varieties of pigmented corn (red and blue Chalqueño varieties) used for brewing (Flores-Calderón et al., 2017; Romero-Medina et al., 2020) and compare it to usual barley malts. More precisely, we wanted to (a) identify the volatile compounds generated during the malting process of the two varieties of pigmented corn grains (red and blue) and the volatiles that come from the grains and remain in the corn malts, (b) identify and compare the volatile compounds in the two pigmented corn malts and two commercial brewing barley malts, and (c) determine the key volatile compounds that can serve as markers of pigmented corn malts.

## 2 | MATERIALS AND METHODS

In this work, six samples were analyzed and compared: two samples of pigmented corn grain (red and blue), two samples of their malted forms, and two samples of commercial barley malts (base and caramel). The abbreviations used in this article to refer to the six samples are given in Table 1. The volatile composition of these

TABLE 1 The six samples and their abbreviations

Sample	Abbreviation
Blue corn grain (unmalted)	BCG
Red corn grain (unmalted)	RCG
Blue corn malt	BCM
Red corn malt	RCM
Barley base malt	BBM
Caramel barley malt	CBM

six samples was obtained using the headspace-solid phase micro-extraction (HS-SPME) technique coupled to gas chromatography (GC) with the mass spectrometry (MS) method. The whole chemical data were statistically analyzed to synthesize and highlight the important results. The different methodological points are described below.

### 2.1 | Chemicals

Reference compounds of the volatiles identified and C<sub>8</sub>-C<sub>40</sub> n-alkane mixture (purity ≥95%, Sigma-Aldrich, Mexico) were obtained commercially: 2-methyl-1-propanol, 3-methyl-1-butanol, 1-pentanol, 2,3-butanediol, 2-furanmethanol, 1-hexanol, 1-heptanol, 1-octen-3-ol, 1-octanol, phenylethyl alcohol, hexanal, benzaldehyde, octanal, citronellal, decanal, undecanal, n-hexane, acetic acid, hexanoic acid, octanoic acid, ethyl acetate, ethyl hexanoate, ethyl octanoate, 2-phenylethyl acetate, butylated hydroxytoluene, 2-heptanone, acetophenone, acetyl furan, furfural, 1,4-dichloro-benzene, 5-methyl-2(3H)-furanone, methyl eugenol, 2-methoxy-phenol, 4-ethyl-phenol, 4-ethyl-2-methoxy-phenol, methyl-pyrazine, dimethyl sulfide, dimethyl disulfide (DMDS), 1,4-cineol, *o*-cymene, limonene, 1,8-cineole, linalool, 1-terpineol, trans-limonene oxide, (-)-camphor, terpinen-4-ol, carvacrol, thymol, caryophyllene.

### 2.2 | Grains and malts

The two red and blue Chalqueño varieties of corn grains were purchased in Milpa Alta, Mexico City. The base and caramel 60 L commercial barley malts (Briess Malt & Ingredients Co., Chilton, WI, USA) were purchased throughout a local brewing store in Mexico City. To obtain the red and blue corn malts (BCMs), both varieties of pigmented corn were subjected separately to a micro-malting following the procedure suggested by Flores-Calderón et al. (2017). Grains were first manually cleaned to remove foreign material. Then, the red and blue corn grains were soaked for 12 and 24 hr, respectively. The samples were germinated for three days to obtain the green malts. To obtain homogenous germination, grains were stirred every 8 hr. The green malts were finally dried in a kiln at 50°C for 48 hr to obtain the corn malts.

### 2.3 | Volatile analysis by HS-SPME-GC-MS

The volatile composition of all the samples of grains and malts was carried out in duplicate by HS-SPME technique coupled to GC with MS method. The six samples of grains (red and blue corn), corn (red and blue), and barley (base and caramel) malts were subjected to the same procedure. The slurry of each sample was made by blending 3 g of ground flour and 8 ml of sodium chloride solution (20%). Each sample was enclosed in 20-ml glass vials which were sealed with a polyethylene and silicone septum cap. Samples were magnetically stirred for 20 min at 20°C ± 1 for sample/headspace equilibration. A 50/30 µm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber (Supelco, Mexico) was used for the extraction and concentration of the volatile compounds. The fiber was exposed to the headspace of the sample's slurry for 60 min with oscillation at 45°C. To prevent contamination from the previous analysis, the fiber was heated in the GC injector at 250°C for 15 min before each use.

GC/MS analyses were conducted using a 7890B/5977A GC-MSD chromatographic system (Agilent Technologies, Palo Alto, CA, USA). Elution and separation of compounds were carried out on an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm film thickness, 190915-433UI). The injector temperature was set at 250°C. The splitless mode was operated in the injector and helium was used as the carrier gas at a flow rate of 1.3 ml/min. The oven temperature was set to 40°C, held for 5 min, raised to 50°C with a heating rate of 2°C/min, then raised 5°C/min to 250°C.

The 5977A MSD (Agilent Technologies, Palo Alto, CA, USA) detector was at 250°C and the quadrupole was operated in the electron-impact mode at 70 eV and in the scan range from 29 to 300 *m/z*, with an ion source temperature of 230°C.

Volatile compounds data were collected with a Mass Hunter GC/MS software (B.07.02.1938). Compounds were identified (Table 2) by comparison of their mass spectra and retention times with both those spectrometric data from the NIST14 MS library database and 55 pure commercial standards. In addition, linear retention indices (LRI) were determined regarding a homologous series of aliphatic hydrocarbons and compared with those reported in the literature. The results are provided in the peak area of the compounds identified.

### 2.4 | Statistical analysis

The data of the chromatographic area of each compound were used to calculate the mean values and the standard deviations. One-way analysis of variance (ANOVA) was performed as well as Fisher's multiple range test (LSD), where significant differences ( $p < .05$ ) were identified between sample means. Then, to explore the differences in volatile composition among the six samples, a principal component analysis (PCA) with Pearson correlation coefficients was performed on the table of volatile compounds (Table 2) containing the means of each compound by each sample. The PCA was also used to identify the key components contained in each sample. All the

statistical analyses were performed using the R Studio Team (version 1.3.959) (RStudio Team, 2020).

## 3 | RESULTS AND DISCUSSION

The complete results of the volatile composition analysis are given in Table 2. A total of 173 volatile compounds were identified from the corn grains and malt samples. To summarize the data and help in the interpretation, the volatiles compounds were categorized into 15 chemical classes: alcohols, aldehydes, alkanes, amides, carboxylic acids, esters, furans, aromatic hydrocarbons, ketones, lactones, phenols, pyrazines-pyrroles, terpenes, sulfur compounds and miscellaneous. For the purpose of this study, the peak areas of the chromatogram were taken as an indirect reflection of the concentration of each compound (Cramer et al., 2005; Dong et al., 2013; Fickert & Schieberle, 1998; Pinho et al., 2006; Saison et al., 2008).

The most relevant chemical classes of all the analyzed samples (grains and malts) were aldehydes, alcohols, esters, ketones, and terpenes which overall reached 61% of the total volatiles. As an overview, the most abundant groups of compounds in all the samples were alcohols, aldehydes, and aromatic hydrocarbons. Among them, the group of aldehydes shows a higher abundance in the samples of malts (corn and barley) than in the unmalted grain samples. On the contrary, terpenes are more abundant in both corn grains than in the malt samples. Additionally, the group of ketones and phenols show more abundance in both red and BCMs than in the rest of the samples. Figure 1 shows the distribution of volatile compounds classes by sample.

### 3.1 | From corn grains to corn malt

Some differences were found in the volatile composition of corn grains compared to their corresponding corn malts, and also between the varieties of corn (blue and red).

It was possible to identify three types of compounds. (a) There was a group of compounds that exist in corn grains itself and remain throughout the malting process; (b) those compounds that were formed during the malting process, (c) volatile compounds that exist in corn and vanish in the malting process. Also, for the first type, some volatiles increased their concentration, others decreased it, and for others no significant change was detected (see Table 2).

A summary of the three types of volatiles will be given highlighting the most relevant of each group. Around 20 compounds were identified in both varieties of corn grains which survive the malting process (type 1). These compounds were: hexanol, 1-heptanol, 1-octen-3-ol, 2-ethyl-1-hexanol, 3-methyl-butanol, 2-methyl-butanol, hexanal, (E)-2-octenal, nonanal, (E)-2-nonenal, 1-octanol, 2-heptanone, 6-methyl-5-hepten-2-one, 3-octen-2-one, acetophenone, (E,E)-3,5-octadien-2-one, limonene, linalool,  $\alpha$ -terpineol, geranyl acetone. Half of them, namely 1-octen-3-ol, 1-octano, 3-methyl-butanol, hexanal, (E)-2-octenal, nonanal, 3-octen-2-one, acetophenone, and

TABLE 2 Volatile compounds identified in pigmented corn grains, com malts, and barley malts

No.	Compound name	LRI <sup>a</sup>	LRP <sup>b</sup>	Blue com grain (BCG)	Red com grain (RCG)	Blue com malt (BCM)	Red corn malt (RCM)	Barley malt (BBM)	Caramel barley malt (CBM)	ID <sup>c</sup>	Flavor <sup>d</sup>
<b>Alcohols</b>											
AU1	2-Methyl-1-propanol	647	609	-	-	-	-	1.8a ± 0.5	-	MS, S	Wine, solvent, bitter <sup>6</sup>
AU2	1-Penten-3-ol	673	659	1.2ab ± 0.5	0.8b ± 0.1	-	-	2.6a ± 1.6	-	MS	Green, vegetable, fruity <sup>3</sup>
AU3	3-Methyl-3-buten-1-ol	746	715	0.4a ± 0.1	-	-	-	-	-	MS	-
AU4	3-Methyl-1-butanol	736	718	2.0b ± 0.4	-	2.3b ± 0.4	1.4b ± 0.5	10.2a ± 2.3	-	MS, S	Whiskey, malt, burnt <sup>6</sup>
AU5	2-Methyl-1-butanol	755	722	1.9b ± 0.5	0.7c ± 0.2	1.0c ± 0.1	-	7.1a ± 0.1	-	MS	Wine, onion <sup>6</sup>
AU6	1-Pentanol	766	753	20.4ab ± 5.8	19.0ab ± 4.7	11.0bc ± 0.5	6.7c ± 1.4	21.7a ± 0.8	4.1c ± 1.5	MS, S	Balsamic <sup>6</sup>
AU7	(Z)-2-Penten-1-ol	769	758	-	-	-	-	5.0a ± 1.7	-	MS	-
AU8	2,3-Butanediol	806	784	1.4b ± 0.3	-	1.3b ± 0.3	1.2b ± 0.6	5.0b ± 1.7	-	MS, S	Fruity, onion <sup>6</sup>
AU9	2-Furanmethanol	851	847	0.3c ± 0.0	-	-	3.5b ± 1.7	2.6b ± 0.2	64.6a ± 2.8	MS, S	Burnt <sup>6</sup>
AU10	1-Hexanol	851	866	58.3b ± 4.7	92.7a ± 29.8	8.5c ± 3.0	3.7c ± 0.1	77.0ab ± 24.7	1.2c ± 0.1	MS, S	Resin, flower, green <sup>6</sup>
AU11	3-Methyl-2-hexanol	906	901	2.2a ± 0.2	-	-	-	-	-	MS	-
AU12	2-Butoxyethanol	909	906	-	1.6a ± 0.01	-	-	2.1a ± 0.9	-	MS	-
AU13	1-Heptanol	962	974	5.0b ± 0.2	6.0b ± 1.0	13.2a ± 2.3	5.7b ± 0.01	4.3b ± 1.8	-	MS, S	Chemical, green <sup>6</sup>
AU14	1-Octen-3-ol	982	982	9.3b ± 0.4	15.4b ± 2.8	32.6a ± 5.9	28.5a ± 3.1	12.3b ± 3.9	14.6b ± 1.0	MS, S	Mushroom, earthy <sup>6</sup>
AU15	6-Methyl-5-hepten-2-ol	994	994	2.2a ± 0.9	2.8a ± 1.0	-	-	-	-	MS	-
AU16	2-Ethyl-1-hexanol	1032	1032	126.7a ± 25.1	94.6b ± 7.0	81.8b ± 19.3	99.5b ± 76.5	180.3a ± 54.2	79b.3 ± 14.4	MS	Rose, green <sup>6</sup>
AU17	Benzyl alcohol	1039	1035	-	-	-	-	2.7a ± 3.0	-	MS	Sweet, flower <sup>6</sup>
AU18	3,5,5-Trimethyl-1-hexanol	1049	1049	-	-	-	-	3.4a ± 1.6	-	MS, T	Green, floral, herbal, cooling <sup>5</sup>
AU19	(E)-2-Octen-1-ol	1069	1070	0.6b ± 0.0	0.6b ± 0.1	-	-	2.2a ± 0.1	-	MS	Green, citrus, vegetable, fatty <sup>5</sup>
AU20	1-Octanol	1072	1073	5.9c ± 0.4	8.9bc ± 1.5	14.9a ± 3.1	10.3b ± 3.2	8.3bc ± 0.3	-	MS, S	Chemical, metal <sup>6</sup>
AU21	Phenylethyl Alcohol	1118	1110	1.1 ± 0.1c	0.5c ± 0.4	2.5b ± 0.7	-	8.6a ± 0.6	-	MS, S	Honey, spice, rose <sup>6</sup>
AU22	1-Nonanol	1154	1172	2.4b ± 0.4	2.5b ± 0.3	1.2c ± 0.7	-	7.6a ± 0.1	-	MS	Fat, green <sup>6</sup>
<b>Aldehydes</b>											
Ad23	2-Methyl-propanal	662	532	-	-	-	12.5b ± 3.5	9.0b ± 1.3	99.8a ± 14.0	MS	Pungent, malt, green <sup>6</sup>
Ad24	3-Methylbutanal	649	631	2.2d ± 1.1	0.9d ± 0.01	12.8cd ± 5.0	1179b ± 35.8	60c.1 ± 15.2	484.6a ± 18.1	MS	Malty
Ad25	2-Methylbutanal	659	640	3.4c ± 2.3	1.7c ± 0.2	4.8c ± 2.5	40.2b ± 9.2	42.7b ± 12.8	473.6a ± 39.8	MS	Malty
Ad26	Pentanal	732	678	4.4b ± 2.6	4.7ab ± 1.7	6.4ab ± 7.9	-	7.0a ± 1.7	8.6a ± 0.01	MS	Almond, malt, pungent <sup>6</sup>
Ad27	2-Methyl-2-pentenal	829	717	-	-	-	1.1b ± 0.2	-	7.3a ± 0.2	MS, T	Pungent, fruity <sup>3</sup>
Ad28	(E)-2-Pentenal	754	739	-	1.1a ± 0.2	-	-	4.1a ± 5.4	-	MS, T	-
Ad29	Hexanal	801	798	38.0c ± 20.0	58.4c ± 10.4	282.3a ± 63.6	153.3b ± 43.6	173.0b ± 6.0	157.5b ± 11.0	MS, S	Grass, tallow, fat <sup>6</sup>

TABLE 2 (Continued)

Compound name	URI <sup>a</sup>	LR <sup>b</sup>	Blue com grain (BCG)	Red com grain (RCG)	Blue com malt (BCM)	Red corn malt (RCM)	Barley malt (BBM)	Camelid barley malt (CBM)	ID <sup>c</sup>	Flavor <sup>d</sup>
Ad30 (E)-2-Hexenal	850	847	-	0.6c ± 0.01	0.7c ± 0.3	1.2c ± 0.5	8.0a ± 0.2	2.8b ± 0.5	MS	Sweet, green apple <sup>5</sup>
Ad31 2-Methyl-2-hexenal	884	86.8	-	-	-	2.5b ± 0.3	-	11.6a ± 2.3	MS	-
Ad32 Heptanal	903	901	-	3.1b ± 0.5	11.6a ± 3.1	6.7ab ± 1.3	9.4a ± 3.5	6.4ab ± 2.2	MS	Fat, citrus, rancid <sup>6</sup>
Ad33 Methional	909	906	0.3c ± 0.0	1.6c ± 0.01	-	8.6b ± 2.8	1.3c ± 0.3	17.8a ± 6.0	MS	Cooked potato <sup>6</sup>
Ad34 Benzaldehyde	960	959	-	8.6c ± 0.3	32.7b ± 5.8	107.6a ± 21.5	24.0b ± 7.7	87.5 ± 28.7	MS, S	Almond, burnt sugar <sup>6</sup>
Ad35 5-Methyl-2-furaldehyde	966	965	-	-	-	-	-	39.8a ± 17.5	MS, T	-
Ad36 2-Ethyl-2-hexenal	964	975	-	-	-	-	-	2.2a ± 0.1	MS, T	-
Ad37 Octanal	1.006	1.002	-	9.9c ± 3.4	38.6a ± 4.8	26.4b ± 6.1	-	-	MS, S	Fat, soap, lemon, green <sup>6</sup>
Ad38 (E,E)-2,4-Heptadienal	1.012	1.009	-	-	-	-	-	12.3a ± 4.0	MS	Fatty, green, oily <sup>5</sup>
Ad39 Benzeneacetaldehyde	1.049	1.043	-	-	14.0c ± 1.9	80.5b ± 15.3	26.9c ± 5.7	171.3a ± 5.7	MS	Honey, floral, rose <sup>5</sup>
Ad40 (E)-2-Octenal	1.060	1.059	2.5e ± 0.0	5.4e ± 0.6	16.0c ± 2.7	21.0b ± 0.1	9.1d ± 0.6	43.9a ± 0.8	MS	Sweet, green, citrus peel, fatty <sup>5</sup>
Ad41 2-Isopropyl-4-methylhex-2-enal	1.097	1.086	-	-	-	-	-	17.7a ± 7.1	MS	Herbal <sup>5</sup>
Ad42 Nonanal	1.104	1.103	4.8c ± 0.1	13.9c ± 3.6	46.5aa ± 8.2	52.7 ± 5.7	28.6b ± 0.2	-	MS	Fat, citrus, green <sup>6</sup>
Ad43 2-Isopropyl-5-methylhex-2-enal	1.106	1.107	-	-	-	4.3b ± 1.5	-	207.0a ± 60.9	MS, T	-
Ad44 (E,Z)-2,6-Nonadienal	1.154	1.153	0.4c ± 0.1	0.6b ± 0.1	-	-	8.2a ± 0.1	-	MS	Cucumber, wax, green <sup>6</sup>
Ad45 (E)-2-Nonenal	1.162	1.159	2.9b ± 0.3	7.0b ± 2.8	5.0b ± 1.7	6.0b ± 0.9	26.0a ± 2.5	7.0b ± 1.3	MS	Cucumber, fat, green <sup>6</sup>
Ad46 Citronellal	1.159	1.195	-	0.9a ± 0.3	-	-	-	-	MS, S	Fat <sup>6</sup>
Ad47 Decanal	1.209	1.205	1.3c ± 0.2	2.2c ± 0.5	7.7ab ± 1.2	9.7a ± 0.7	5.9b ± 1.8	-	MS, S	Soap, orange peel, tallow <sup>6</sup>
Ad48 2,5-Dimethylbenzaldehyde	1.208	1.212	-	-	6.3a ± 1.6	7.2a ± 1.0	-	-	MS, T	-
Ad49 (E,E)-2,4-Nonadienal	1.200	1.212	-	0.7a ± 0.3	-	-	1.0a ± 0.3	-	MS	Watermelon <sup>6</sup>
Ad50 (E)-2-Decenal	1.262	1.261	-	-	2.0b ± 0.8	-	-	3.2a ± 0.2	MS	Tallow <sup>6</sup>
Ad51 2-Phenyl-2-butenal	1.281	1.271	-	-	-	7.3b ± 4.4	-	28.9a ± 1.6	MS	Musty, floral, honey <sup>5</sup>
Ad52 Undecanal	1.308	1.319	5.9a ± 1.7	-	-	-	1.1b ± 0.2	-	MS, S	Waxy, soapy <sup>5</sup>
Ad53 2-Butyl-2-octenal	1.389	1.373	-	3.4c ± 0.9	8.7a ± 2.4	6.8b ± 1.2	-	-	MS	-
Ad54 4-Methyl-2-phenyl-2-pentenal	1.383	1.374	-	-	-	-	-	18.8a ± 1.4	MS	Sweet, floral, honey, powdery, and cocoa-like <sup>3</sup>
Ad55 5-Methyl-2-phenyl-2-hexenal	1.482	1.465	-	-	-	3.6b ± 0.6	0.6c ± 0.01	6.0a ± 0.4	MS	Bitter cocoa, honey, aldehydic, coffee, and nutty <sup>5</sup>
Alkanes										
Ak56 n-Hexane	600	575	8.0a ± 1.8	4.6b ± 0.2	-	-	-	-	MS, S	-

(Continues)

TABLE 2 (Continued)

Compound name	LRI <sup>a</sup>	LRI <sup>b</sup>	Blue com grain (BOG)	Red com grain (RCG)	Blue com malt (BCM)	Red com malt (RCM)	Barley malt (BBM)	Caramel of barley malt (CBM)	ID <sup>c</sup>	Flavor <sup>d</sup>
Alk57 Undecane	1,100	1,101	-	-	-	2.3a ± 0.5	-	-	MS, S	Alkane <sup>6</sup>
Alk58 Dodecane	1,200	1,199	-	-	-	3.6a ± 1.3	-	-	MS, S	Alkane <sup>6</sup>
Alk59 Tetradecane	1,400	1,399	-	-	2.8b ± 0.2	5.7a ± 2.2	-	-	MS, S	Alkane <sup>6</sup>
Alk60 Pentadecane	1,500	1,501	-	0.4b ± 0.01	0.5b ± 0.1	2.8a ± 1.4	-	-	MS, S	Alkane <sup>6</sup>
Alk61 Hexadecane	1,600	1,600	-	1.1a ± 0.01	-	-	-	-	MS, S	Alkane <sup>6</sup>
<b>Amides</b>										
Am62 Benzeneacetamide	1,393	1,394	-	-	-	6.6a ± 5.8	-	-	MS, T	
<b>Carboxylic acids</b>										
Ca63 Acetic acid	600	573	2.9ab ± 1.5	1.5ab ± 0.2	2.4ab ± 1.2	9.2a ± 4.2	7.1ab ± 6.4	-	MS, S	Sour <sup>6</sup>
Ca64 3-Methyl- pentanoic acid	946	997	-	2.3 ± 0.8	3.7 ± 1.4	-	-	-	MS	Sour, cheesy, fresh with fruity notes <sup>5</sup>
Ca65 Hexanoic acid	1,019	1,017	1.1b ± 0.4	1.9b ± 1.2	2.2b ± 1.1	-	20.0a ± 0.9	-	MS, S	Fatty, sour, sweat, cheese <sup>6</sup>
Ca66 Heptanoic acid	1,080	1,090	-	-	-	2.8a ± 1.4	-	-	MS	
Ca67 2-Ethyl-hexanoic acid	1,128	1,127	-	-	-	-	-	2.4a ± 1.4	MS	
Ca68 Octanoic acid	1,279	1,180	-	-	-	3.0a ± 0.2	2.1a ± 0.3	-	MS, S	Sweat, cheese <sup>6</sup>
<b>Esters</b>										
Es69 Ethyl acetate	628	600	-	-	8.0a ± 7.6	-	8.0a ± 2.2	-	MS, S	Pineapple <sup>6</sup>
Es70 Butyl acetate	812	813	-	0.4a ± 0.1	-	-	-	-	MS	Sweet smell of banana <sup>6</sup>
Es71 Ethyl isovalerate	856	849	-	-	-	-	1.4a ± 0.1	-	MS	Fruity, sweet, apple, pineapple <sup>5</sup>
Es72 Methyl hexanoate	1,000	932	-	1.1a ± 0.3	-	-	-	-	MS	Fruity, fresh, sweet <sup>6</sup>
Es73 Ethyl hexanoate	1,002	1,003	-	-	-	-	14.7a ± 4.4	-	MS, S	Apple peel, fruit <sup>6</sup>
Es74 Hexyl acetate	1,014	1,016	-	0.7a ± 0.1	-	-	-	-	MS	Fruity, herb <sup>6</sup>
Es75 Benzyl acetate	1,162	1,164	-	-	-	-	2.3a ± 0.1	-	MS	Fresh, boiled vegetable <sup>6</sup>
Es76 3,5-Trimethylhexyl acetate	-	1,174	-	-	1.5b ± 0.6	-	4.4a ± 0.2	-	MS, T	Sweet, cumin, woody, fruity, spicy <sup>5</sup>
Es77 Methyl salicylate	1,197	1,192	-	0.8b ± 0.2	2.2ab ± 0.8	2.6a ± 1.0	1.8ab ± 0.6	-	MS	
Es78 Ethyl octanoate	1,198	1,198	-	0.7b ± 0.4	1.7ab ± 0.5	2.6a ± 0.9	-	-	MS, S	Fruity, fat <sup>6</sup>
Es79 2-Phenylethyl acetate	1,260	1,257	-	-	-	1.3a ± 0.5	-	-	MS, S	Rose, honey, tobacco <sup>6</sup>
Es80 Phenylethyl valerate	-	1,261	-	-	-	-	0.6a ± 0.2	-	MS, T	Floral <sup>5</sup>
Es81 Pentyl hexanoate	1,289	1,288	-	1.1a ± 0.01	1.0a ± 0.2	-	-	-	MS	
Es82 Amyl hexanoate	1,287	1,288	-	-	-	-	0.7a ± 0.2	-	MS	Fruity, sweet, green <sup>5</sup>

TABLE 2 (Continued)

Compound name	LR <sup>a</sup>	LR <sup>b</sup>	Blue com grain (BCG)	Red com grain (RCG)	Blue com malt (BCM)	Red corn malt (RCM)	Barley malt (BBM)	Caramel barley malt (CBM)	ID <sup>c</sup>	Flavor <sup>d</sup>
Es83 Ethyl nonanoate	1.294	1.296	-	-	1.5b ± 0.3	2.4a ± 2.0	-	-	MS	Waxy, cognac, fruity, apple, and banana <sup>3</sup>
Es84 Butyl carbitol acetate	1.365	1.368	-	2.1bc ± 0.1	3.5b ± 1.0	5.5a ± 0.7	1.4c ± 0.1	-	MS, T	
Es85 3-hydroxy-2,4-trimethylpentyl isobutyrate	1.387	1.373	3.3b ± 1.9	-	-	-	7.0a ± 0.5	-	MS, T	
Es86 2-Ethylhexyl salicylate	1.816	1.806	-	-	-	-	-	0.9a ± 0.3	MS	Floral <sup>3</sup>
<b>Furans</b>										
Fu87 2-Methyl-furan	603	592	3.4ab ± 2.1	2.7b ± 0.2	-	-	-	7.5a ± 3.8	MS	
Fu88 2-Ethyl-furan	702	695	-	0.9b ± 1.0	-	-	2.3a ± 0.6	2.9a ± 0.2	MS	Burnt, ethereal, sweet <sup>3</sup>
Fu89 2,5-Dimethyl-furan	706	694	-	-	-	-	-	1.0 ± 0.01	MS	Chemical, meaty, gravy <sup>3</sup>
Fu90 2-Vinylfuran	765	710	-	-	-	-	-	0.5 ± 0.01	MS	
Fu91 Acetyl-furan	893	911	-	-	-	-	-	98.6 ± 37.0	MS, S	Balsamic <sup>6</sup>
Fu92 Furfural	829	828	0.5 ± 0.0	1.3 ± 0.1	5.5 ± 1.9	41.0 ± 9.1	16.2 ± 2.8	392.0 ± 118.6	MS, S	Bread, almond, sweet <sup>6</sup>
Fu93 2-Pentyl-furan	996	991	8.1 ± 2.0	8.2 ± 1.4	16.8 ± 3.1	13.0 ± 0.1	12.6 ± 5.5	17.0 ± 10.6	MS	Fruity, green <sup>3</sup>
Fu94 3-Phenyl-furan	1.225	1.219	-	-	-	6.0 ± 2.4	-	8.1 ± 3.5	MS	
Fu95 5-Hydroxymethylfurfural	1.233	1.227	-	-	-	-	-	5.5a ± 1.3	MS	
Fu96 2-Hexanoylfuran	1.239	1.266	-	-	-	-	-	5.7a ± 0.1	MS	Sweet, fruity, ketonic, green <sup>3</sup>
<b>Aromatic hydrocarbons</b>										
Ar97 Toluene	773	750	5.0a ± 0.6	2.7b ± 0.6	0.5c ± 0.3	1.8b ± 0.2	2.8b ± 0.3	2.1b ± 0.3	MS, T	Paint <sup>6</sup>
Ar98 m-Xylene	802	855	-	0.5b ± 0.01	0.8ab ± 0.2	-	1.3a ± 0.5	-	MS	Plastic <sup>6</sup>
Ar99 p-Xylene	860	861	2.2a ± 1.0	1.3a ± 0.01	2.1a ± 0.5	-	-	-	MS	Geranium <sup>6</sup>
Ar100 Styrene	893	884	-	-	-	3.1a ± 0.1	-	-	MS	Balsamic, gasoline <sup>6</sup>
Ar101 1,4-Dichlorobenzene	1.015	1.006	101.9 ± 13.1	152.3a ± 1.4	161.0a ± 5.7	248.0a ± 147.4	200.4a ± 71.1	175.5a ± 32.7	MS, S	Mothball-like <sup>7</sup>
Ar102 Maltol	1.088	1.143	-	-	-	-	-	24.6a ± 3.2	MS	
Ar103 Naphthalene	1.214	1.179	-	-	-	3.1a ± 0.1	-	-	MS	Tar <sup>6</sup>
Ar104 Benzothiazole	1.240	1.219	-	-	-	-	1.6a ± 0.4	-	MS	Gasoline, rubber <sup>6</sup>
Ar105 Anethole	1.283	1.265	0.6b ± 0.2	8.6a ± 1.5	-	-	-	-	MS	Sweet, anise, liquorice, medicinal <sup>3</sup>
Ar106 Indole	1.292	1.291	-	-	-	6.8 ± 7.9	-	-	MS	Mothball, burnt <sup>6</sup>
Ar107 Butylated Hydroxytoluene	1.497	1.518	0.9b ± 0.9	14.2a ± 8.8	0.8b ± 0.2	4.8b ± 3.1	-	-	MS, S	Phenolic <sup>7</sup>
<b>Ketones</b>										
Ke108 2,3-Butanedione	593	564	-	-	2.2a ± 1.9	-	-	-	MS	Butter <sup>6</sup>

(Continues)

TABLE 2 (Continued)

Compound name	LRI <sup>a</sup>	LRI <sup>b</sup>	Blue com grain (BCG)	Red com grain (RCG)	Blue com malt (BCM)	Red com malt (RCM)	Barley malt (BBM)	Caramel barley malt (CBM)	ID <sup>c</sup>	Flavor <sup>d</sup>
Ke109 2-Butanone	597	569	-	-	-	2.8a ± 1.2	-	-	MS	Ether <sup>6</sup>
Ke110 2-Pentanone	685	667	-	0.7a ± 0.2	-	-	-	-	MS	Fruity, woody and pungent notes <sup>5</sup>
Ke111 Acetoin	718	697	-	-	-	-	-	1.4a ± 0.1	MS	Butter, cream <sup>6</sup>
Ke112 2-Hexanone	792	784	0.5b ± 0.01	1.2a ± 0.2	-	-	-	-	MS	Ether <sup>6</sup>
Ke113 3-Heptanone	887	884	2.4a ± 0.9	2.9a ± 0.2	-	-	2.9a ± 0.6	-	MS	Fruity ketonic, sweet, musty cheese-like <sup>5</sup>
Ke114 2-Heptanone	895	888	5.4bc ± 0.2	16.2a ± 4.1	12.ab2 ± 3.4	5.4bc ± 0.5	9.2abc ± 5.1	3.7c ± 1.1	MS, S	Soap <sup>6</sup>
Ke115 Di-tert-butyl ketone	930	944	-	-	-	-	-	0.5a ± 0.1	MS	-
Ke116 2,6-Dimethyl 3-heptanone	985	955	-	-	-	3.3b ± 0.8	-	22.1a ± 7.2	MS	-
Ke117 2,3-Octanedione	987	987	-	-	-	-	2.7a ± 0.9	-	MS	-
Ke118 6-Methyl-5-hepten-2-one	988	990	5.3a ± 0.5	6.6a ± 1.3	5.4a ± 1.2	2.3a ± 0.8	7.2a ± 3.1	8.4a ± 7.8	MS	-
Ke119 3-Octen-2-one	1,040	1,041	3.0c ± 0.4	7.3b ± 0.7	20.8a ± 3.8	22.3a ± 2.2	5.7b ± 2.2	-	MS	Nutty <sup>6</sup>
Ke120 Acetophenone	1,041	1,064	1.8b ± 0.4	2.2b ± 0.3	4.6b ± 1.9	21.6a ± 8.6	5.1b ± 1.0	-	MS, S	Mold, flower, almond <sup>6</sup>
Ke121 Furyl hydroxymethyl ketone	1,087	1,080	-	-	-	-	-	1.6a ± 0.4	MS, T	-
Ke122 (E)-3,5-Octadien-2-one	1,098	1,093	1.1c ± 0.2	4.0b ± 0.9	5.2b ± 1.4	8.1a ± 0.7	5.3b ± 0.1	3.4b ± 1.3	MS	Fruity, green, grassy <sup>5</sup>
Ke123 Isophorone	1,124	1,117	0.7a ± 0.1	-	-	-	-	-	MS	Cooling, woody, sweet, green, fruity, and musty <sup>5</sup>
Ke124 2-Tridecanone	1,494	1,499	6.8a ± 8.6	-	-	-	-	-	MS	Fatty, waxy, mushroom, coconut <sup>7</sup>
Ke125 2-Pentadecanone	1,698	1,698	-	-	-	-	-	0.7a ± 0.3	MS	Fresh, jasmine, celery <sup>5</sup>
<b>Lactones</b>										
La126 5-Methyl-2(3H)-Furanone	869	864	-	-	-	-	-	0.7a ± 0.2	MS, S	-
La127 5-Ethylidihydro-2(3H)-furanone	1,056	1,054	0.6b ± 0.01	0.6b ± 0.1	1.a2 ± 0.5	-	1.2a ± 0.6	-	MS	Sweet, creamy, tobacco <sup>5</sup>
La128 γ-Nonalactone	1,362	1,361	1.1b ± 0.2	0.9b ± 0.3	3.0a ± 1.2	-	3.5a ± 0.8	-	MS	Sweet, creamy, coconut, buttery <sup>5</sup>
<b>Miscellaneous</b>										
M129 2,4,5-Trimethyl-1,3-Dioxolane	745	713	-	-	-	-	0.9b ± 0.1	1.7a ± 0.1	MS, T	-
M130 3-Methyl-butanenitrile	737	715	-	-	-	-	0.7a ± 0.01	-	MS, T	-
M131 Hexanenitrile	880	875	-	0.8b ± 0.01	-	-	3.7a ± 0.9	-	MS, T	-
M132 Methyl Eugenol	1,410	1,404	1.1a ± 0.1	-	-	-	-	-	MS, S	Spicy, cinnamon, clove, musty, vegetative, waxy, and peppery <sup>5</sup>

TABLE 2 (Continued)

Compound name	LR <sup>a</sup>	LR <sup>b</sup>	Blue com grain (BCG)	Red com grain (RCG)	Blue com malt (BCM)	Red com malt (RCM)	Barley malt (BBM)	Caramel barley malt (CBM)	ID <sup>c</sup>	Flavor <sup>d</sup>
<b>Phenols</b>										
Ph133 Phenol	980	988	-	0.1b ± 0.01	-	5.8a ± 0.01	-	-	MS	Phenol <sup>6</sup>
Ph134 2-Methoxy-phenol	1,089	1,087	-	-	3.5a ± 0.7	-	-	-	MS, S	Smoke, sweet, medicine <sup>6</sup>
Ph135 4-Ethyl-phenol	1,165	1,167	-	0.6b ± 0.01	3.6a ± 1.1	2.7a ± 1.2	-	-	MS, S	Smoke, phenolic <sup>6</sup>
Ph136 4-Ethyl-2-methoxy-phenol	1,287	1,277	-	0.8c ± 0.1	42.5a ± 9.4	10.3b ± 1.1	-	-	MS, S	Spice, clove <sup>6</sup>
Ph137 2-Methoxy-4-vinylphenol	1,323	1,312	-	-	3.2b ± 0.9	11.6a ± 1.6	-	-	MS	Clove, curry <sup>6</sup>
Ph138 2,4-Di-tert-butylphenol	1,518	1,512	-	-	0.8a ± 0.3	-	-	-	MS	Phenolic <sup>5</sup>
<b>Pyroles and pyrazines</b>										
Py139 1-Ethyl-1H-pyrrole-2-carboxaldehyde	1,046	1,048	-	-	5.9b ± 1.5	31.0a ± 7.7	-	-	MS, T	Burnt, roasted, smoky <sup>5</sup>
Py140 Tetramethyl-pyrazine	1,075	1,085	2.0a ± 0.1	-	-	-	-	-	MS	Nutty, musty, vanilla with dry brown cocoa <sup>5</sup>
Py141 Pyrrole	751	740	-	-	-	1.9a ± 1.3	-	-	MS	Nutty <sup>6</sup>
Py142 Methyl-pyrazine	826	817	-	-	-	6.1a ± 2.0	2.6b ± 0.01	-	MS, S	Nutty, cocoa, roasted, chocolate, peanut <sup>5</sup>
Py143 2,5-Dimethyl-pyrazine	913	908	-	-	-	4.6a ± 1.6	-	-	MS	Cocoa, nutty, peanut <sup>5</sup>
Py144 2,6-Dimethyl-pyrazine	909	910	-	-	-	6.5a ± 2.2	6.2a ± 0.2	-	MS	Cocoa, roasted, nuts <sup>5</sup>
Py145 Vinylpyrazine	940	929	-	-	-	1.4a ± 0.4	-	-	MS, T	-
Py146 2-Ethyl-6-methyl-pyrazine	986	995	-	-	-	10.3a ± 1.9	-	-	MS	Roasted potato <sup>6</sup>
Py147 2-Ethyl-5-methyl-pyrazine	997	998	-	-	-	7.3a ± 1.7	-	-	MS	Nutty, roasted, coffee, cocoa <sup>5</sup>
Py148 Trimethyl-pyrazine	1,005	999	-	-	-	4.9a ± 0.9	-	-	MS	Nutty <sup>5</sup>
Py149 2-Acetylpyrrole	1,045	1,064	-	-	-	-	15.9a ± 2.0	-	MS	Nutty, walnut, bread <sup>6</sup>
Py150 3-Ethyl-2,5-dimethyl-pyrazine	1,078	1,079	-	-	3.0a ± 0.5	-	4.1a ± 1.2	-	MS	Potato, cocoa, roasted nutty <sup>6</sup>
Py151 2-Propionylpyridine	-	1,135	-	-	-	-	3.2a ± 1.1	-	MS, T	-
Py152 2,3-Diethyl-5-methyl-pyrazine	1,159	1,155	-	-	-	2.3a ± 1.2	-	-	MS	Musty, nut sion, earthy, toasted <sup>6</sup>
Py153 3,5-Diethyl-2-methyl-pyrazine	1,156	1,157	-	-	-	2.1a ± 0.1	-	-	MS	Nutty, meaty, vegetable <sup>5</sup>
Py154 1-(2-furanyl)methyl-1H-pyrrole	1,185	1,182	-	-	-	-	3.7a ± 1.1	-	MS	Vegetative, cereal, bready, radish, mushroom <sup>5</sup>
Py155 2-Isoamyl-6-methylpyrazine	1,249	1,250	-	-	-	6.3a ± 1.7	1.8b ± 0.8	-	MS	Roasted potato <sup>5</sup>
<b>Sulphur compounds</b>										

(Continues)

TABLE 2 (Continued)

Compound name	LRI <sup>a</sup>	LRI <sup>b</sup>	Blue com grain (BOG)	Redcom grain (RCG)	Blue com malt (BCM)	Red corn malt (RCM)	Barley malt (BBM)	Caramel barley malt (CBM)	ID <sup>c</sup>	Flavor <sup>d</sup>
Su156 Dimethyl sulfide	505	502	10.5b ± 2.5	13.5a ± 2.5	-	-	-	18.0a ± 3.3	MS, S	Cabbage, sulphur, gasoline <sup>e</sup>
Su157 Dimethyl disulfide	785	727	-	1.5c ± 0.4	1.5c ± 0.01	4.3b ± 0.9	-	9.6a ± 2.4	MS, S	Onion, cabbage, putrid <sup>e</sup>
Su158 Dimethyl trisulfide	974	965	-	-	1.2b ± 0.6	6.7a ± 2.4	-	-	MS	Sulphur, fish, cabbage <sup>e</sup>
<b>Terpenes and Terpenoids</b>										
Te159 1,4-Cineol	1,018	1,013	-	1.4a ± 0.1	-	-	-	-	MS, S	Spice <sup>e</sup>
Te160 o-Cymene	1,020	1,022	3.5a ± 1.7	-	-	-	1.2b ± 1.1	-	MS, S	-
Te161 Limonene	1,033	1,026	3.4ab ± 1.3	2.3b ± 0.1	1.9b ± 0.01	4.6a ± 0.9	1.5b ± 0.9	-	MS, S	Lemon, orange <sup>e</sup>
Te162 1,8-Cineole	1,030	1,028	8.4a ± 0.1	7.8b ± 0.4	-	-	-	-	MS, S	Mint, sweet <sup>e</sup>
Te163 Linalool	1,100	1,098	3.9a ± 0.2	1.0d ± 0.1	-	2.8b ± 0.2	1.9c ± 0.1	-	MS, S	Flower, lavender <sup>e</sup>
Te164 1-Terpinenol	1,137	1,133	1.2a ± 0.1	0.5b ± 0.2	-	-	1.2a ± 0.1	-	MS, S	Lilac <sup>e</sup>
Te165 trans-Limonene oxide	1,132	1,136	-	0.6b ± 0.3	-	-	1.2a ± 0.4	-	MS, S	Fruity <sup>e</sup>
Te166 (-)-Camphor	1,139	1,141	1.9b ± 0.7	2.1b ± 0.4	-	-	4.1a ± 0.3	-	MS, S	Camphor <sup>e</sup>
Te167 Terpinen-4-ol	1,179	1,175	1.9a ± 0.1	-	-	-	-	-	MS, S	Turpentine, nutmeg, must <sup>e</sup>
Te168 α-Terpineol	1,195	1,189	3.2a ± 1.7	2.3a ± 0.5	3.1a ± 1.2	3.0a ± 0.1	4.3a ± 1.8	3.2a ± 1.1	MS	Oil, anise, mint <sup>e</sup>
Te169 Carvacrol	1,298	1,300	2.1a ± 0.2	-	-	-	-	-	MS, S	Spicy, herbal, phenolic, medicinal, and woody <sup>e</sup>
Te170 Thymol	1,297	1,309	0.8a ± 0.3	0.8a ± 0.8	-	-	-	-	MS, S	Phenolic, medicinal, woody, and spicy <sup>e</sup>
Te171 β-Bisabolene	1,498	1,509	-	-	2.1a ± 0.7	-	-	-	MS, T	Balsamic <sup>e</sup>
Te172 Geranyl acetone	1,460	1,453	0.3b ± 0.1	0.6b ± 0.2	1.2b ± 0.3	2.3a ± 0.9	1.1b ± 0.1	1.3b ± 0.1	MS	Floral <sup>e</sup>
Te173 Caryophyllene	1,467	1,420	0.8a ± 0.3	-	-	-	-	-	MS, S	Wood, spice <sup>e</sup>

Note: Chromatographic peak area ( $\times 10^4$ ) of the volatile compounds. Results are expressed as mean  $\pm$  standard deviation ( $n = 2$ ). Means with different letters in the same row are significantly different ( $p < 0.05$ ).

-: no detected.

<sup>a</sup>LRI, linear retention index (NIST values; <http://webbook.nist.gov/chemistry/name-ser.html>).

<sup>b</sup>LRI, linear retention index on HP-5MS column (Agilent Technologies), calculated using n-alkanes C<sub>8</sub>-C<sub>40</sub> and found to be comparable with NIST values (<http://webbook.nist.gov/chemistry/name-ser.html>).

<sup>c</sup>ID, identification used as confirmation of compounds per: MS, library match; S, standards; T, tentative.

<sup>d</sup>Flavor descriptors according to <sup>5</sup>The Good Scents Company (<http://www.thegoodscentscompany.com/>), <sup>6</sup>Flavornet (<http://www.flavornet.org/flavornet.html>) and <sup>7</sup>Pherobase (<http://www.pherobase.com/>).



FIGURE 1 Distribution of chemical groups compounds by sample. BBM, barley base malt; BCG, blue corn grain; BCM, blue corn malt; CBM, caramel barley malt; RCG, red corn grain; RCM, red corn malt

(E,E)-3,5-octadien-2-one showed a significantly increase in their concentrations from grains to malts. The presence of aldehydes has been reported before in barley and wheat flours and (E)-2-nonenal and 1-octen-3-ol have been identified as aroma-active compounds. Also, hexanal, a product of the oxidative degradation of linoleic acid (Cramer et al., 2005; Sayaslan et al., 2000), was the most abundant volatile detected in corn grains and corn malts. Regarding the group of terpenes, limonene has been reported in beers made with pigmented corn malts (Romero-Medina et al., 2020), as well as in some sweet corn products and corn grains adding lemon/orange flavor to the products (Buttery et al., 1994). Some authors reported that this monoterpene seems to have an insecticidal and antimicrobial activity which could be used by the grain for its own protection (Dambolena et al., 2008; de Lucca et al., 2012).

When comparing blue grains and red grains, a significant difference in the group of phenol compounds was found. For instance, phenol, 4-ethyl-phenol, and 4-ethyl-2-methoxy-phenol were found in the red corn grains while blue corn grains showed an absence of phenol compounds. However, these compounds were identified in the BCM, suggesting that were formed during the malting process

(type 2). Also, for the red corn, a significant increase of them was also shown from grains to malt.

In addition, compounds such as dimethyl sulfide, 1,8-cineole, (-)-camphor, 1-terpineol, and thymol comprise the third type of volatiles that were vanished during the malting process for both varieties of corn.

In general, the volatiles that exist in the grains and survive all the malting steps (type 1) and those formed during the malting process, are the ones of the main interest for brewers as the volatile profile of malt will influence to large extent the flavor stability of beer (Dong et al., 2013).

### 3.1.1 | Volatile profiles of pigmented corn and barley malts

As no report of the composition of pigmented corn malts exists in the literature and as barley malt stays the most widely used cereal for beer, for this study, the volatile profiles of the two barley malts served as references to compare with the chemical analysis of the

two pigmented corn malts. The results were also compared to the results of the few studies conducted on the volatile composition of brewing barley cultivars (Cramer et al., 2005; Dong et al., 2015), malted barley, and roasted malts (Beal & Mottram, 1994; Yahya et al., 2014).

Similar volatile profiles were found in all the samples of corn and barley malts (Figure 1). Aldehydes were the predominant group of volatiles on both types of malts (corn and barley), followed by alcohols, ketones, and esters. The greatest content of volatile compounds was found in the samples of barley base malt (BBM) and red corn malt (RCM) as can be seen in Table 2. Among these volatiles, eight compounds corresponding to the group of alkanes and phenols were only identified in the pigmented corn malts.

Among aldehydes, nine namely 3-methyl-butanal, 2-methyl-butanal, hexanal, (E)-2-hexenal, heptanal, benzaldehyde, benzeneacetaldehyde, (E)-2-octenal, (E)-2-nonenal, were common to corn and barley malts. Of them, 3-methyl- and 2-methyl-butanal showed more abundance in the caramel barley malt (CBM) than in the other malt samples. This behavior is consistent with Gijs et al. (2000) who found that the higher the color of malts, the higher the concentrations of these compounds. These two compounds have been associated to be the major contributors to the overall malty and toasted flavors in brewing malts (Beal & Mottram, 1994).

Except for the BCM, 2-methyl-propanal and methional were detected in all the malt samples. These volatiles along with 3-methyl and 2-methyl-butanal belong to the group of compounds known as Strecker aldehydes, which are products of the Strecker degradation reaction that converts  $\alpha$ -amino acids into aldehydes of significant flavor value (Rizzi, 1999). These compounds have been associated with fruity, roasted, and vegetable-like aromas and flavors.

Methional has been identified in corn-based products such as tortillas and tortilla chips, and its presence contributes to the characteristic aroma and flavor of those products (Buttery & Ling, 1995, 1998). To our knowledge, methional has only been reported in caramel malt as a contributor to the overall malt flavor (Fickert & Schieberle, 1998). The importance of this compound lies in its capacity to induce the formation of other compounds that could influence the flavor of the beer. For instance, methional is a precursor of DMDS and dimethyl trisulfide (DMTS), both responsible for undesirable cooked vegetable flavors in fresh and aged beers (Gijs et al., 2000; Liu et al., 2013).

Alcohols such as 1-pentanol, 1-hexanol, 1-octen-3-ol, and 2-ethyl-1-hexanol were identified in both pigmented corn and barley malts. Of those, 1-octen-3-ol and 1-hexanol are considered as potential aroma-active compounds and have been identified in barley and wheat cultivars, as well as in barley malt (Dong et al., 2013). But so far to our knowledge, there are no reports involving malted corn. Alcohols as well as aldehydes are compounds associated with enzymatic activity and are formed from amino acid degradation or lipid oxidation (Zhou et al., 1999).

Different behaviors were observed in the group of esters, although both types of malts samples, corn, and barley malts, were

characterized by a low abundance (peak area) of this group of volatiles. For instance, an absence of esters was observed in the CBM. In contrast, BBM was the sample with a higher number of esters, followed by blue and RCMs. The low abundance of these compounds is expected since esters are mainly formed during the fermentation process, but their presence in malts could be attributed to the esterification of alcohols and fatty acids (Thompson-Witrick & Pitts, 2020). Usually, esters determine the aroma of the fermented beverage to a large extent contributing to the fruity and floral flavors (Meussdoerffer & Zarnkow, 2009; Taylor & Organ, 2009).

Among esters, ethyl octanoate and ethyl nonanoate were found only in red and BCMs. Ethyl octanoate has been reported as a constituent of corn oil and imparts an apple and fruity flavor (Taylor & Organ, 2009). The presence of some methyl esters such as methyl hexanoate has been reported in wheat cultivars (Mattiolo et al., 2017; Seitz, 1995). As far as we know, there are no reports of the presence of esters in corn and barley cultivars, and neither in barley malts.

Concerning the group of ketones, 2-heptanone, 6-methyl-5-hepten-2-one, and (E,E)-3,5-octadien-2-one were found in corn and barley malt samples. Three-octen-2-one and acetophenone were only found in RCM, BCM, and BM. Of them, 2-heptanone and 6-methyl-5-hepten-2-one have been previously reported in corn products, barley cultivars, and barley malt (Dong et al., 2013). These compounds are formed by the oxidation of lipids and could contribute to a buttery, sulfurous, and pungent odor in the final malt flavor (Dong et al., 2013).

Another important contributor of the malt flavor is given by the group of volatiles known as pyrazines, which are produced by the reaction of carbohydrates and amine compounds (Lasekan & Feuaio-Teixeira, 2004). Surprisingly, pyrazines were mostly found in the RCM. Only three of them, namely methyl-pyrazine, 3-ethyl-2,5-dimethyl-pyrazine, and 2-isoamyl-6-methylpyrazine were also identified in the CBM. These compounds are formed during the kiln-dried step of the malting process and are responsible for the nutty, roasted, and toasted flavors in brewing malts.

The main differences between the corn and barley malts were found in the group of phenols. For instance, six volatile phenol compounds were found only in both red and BCMs. These components were phenol, 2-methoxy-phenol, 4-ethyl-phenol, 4-ethyl-2-methoxy-phenol, 2-methoxy-4-vinylphenol, and 2,4-di-tert-butylphenol. According to Seitz and Ram (Seitz & Ram, 2000), 2-methoxy-phenol has been found in corn grains (yellow variety) and has been linked to a strong smoky odor. Moreover, the presence of phenol compounds, especially 2-methoxy-phenol, 4-ethylphenol, and 2-methoxy-4-vinylphenol, have been considered as markers in malts that were submitted to a roasting process (Scholtes et al., 2014). However, in this study, neither of those compounds were found in the CBM sample.

Four-ethyl-2-methoxy-phenol and 2-methoxy-4-vinylphenol were the most abundant (peak area) phenols in BCM and RCM, respectively. Buttery and Ling (R.G. Buttery & Ling, 1995) reported that

4-ethyl-2-methoxy-phenol is one of the major components in products like corn tortillas and tortilla chips. Four-ethyl-phenol, which was identified in both BCM and RCM, and 4-ethyl-2-methoxy-phenol were previously reported in beers made with red and blue corn (Romero-Medina et al., 2020). Volatile phenol compounds in corn malts might be produced by decarboxylation of ferulic and p-coumaric acid, two polyphenolic compounds reported in white and blue varieties of corn (Del Pozo-Insfran et al., 2006).

The presence of phenol volatiles in corn malts could serve as a fingerprint of pigmented corn malts and can be considered as key odorant compounds as it has proved significantly that it influences the volatile profile beers made with pigmented corn (Romero-Medina et al., 2020).

Among the 15 terpenes identified, only  $\alpha$ -terpineol and geranyl acetone were common to the 4 types of malts (BCM, RCM, BBM, and CBM). On the contrary, limonene and linalool were identified in the corn malts and BBM. The number and abundance of terpene compounds were the highest in BBM followed by RCM and BCM. Terpenes, which are formed in plant metabolism, have been associated with citrus and fruity-like flavors, especially limonene and linalool (Bettenhausen et al., 2018), and their presence could be beneficial to the flavor profile of beers. Also, these are commonly known as the most important odor compounds of many plants and corn-based products (Buttery et al., 1994).

The final part presents the results of the statistical analysis carried out on the whole data. Its aim was to consolidate the results detailed in the two previous parts and help us to bring out the key compounds of pigmented corn malts.

### 3.2 | Principal component analysis

To better determine which volatiles contributed the most to the differences among the two varieties of corn grains (RCG, BCG) and the four types of malts (RCM, BCM, BBM, CBM), and to visualize the differences and similarities among them, PCA was applied to all the 173 volatiles identified (Table 2). Figure 2 is a PCA biplot of the six samples of grains and malts based on their volatile composition. The PCA explained 59.47% of the total variance, comprising 31.08% from the first principal component (Dim1) and 28.39% from the second component (Dim2). In Dim1 of Figure 2a, the barley malt samples (BBM and CBM) were clearly separated based on the thermal processing steps that were submitted (BBM-kilning and CBM-roasting). BBM is mainly characterized by alcohols such as 1-octanol, 2-methyl, and 3-methyl-1-butanol, phenylethyl alcohol, (E)-2-octen-1-ol, 2-ethyl-1-hexanol, 1-penten-3-ol, 1-hexanol, 1-nonanol, and 1-pentanol, as well as by two terpenes namely 1-terpineol and (-)-camphor, and one lactone namely  $\gamma$ -nonalactone. In contrast, CBM is mostly characterized by the group of aldehydes, followed by furans, ketones, and pyrroles. Of them, volatiles like 2-phenyl-2-butenal, 2-methyl-2-hexenal, 2-methyl-2-pentenal, 2-octenal, methional, 3-methyl-butanal, furfural, and benzaldehyde, along with DMDS and 2-furanmethanol are the main contributors to differentiate this type of malt.

The corn malt samples were separated depending on the variety of corn (red or blue), and it seems that RCM is better represented in the Dim2 than BCM. Volatiles such as ethyl caprylate, ethyl nonanoate, 2-methoxy-4-vinylphenol, phenol, 4-ethyl-phenol, limonene,

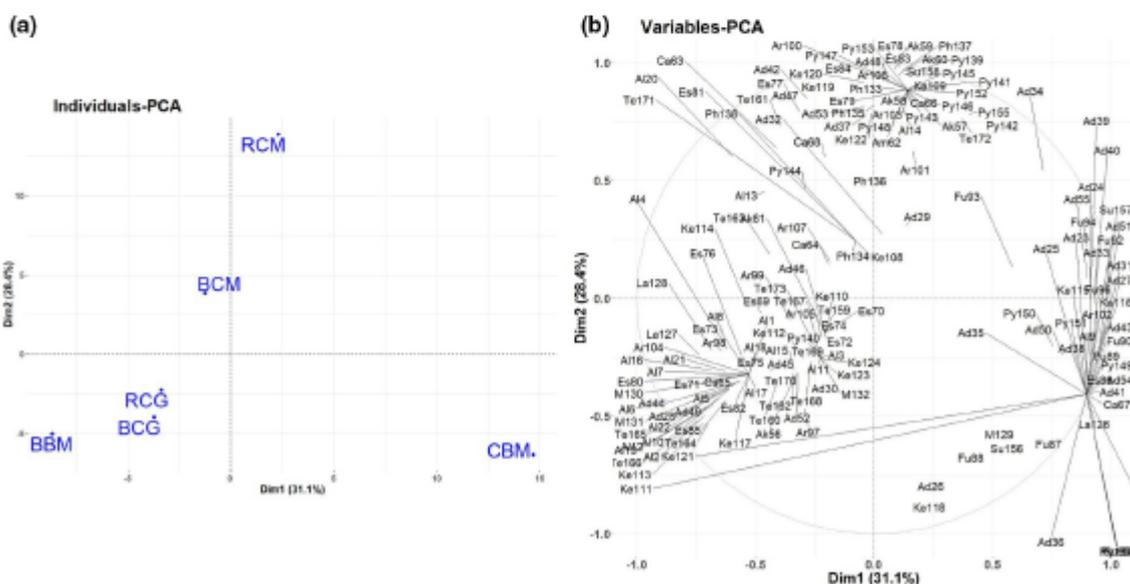


FIGURE 2 PCA of the volatile compounds of the corn grains and corn and barley malts. (a) score plot for the first and second principal components, BBM, barley base malt; BCG, blue corn grain; BCM, blue corn malt; CBM, caramel barley malt; RCG, red corn grain; RCM, red corn malt; (b) loading plot for the first and second principal components, the numbers are consistent with the data in Table 2

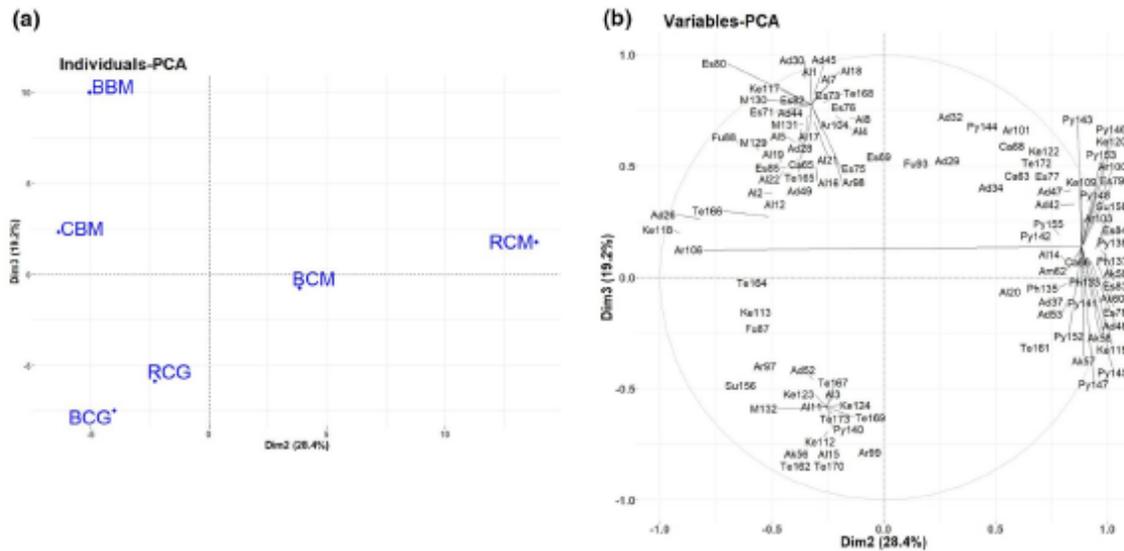


FIGURE 3 PCA of the volatile compounds of the corn grains and corn and barley malts. (a) score plot for the second and third principal components, BBM, barley base malt; BCG, blue corn grain; BCM, blue corn malt; CBM, caramel barley malt; RCG, red corn grain; RCM, red corn malt.; (b) loading plot for the second and third principal components, the numbers are consistent with the data in Table 2

geranyl acetone, DMTS, acetophenone, and 3-octen-2-one are responsible for the differentiation of RCM. These results showed that corn malts (RCM and BCM) are different from each other and can be clearly distinguished from barley malts.

To explain the differences in the grain samples (RCG, BCG), a PCA (Figure 3) was plotted with the second and third principal components (Dim2 and Dim3). Only the variables that have contributed most to the differences were plotted. Collectively, Dim1, Dim2, and Dim3 accounted for 78.67% of the total variation. Grain corn samples (RCG and BCG) were more clearly separated on Dim3, which accounted for 19.2% of the variation. In the PCA scores plot (Figure 3a), Dim3 is related to the malting process. Red and blue corn grains were differentiated from both barley malt samples. In addition, separation along Dim 2 on the score plot is due to the difference in the type of malt (corn and barley).

Corn grains were mostly characterized by terpenes such as terpinene-4-ol, 1,8-cineole, carvacrol, and thymol. Among them, carvacrol and thymol have shown antioxidant properties in some oregano species and sunflower seeds (Dambolena et al., 2008). As mentioned before, the presence of these monoterpenes in corn grains might be explained since these compounds are used for protection to prevent lipid oxidation and the development of rancid flavors in kernels (de Lucca et al., 2012). Dimethyl sulfide was another volatile that contributes to characterizing corn grains (RCG and BCG).

As mentioned before, despite corn malts having a similar profile as barley malts, some volatiles help to differentiate between them, especially volatiles of the group of phenols, pyrazines, and pyrroles.

## 4 | CONCLUSION

Recent studies have demonstrated the feasibility of using malted pigmented corn to produce beers (Flores-Calderón et al., 2017; Romero-Medina et al., 2020). The main objective of this study was to characterize for the first time the volatile composition of the red and blue pigmented corn varieties used for brewing and compare it to base and CBMs.

It was possible to identify certain groups of compounds that allow the differentiation between corn and barley malts, as well as between varieties of pigmented corn. For barley malts, BBM is mainly characterized by the presence of alcohols (responsible for the floral, sweet, and alcoholic flavors), and BCM is better characterized by aldehydes and furans, which usually are responsible for the green, fresh, and grass aroma of the malt. Among the corn malts, RCM is represented by phenols and terpenes such as 2-methoxy-4-vinylphenol, phenol, 4-ethyl-phenol, limonene, and geranyl acetone. Moreover, the results showed the volatiles that exist in each variety of grain itself and remained or vanished during the malting process as well as those volatiles that are formed during malting. Some compounds of the group of phenols and terpenes such as 4-ethyl-phenol, 4-ethyl-2-methoxy-phenol, limonene, geranyl acetone, and  $\alpha$ -terpineol might be considered as flavor markers of the red and blue corn grains and corn malts. The results show the contribution of the corn grains in the volatile profile of corn malts, which helps to understand its eventual influence on the beer flavor profile made with these grains.

Even though there are certain volatile compounds in common between barley and corn malts, there are also some compounds that are characteristics of these varieties of corn and make it possible to

differentiate them among barley malts. Thus, these results confirm that its use in the brewing industry as the main ingredient is feasible as has been shown in previous studies (Flores-Calderón et al., 2017; Romero-Medina et al., 2020). As these varieties of pigmented corn and their respective malts have been characterized for the first time, this work will lay the groundwork for the use of pigmented corn for brewing purposes, bringing an added value to these varieties of corn.

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#### CONFLICT OF INTEREST

The authors have declared no conflicts of interest.

#### AUTHOR CONTRIBUTIONS

**Angélica Romero-Medina:** Conceptualization; Formal analysis; Investigation; Methodology; Writing-original draft; Writing-review & editing. **Mima Estarrón-Espinosa:** Conceptualization; Formal analysis; Methodology; Writing-review & editing. **José Ramón Verde-Calvo:** Conceptualization; Formal analysis; Methodology; Supervision; Writing-review & editing. **Maud Lelièvre-Desmas:** Writing-review & editing. **Héctor B. Escalona-Buendía:** Conceptualization; Formal analysis; Methodology; Supervision; Writing-original draft; Writing-review & editing.

#### DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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### *10.3 Conclusion*

This study revealed for the first time, the volatile composition of both varieties of pigmented corn, blue and red of the Chalqueño race. The volatile composition of both is similar, with some discrepancies for certain compounds. Thus, it is possible to differentiate both varieties according to their volatile composition.

Regarding the samples of malts, the characterisation of the two corn malts (blue and red corn) was made for the first time. As well as with their unmalted grains, corn malts show differences in their volatile composition that make it possible to differentiate between the two types of malts (barley and corn). Compared to barley malts, corn malt samples show a similar volatile composition. However, there are certain compounds, especially in the phenols and terpenes groups, that could be used as fingerprints between barley malts and corn malts.

As malt is known as the “soul of beer” because it largely determines a beer’s colour, flavour and strength, the study of the volatile composition of corn malts turns essential to predict the flavour of the final beers. Thus, with this study, we conclude that corn malt is a feasible option to be used in brewing as its volatile profile could contribute to adding flavours that reflect the character of the corn brew.

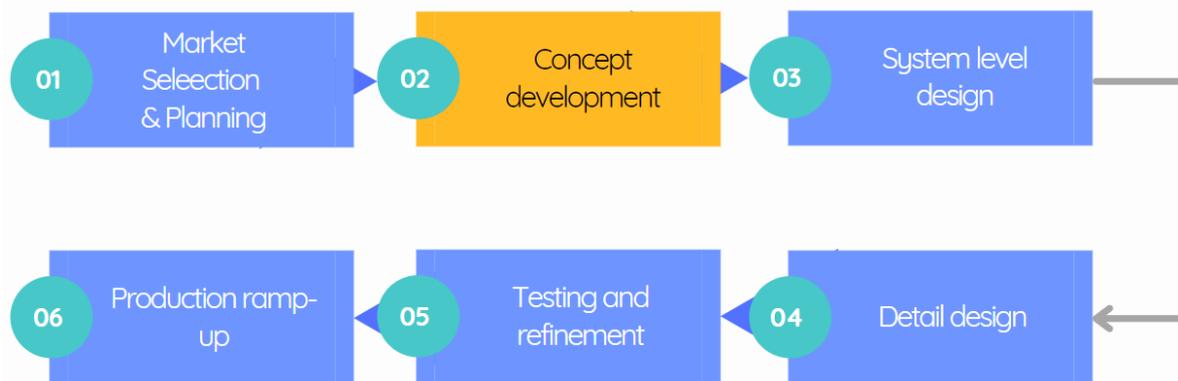


## CHAPTER II

**Pigmented corn beers, a matter of concept**

"De maíz amarillo y de maíz blanco hizo su carne; de masa de maíz se hicieron los brazos y las piernas... Únicamente masa de maíz entró en la carne de nuestros padres, los cuatro hombres que fueron creados..."  
-Popol Vuh

## 11 Chapter II- Pigmented corn beers, a matter of concept



Study 2 comprises the second phase of the “New Product Development Process”. Usually in academic research, this phase is not explored. This phase should have an active role in science, and it is here where the use of sensory science is essential. Concept development and concept testing helps to understand consumer reaction to the product idea and allow to estimate purchase intent and along with that, product success.

### 11.1 Introduction

The craft beer industry is characterised by a growing, versatile and fast-changing market. As a result, brewers have been forced to develop new products and even new brewing techniques to address the consumer’s needs. Despite that this sector of the brewing industry is one of the most innovative and creative when it comes to creating beers, not all beers will succeed in the marketplace. The success of new products depends on both, the brewing process and the marketing strategy. Thus, the creation of a new product concept that reflects and meets the needs of the potential consumers is highly important to improve their chances of success in the marketplace (Ayağ, 2016).

A product concept is a statement with a detailed description of an idea that maximises the best qualities and features of the product described from the perspective of the target consumer. The concept gradually develops alongside the product based on the analysis of the prototypes features and the final product (Hartson & Pyla, 2019).

The concept development process involves the following steps: identifying consumer needs, establishing target specifications, concept generation, concept selection, concept testing, setting final specifications and prototype development (Figure 3) (Ayağ, 2016).

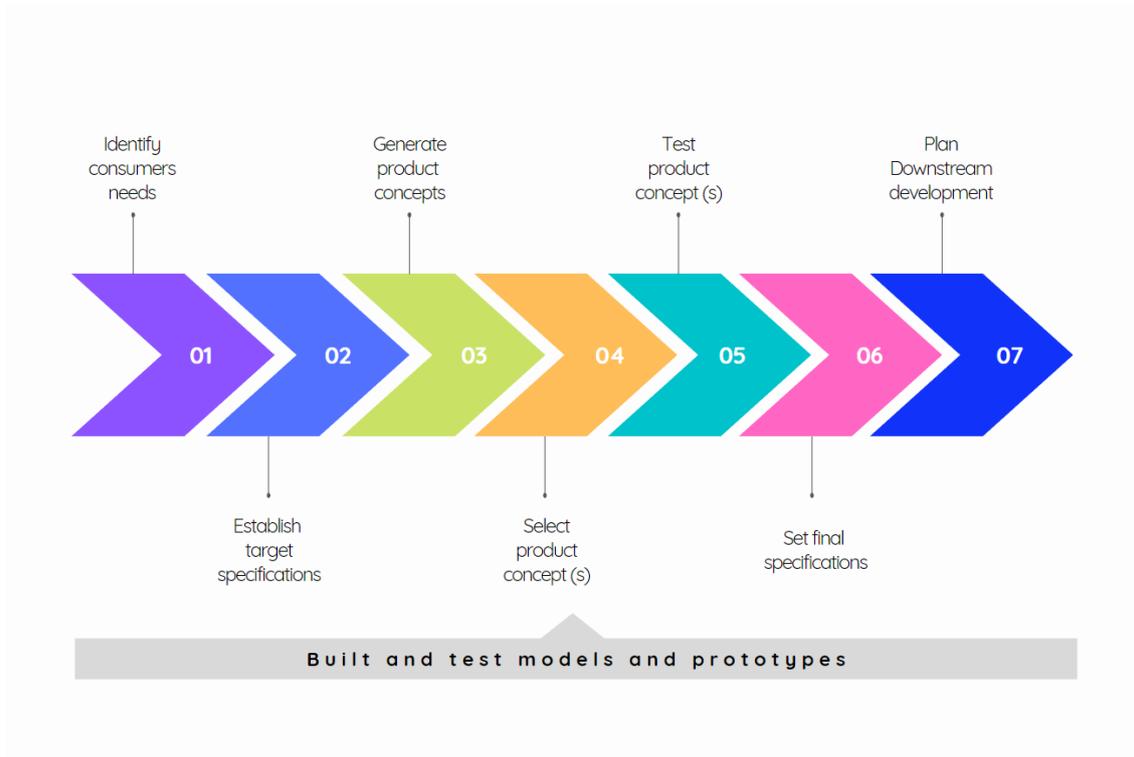


Figure 3. The concept development process (Ayağ, 2016; Ulrich & Eppinger, 2018).

Concepts can be presented as a simple factual statement, as a commercialised concept with persuasive claims or as a full mock advertisement (Lees & Wright, 2004).

An effective product concept should have certain characteristics (Earle et al., 2001; Hartson & Pyla, 2019):

- Be concise, explain what the product is, with no technical details.
- Use common language and attractive descriptions to keep consumers' interest.
- Show at what product category it belongs.
- Be realistic and believable, show the major and real qualities and features of the product.
- Describe the uses and the emotional impact, what experience will the product provide to the consumer over existing products.
- Avoid brand names.

The development of the product concept should consider the major characteristics, benefits and strengths of the product in all aspects. These benefits and strengths may have been built through the sensory (colour, flavours, textures) properties, the chemical functional benefits (nutrients, antioxidants, vitamins, etc) and the nutritional value. Additionally, these

characteristics can reflect the emotional benefits and appeal to the consumer's emotional state, needs and wants (Earle et al., 2001).

Once the concept has been created and selected, the next step is to test it with potential consumers. It is highly recommended to test several product concepts to find the most adequate and the one that suits the best consumers' needs. The consumer testing can be done by focus groups or by consumer survey (30-200 consumers). This evaluation aims to know the consumers' acceptability and liking of the product but also is intended to know the believability and uniqueness of the concept as well as to predict the consumer's intention to buy the final product.

In addition, a cross-cultural approach was added to the study with the aim to explore an alternative potential market for the "corn beers" and to explore the differences and similarities between two countries that are experiencing a similar incursion in the craft beer movement, but at the same time, has a different history in relation with the consumption of corn.

To this end, the main objective and sub-objectives in this phase were:

**Objective 2:** Evaluate the perception of potential consumers towards the concept of corn beer in order to know the weaknesses and strengths of the product to produce a beer(s) that meet the expectations of the consumer.

- Objective 2.1: Develop two types of concepts and determine their perception and relevance among two potential beer consumers market (Mexican and French)
- Objective 2.2: Obtain the sensory characteristics that the consumer expects to find in the corn beer with the end of being used in the formulation stage of the product.

**Hypothesis:**

The concept of "corn beer" could have some the acceptance of a foreign market that has passed through the boom of craft beers, but it will be more appreciated by Mexican beer consumers due to its relation with corn-based products.

## 11.2 Methodology

For the present study, two types of concepts of the 'pigmented corn beers' were created and tested with potential beer consumers following the concept developing process described by Ayağ (2016). The two concepts were identified as: 'functional' and 'emotional' respectively. The 'functional' concept highlights the beneficial characteristics of the beers due to the antioxidants that came from the pigmented corns. On the other hand, the 'emotional' concept highlights the traditions and the use of native varieties of corn and appeals to the emotional response of the consumers (Figure 5).

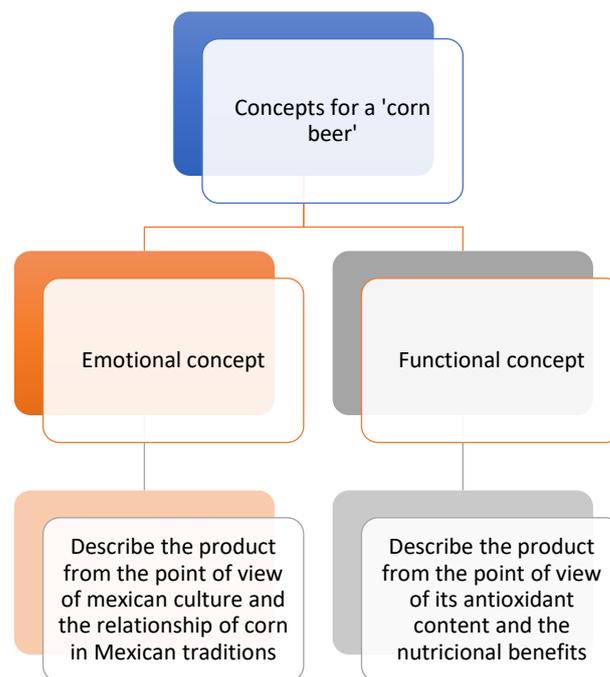


Figure 4. Description of the concepts of pigmented corn beer

As the process of creation, a new product concept involves the feedback of the consumers, this study was done in two phases.

Phase I: The two concepts (both in the Spanish language) were tested by 94 participants in Mexico City. This served us to have an idea if the concepts meet the consumers' needs and helped us to modify the concepts in an early stage of the process.

Phase II: Once the two concepts were tested and modified, both concepts were evaluated by beer consumers of two different countries (Mexico and France).

The process of the creation and evaluation of the concepts is described according to the phases and is listed below:

### Phase I:

**a. Identification of consumers' needs-** as mentioned before, the craft beer industry is an emergent market that is characterised by a highly competitive, where innovation plays a crucial role. As a result, craft beer consumers are classified as the most demanding consumers in terms of desired quality. This segment of consumers are always seeking novelty and unconventional beers and their interest grows in those products that differ from others (Carvalho et al., 2018; Gómez-Corona, Escalona-Buendía, et al., 2016). In addition, there is a trend among consumers to look up products made with local and traditional ingredients of their region or country. A list of the identified consumers' needs and wants in the craft beer segment is summarised below (Carvalho et al., 2018; Gómez-Corona, Escalona-Buendía, et al., 2016):

- Traditional ingredients
- High-quality ingredients
- New flavours
- New tasting experiences
- Novel beers
- Innovative ingredients
- Distinctive products

**b. Establishing target specifications:** Mexican consumers of either industrial or craft beers in the age range of 18-50 years old.

**3. Concept(s) generation and selection:** As mentioned before, two concepts (emotional and functional) were created based on the consumers' needs. Both concepts were written in the Spanish language.

- ***Emotional concept:*** The creation of the emotional concept considered the cultural and social representation of the corn in Mexican's life. The concept describes the consumption of beer as a tradition as well as to eat corn. The flavours and its ingredients were described as those typical in Mexican gastronomy, emphasizing that the main ingredient- corn - is native and 100% from Mexico (Figure 5).

***Cerveza de maíz, recuperando nuestras tradiciones***

Para mí el maíz es parte importante de mis orígenes y tradiciones así como lo es tomar una buena cerveza. ¿No sería increíble una cerveza con sabor a México?

Deleita tus sentidos con una cerveza de maíz que combina el sabor distintivo del maíz con el toque amargo de una cerveza y regresa a tus orígenes.

La cerveza de maíz es la única cerveza elaborada con maíz 100% originario de México, con un balanceado sabor a maíz y ligero toque picante que te devuelve a tus orígenes, la tradicional mexicana.

*Figure 5. Emotional concept (Phase I)*

- **Functional concept:** The creation of the functional concept took into account the antioxidant properties of the beer due to the varieties of corn used in the brewing process are an important source of anthocyanins and polyphenols, compounds that enriched the antioxidant capacity of the beer. The corn beer was described from this point of view emphasizing the benefits that consumption this beer could have in health (Figure 6).

***Cerveza de maíz, más que una cerveza, un estilo de vida***

Tomar cerveza es algo que disfruto, me gustaría que una cerveza además de rica y refrescante me ayudara a cuidar mi salud.

Tomar cerveza de maíz no solo me da las sensaciones refrescantes de una cerveza, además da beneficios a mi salud.

La cerveza de maíz es la primera cerveza en su tipo creada por investigadores mexicanos, al contener maíz es rica en antioxidantes naturales que te ayudarán a proteger a tu cuerpo del envejecimiento causado por el estrés diario.

*Figure 6. Functional concept (Phase I)*

4. **Concept evaluation:** All participants had to answer a questionnaire to collect information about their beer consumption habits and about one of the two concepts. The test was performed over two consecutive days. One-half of the participants tested the 'emotional' concept, and the other half tested the 'functional' concept. The order of the presentation was randomised.

Consumers were asked to rate some aspects of the concepts. the overall liking of the concept was scored on a 9-point hedonic scale (from 1= dislike extremely to 9 = like extremely). Importance, uniqueness, credibility, appropriateness and purchase intention were rated on a

5-point scales (See appendix 1). Additionally, consumers were asked for a free word association, which is a technique where respondents can show their opinions, attitudes and perceptions (Guerrero et al., 2010; Vanhonacker et al., 2010). Consumers were asked to write a) the first three words that came to their mind after reading the beer concept, and b) the flavours they expect to be found in the kind of beer related to each concept. The final section consisted of questions about their socio-demographic characteristics such as age, gender, occupation, and educational level.

### **Data analysis**

Analysis of variance (ANOVA) was performed on the scores of each parameter evaluated considering the concept, gender and the interaction concept \* gender as fixed sources of variation. When differences were significant ( $p < 0.05$ ), honestly significant differences were calculated using Tukey's test.

For the free association test, the words were organized and were renamed if they have the same meaning, for example, 'maíz', 'maices', 'elote' were renamed as *corn*. Then words with similar meanings were grouped into categories, and these categories were also grouped into dimensions. Frequencies of each word were calculated and word cloud by concept was made. The categories and dimensions were analysed by concept using a Chi-squared test per cell.

## **11.3 Results**

### **(Phase I)**

A total of 188 participants (students, University members) were recruited from the university (Universidad Autonoma Metropolitana). One-half of the participants evaluated the emotional concept and the other half of the functional concept. Of the 188 participants, 48% were male and 52% were female. The age of the participants ranged from 18 to 57 years old (21 years old average). About 55% of the participants were industrial beer consumers, 40% used to drink both craft and industrial beers, and 5% were craft beer consumers. 40% prefer national brands while drinking both national and foreign beer brands. Regarding the frequency of consumption, 42% of participants consumed craft beer once a month and only 3% drink it once a week. Contrary, 43% of participants consumed industrial beer once a month, 17% once a week and 7% drink it more than two times a week. 61% of participants spent \$1-150 MXN per month on industrial beer vs 34% of participants who spend the same amount buying craft beers.

Table 3 summarises all participants characteristics collected for the study.

*Table 3. Socio-demographic characteristics of the participants (N=188)*

	Variable	Categories	Frequencies	%
Frequency of consumption	Concept	Functional	94	50.0
		Emotional	94	50.0
	Gender	Male	91	48.4
		Female	97	51.6
	Age	< 20 years old	107	56.9
		20-30 years old	72	38.3
		> 30 years old	9	4.8
	Occupation	Student	181	96.3
		Employee	7	3.7
	Type of beer preferred	Industrial	103	54.8
		Craft	10	5.3
		Both	75	39.9
	Origin of beer preferred	National	76	40.4
		Foreign	12	6.4
		Both	100	53.2
	Industrial beer	Once a week	32	17.0
		More than once a week	14	7.4
		Once a month	80	42.6
Twice a month		60	31.9	
Never		2	1.1	
Craft beer	Once a week	6	3.2	
	Once a month	79	42.0	
	Twice a month	12	6.4	
	Never	91	48.4	
Money spent per month buying beer	\$-industrial beer	\$0	5	2.7
		\$1 a \$150	115	61.2
		\$151 a 250	40	21.3
		\$251 a 350	15	8.0
		> \$350	13	6.9
	\$-craft beer	\$0	92	48.9
\$1 a \$150		64	34.0	
\$151 a 250		16	8.5	
\$251 a 350		10	5.3	
> \$350		6	3.2	

ANOVA explored the consumer responses of the concept parameters evaluated considering the effects of beer concept and gender and their two-way interactions (Figure 7, Table 4).

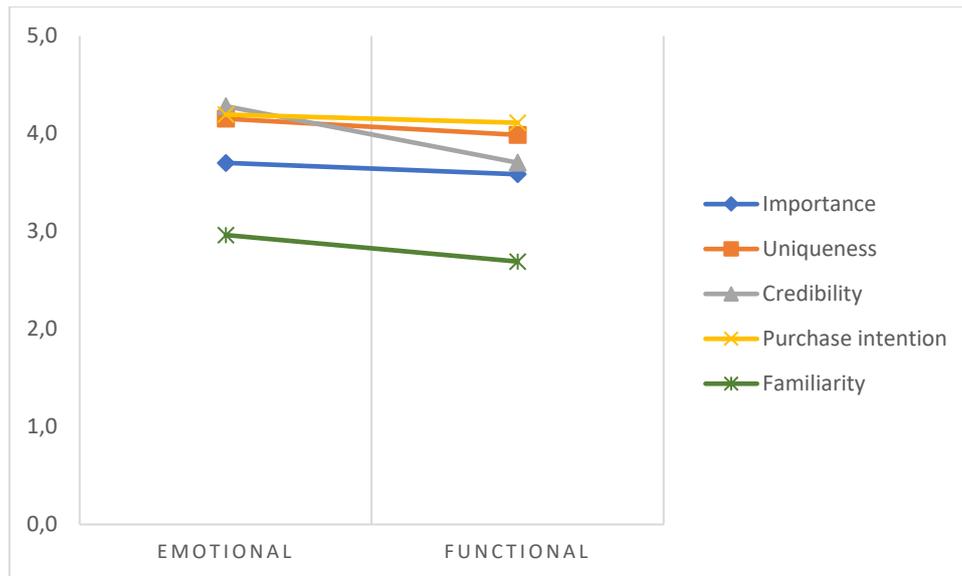


Figure 7. Main effects: estimated means of importance, uniqueness, credibility, purchase intention and familiarity as a function of product concept.

Table 4. Mean values for each variable of the affective test (N=188)

Variable	Concept		Gender	Concept*Gender
	Emotional	Functional	p-value	p-value
<b>Overall liking<sup>1</sup></b>	7.7a	7.7a	0.111	0.018
<b>Importance<sup>2</sup></b>	3.6a	3.6a	0.090	0.437
<b>Uniqueness<sup>2</sup></b>	4.1a	4.0a	0.785	0.067
<b>Credibility<sup>2</sup></b>	4.0a	3.7b	0.741	0.413
<b>Familiarity<sup>2</sup></b>	2.7a	2.6a	0.556	0.268
<b>Purchase intention<sup>2</sup></b>	4.3a	4.2a	0.073	0.915

Means with different letters within columns are significantly different according to Tukey's test ( $p < 0.05$ ).

<sup>1</sup> Evaluated on a nine-point hedonic scale.

<sup>2</sup> Evaluated on a five-point hedonic scale.

No significant differences were found between concepts in terms of overall liking (rated as "like very much"), importance (rated as "very important"), uniqueness (rated as "very different"), familiarity (rated as "neither easy nor hard" to identify me with the concept) and purchase intention (rated as "probably will buy it"). The only significant difference was found in credibility, where the emotional concept was more reliable than the functional concept. For the factor 'gender' no significant differences were found between concepts. In addition, there were significant differences in the interaction concept \* gender in terms of overall liking, where women rated as "like very much" for the functional concept and men rated as "like moderately".

## Free word association

A word cloud by concept was made using the text mining method in R software. Wordcloud is a technique that allows us to compare related words graphically by analysing frequencies. The more mentions the bigger is the size of the word (Sánchez-Vega et al., 2020).

A total of 532 different words were mentioned for both emotional and functional concepts. The words were grouped into 14 categories, which were regrouped into 8 dimensions (Table 5). For both concepts, the *culture* dimension grouped the most significant proportion of words (25%), mainly related to *traditions and identity* categories, followed by, *emotions* (22%), *sensory characteristics* (19%), and *health* (17%) categories. The Chi-squared test indicated that the frequency of mention of these dimensions and their categories were significantly different in both concepts ( $p < 0.001$ ).

Table 5. Frequency of mention of the dimensions and categories for the emotional and functional concepts.

Dimensions	Categories	Percentage of mention	
		Emotional concept	Functional concept
Culture		43 (+)***	7 (-)***
	Historical aspects	2	1
	Identity	30 (+)***	4 (-)***
	Traditions	11 (+)***	2 (-)***
Economical		2	3
	Economic aspect	2	3
Emotions		20	24
	Valorisation	20	21
	Feelings	1	3
Food relations		13	9
	Beverages	4	3
	Food	9	6
Health		2 (-)***	31 (+)***
	Nutrition	2 (-)***	9 (+)***
	Health	0 (-)***	22 (+)***
Process		4	3
	Process aspects	4	3
Rurality		2	0(-)***
	Agricultural	2	0 (-)***
Sensory characteristics		14 (-)**	23 (+)*
	Flavour	7 (+)**	2 (-)**
	Hedonic	7 (-)***	21 (+)***

Effect of the chi-square per cell, (+) or (-) indicate that the observed value is higher or lower than the expected theoretical value. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p, 0.001$ .

## Emotional concept

Word clouds show that *Mexico* is the central element that consumers associate with this concept. The dimension “*culture*” stands out followed by *sensory characteristics* and *emotions*. Within the “*culture*” dimension, the categories: *identity* and *traditions*, which includes the words *Mexico*, *origin*, *tradition*, *traditional*, *Mexican*, and *culture*, were the most frequently mentioned. The second most salient category was related to *sensory characteristics*, which was mainly associated with the “*hedonic*” category, with words such as *tasty* (Figure 8a).

The most frequent words when consumers were asked about flavours that they expect to find in this beer were: *beer*, *bitter*, *chocolate*, *corn*, *spicy*, *sweet*, *bittersweet*, *chili*, *tortilla*, and *fruits* (Figure 8b).

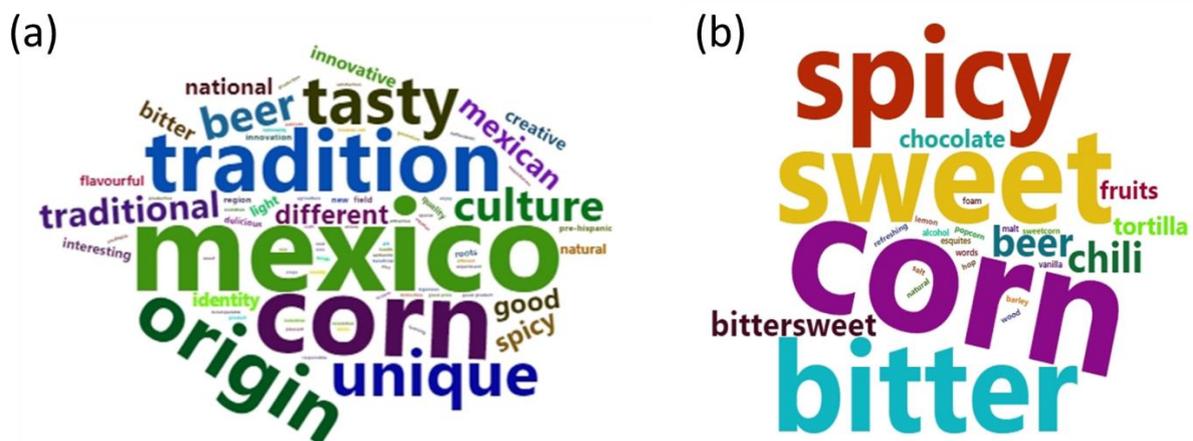


Figure 8. Word clouds related to the “Emotional beer concept”. a) Words related to the emotional concept, b) Flavours expected to be found in a beer-related to the emotional concept

## Functional concept

On the other hand, for the functional concept, the dimension *health* was the most representative, followed by *sensory characteristics*, and *emotions*. Words as *healthy* and *health* (health category) were the main ideas related to this beer concept (Figure 9a). Also, for the “*hedonic*” category within the *sensory characteristics* dimension, the words *tasty* and *refreshing* were the most frequently mentioned.

The words related to the flavours they expect to find in the beer with a functional concept were *sweet*, *corn*, *bitter*, *tortilla* and *fruits* (Figure 9b).

(a)



(b)



Figure 9. Word clouds related to the 'functional beer concept'. a) Words related to the functional concept, b) Flavours expected to be found in the beer-related to the functional concept

It was surprising to find that for both emotional and functional concepts, the taste *sweet* was frequently mentioned. This could be associated with the sweet taste found in corn products such as 'esquites' and tortilla. The words related to flavours such as *corn*, *tortilla*, and *bitter* were also related to both beer concepts. As corn is the main ingredient in these beers, it was expected that consumers relate these flavours to these beers. *Bitter* is a characteristic taste of beers and consumers also expect to find this taste in both beers. On the other hand, it is particularly interesting that the flavour *spicy* was only mentioned for the beer-related to the emotional concept. This could be explained as "*spicy*" is a traditional flavour in Mexican gastronomy, thus consumers have affectivity and symbolic value for this flavour.

### Conclusions (Phase I)

In general, few differences are seen between both concepts. The fact that no significant differences were found on the overall liking between functional and emotional concepts indicates that both concepts are well accepted by consumers. However, the emotional concept is more credible than the functional concept. Also, both concepts bring out positive related words. The emotional concept appeals to the emotional side of Mexicans, related to its identity, culture and traditions. Contrary, the functional concept emphasizes the healthy properties that this beer could have. Even though the functional concept increased scepticism on consumers it does not negatively influence overall liking or purchase intention. Some limitations in this study were the target population and the limited selection of product concepts.

### Phase II

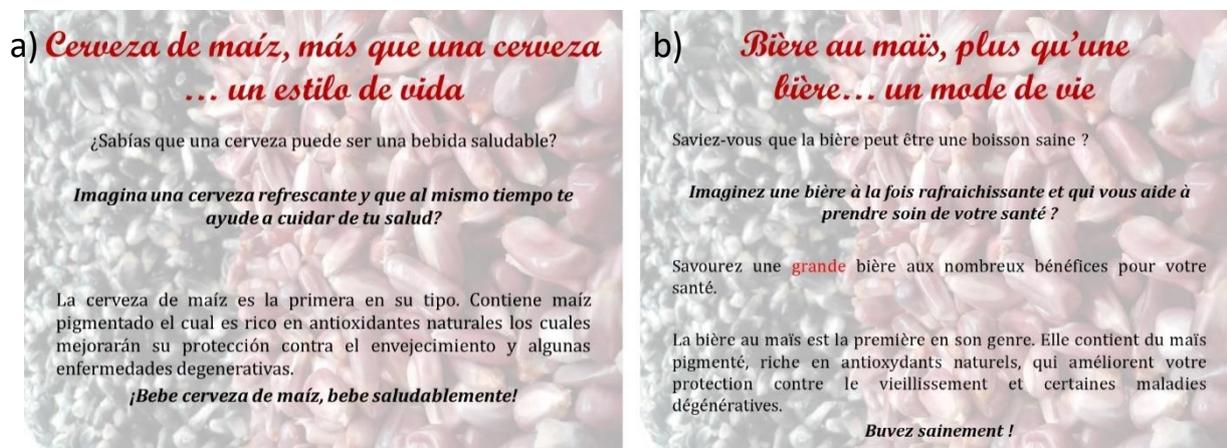
As mentioned before, the concept testing consisted of two phases. Phase II was done once the two concepts (emotional and functional) were tested and the concepts were slightly modified according to the results of the word association. For both concepts, the most frequent

words mentioned in the free word association test were taken into account to re-write the new concepts (Figure 10 and 11). To translate the concepts and the questionnaire to French, a double translation approach was used, consisting of two steps: one person translated the concept/questionnaire from Spanish/French to English; afterwards that concept/questionnaire was given to another person who translated from English to its original Spanish/French language. When a perfect match was found, the translated concept/questionnaire was kept. The three versions of each concept are given in Appendix 2.

Figure 10. Emotional beer concepts. a) Spanish version, b) French version.



Figure 11. Functional beer concepts. a) Spanish version, b) French version



This study was designed as an online survey in Mexico and France in Summer 2016 using Google Forms. Respondents were reached and invited via social networks starting with students at Universidad Autonoma Metropolitana (Mexico) and at 'Institut Supérieur d'Agriculture-Lille (France), and progressively enlarging the number of participants. The study took place at the same time in both countries.

A total of 452 participants took the survey. Of them, 277 were recruited from Mexico, and 175 were from France. To participate, respondents had to be at least 18 years old, had to state to

consume beer (craft or industrial beer) at least once a year, and had to be Mexican and French citizens. Respondents were asked to voluntarily participate, and instructions were provided online; no incentives were offered for participating in the survey.

The questionnaire explores consumption habits related to drinking beer, and the new versions of both emotional and functional concepts tested before (phase I) were also tested. Both concepts were presented randomized to each respondent to avoid bias. Participants had to rate for each concept the overall liking, importance, credibility, perceived innovativeness and purchase intention (See appendix 3). Also, a free word association test was performed after reading each concept.

## Results (Phase II)

The survey was responded by 277 people from Mexico and 175 people from France. The same proportion of females and males took the survey in both countries (~50% female, ~50% male). The average age range of participants was 26-35 years old, and most of them had a university education level (95%-Mexico, 86%-France). In Mexico, 38% of participants have a professional activity as *student* and 33% as a *professional*. In France, 74% of the participants referred to be an employee and only 8% and 4% have *professional* and *student* activities, respectively. For both countries, >80% of participants are not related to the beer industry (Table 6).

Table 6. Socio-demographic characteristics of survey participants from Mexico and France.

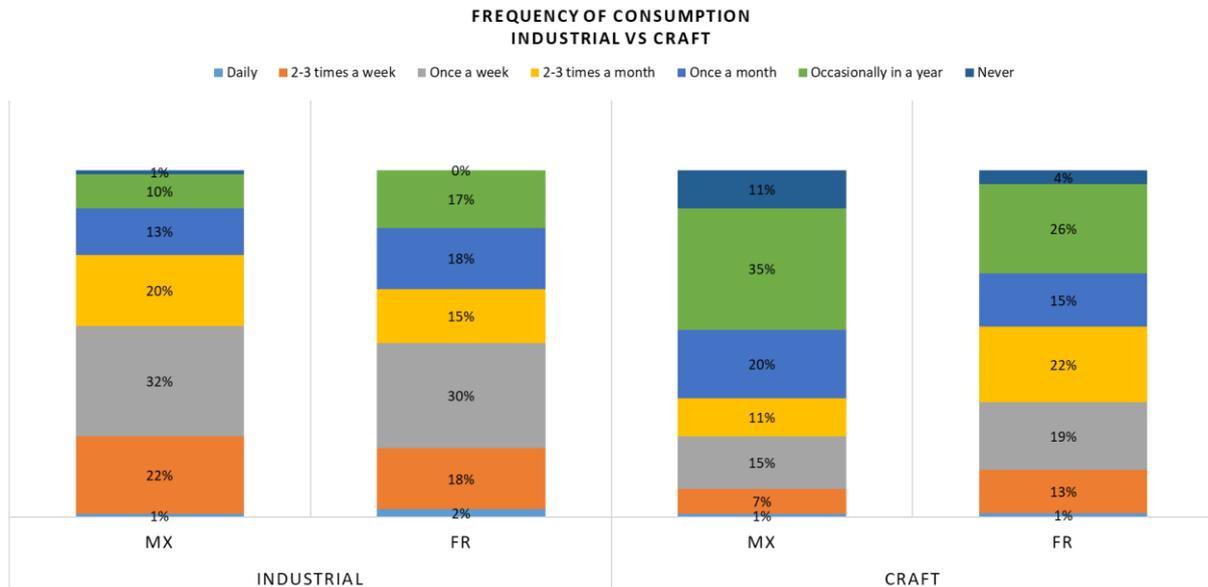
	Mexico	France	Professional activity	Mexico	France
Gender	%	%		%	%
Female	50.9a	52.0a	Artist	0.7a	0.0a
Male	49.0a	48.0a	Employee	14.8a	74.2b
Age	%	%	Freelance	0.7a	0.5a
36-45	34.2b	7.4a	In search of	7.2b	0.5a
26-35	46.2a	42.2a	Merchant	0.7a	1.7a
46-55	10.4a	33.7b	No activity	2.1a	2.2a
18-25	6.1a	8.5a	Professional	33.9b	8.5a
+56	2.8a	8.0b	Retired	0.7a	3.4a
Education level			Student	38.6b	4.5a
Elementary	0.0a	4.0b	Worker	0.3a	4.0b
High school	5.4a	9.7a			
University	94.5b	86.2a			
Do you or someone in your family have an occupation related to beers?					
Yes	10.8a	13.1a			
No	89.1a	86.8a			

Different lowercase letters between columns indicate significant differences between demographic characteristics according to the Chi-square test ( $P < 0.05$ ) of K proportion with the Marascuilo procedure.

Data on beer consumption concerning the question “How frequently do you drink each of these two types of beer?” reveal that more than 30% of participants use to drink industrial beer at

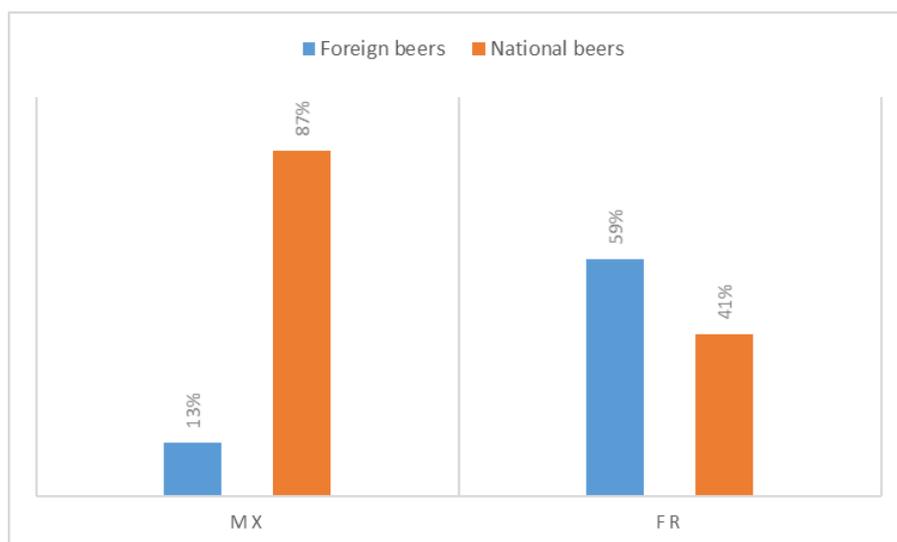
least *once a week* both in Mexico (20.5%) and in France (30.3%) vs craft beer. Contrary, for craft beer, 35% of Mexican participants use to drink craft beer *occasionally in a year*, while 21% of French participants use to drink it *2-3 times a month* (Figure 12).

Figure 12. Comparison of frequency of consumption for industrial and craft beers in Mexico and France.



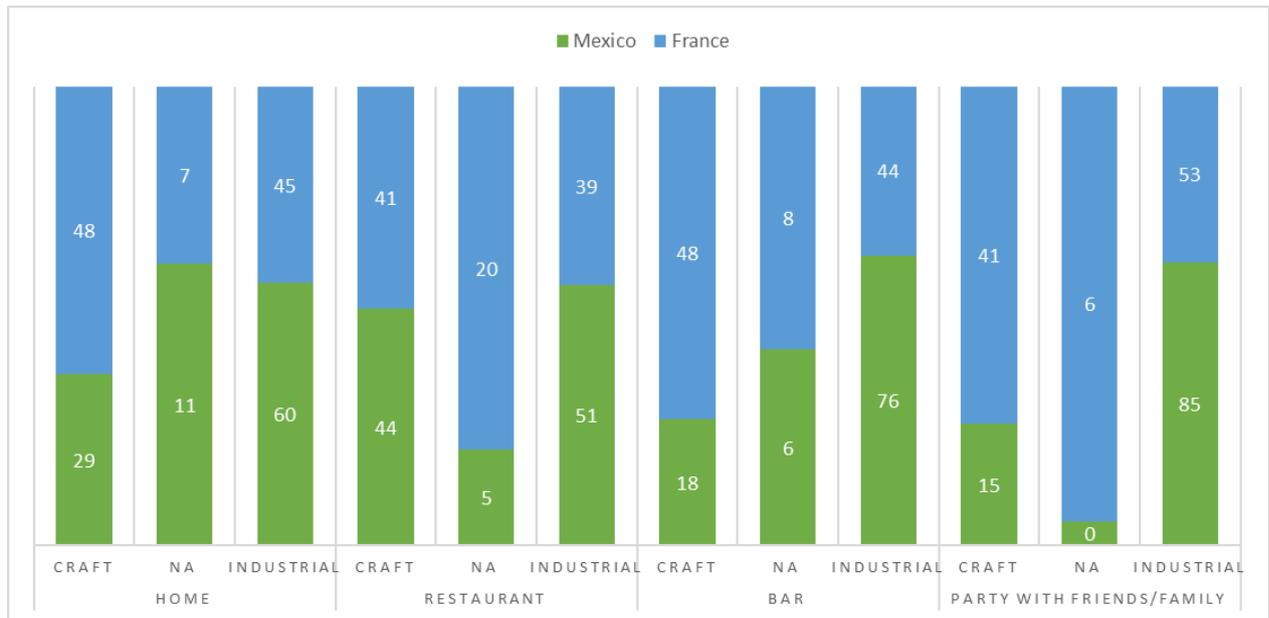
For the situation *at the restaurant*, the same proportion of participants (>40%) mentioned drinking *craft* beer rather than *industrial* beer. In general, participants consume more industrial beer at this place. Regarding the question “*Generally speaking, do you prefer drinking..?*”, 87% of Mexican consumers prefer drinking national beers vs 59% of French consumers who prefer foreign beers (Figure 13).

Figure 13. Preference of drinking (national vs foreign beers) in Mexico and France



For the question “For each of the following situations, indicate the kind of beer you generally drink”, the study reveals that participants from France consume more craft beer at home (48%), at the bar (28%), and parties (41%) than Mexicans (28%, 18%, and 14%, respectively) (Figure 14).

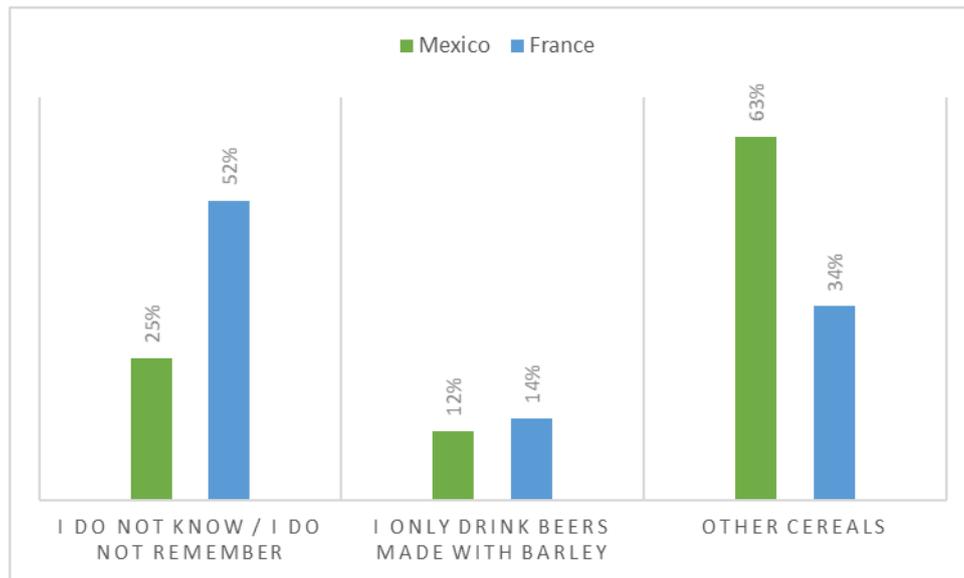
Figure 14. Kind of beer that consumers usually drink in each situation in Mexico and France.



Numbers are given in percentage. NA – I do not consume beer in this place.

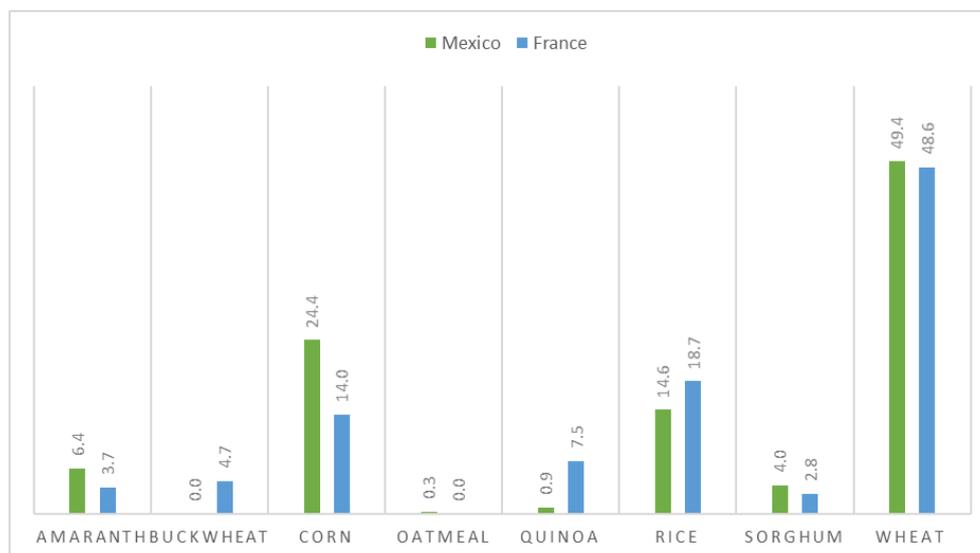
Regarding the question “Beer is a fermented beverage generally made with barley. Have you ever drunk beers made with other cereals?”, there was a significant difference between Mexico and France. 25% of participants from Mexico mentioned I do not know/ not remember if they drink beer made with other cereals, while 12% of participants mentioned they only drink beers made with barley, and the other 63% of them mentioned to drink beer made with other cereals. In contrast, 52% of French participants mentioned that they do not know/ remember to drink beer made with other cereals, 14% mentioned they only drink beers made with barley, and the other 34% drink beers made with other cereals (Figure 15). These results could infer that Mexicans have more knowledge about the type of beer they are drinking than French.

Figure 15. Have you ever drunk beers made with other cereals?



The most mentioned cereals in both Mexico and France were wheat (49%-Mx, 48%-Fr), followed by corn (24%-Mx, 14%-Fr), and rice (14%-Mx, 18%-Fr). Oatmeal was only mentioned by Mexicans while buckwheat was only mentioned by French participants (Figure 16).

Figure 16. Cereals mentioned by Mexican and French participants



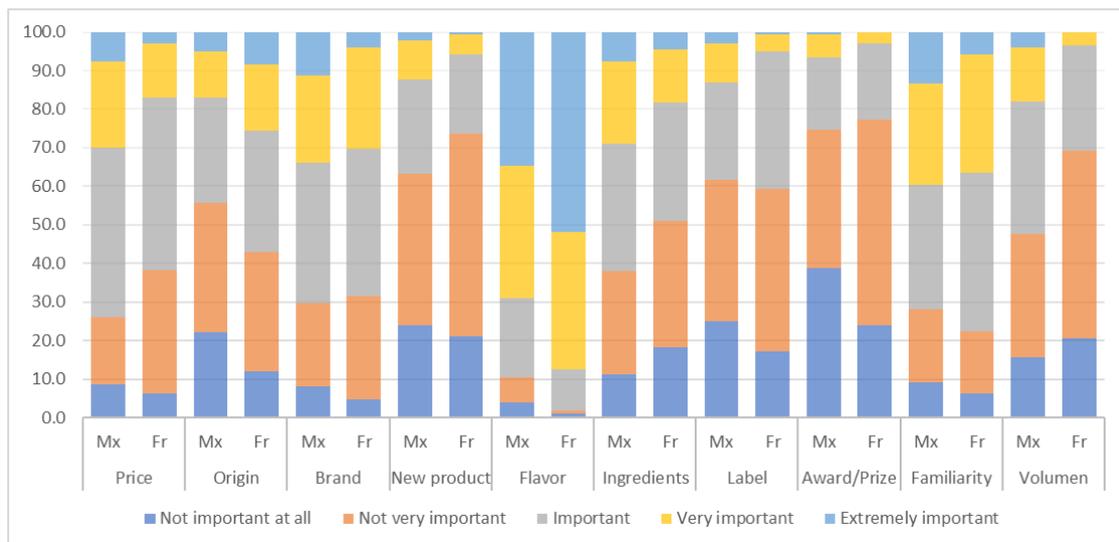
Numbers are given as a percentage of the total mentions by country.

About the purchase habits, three questions were addressed. First, we asked *who buys the beers that you consume?* and 68% of Mexican vs 75% of French respondents said, *“me or another person, depending on the situation”*. Then, for the question *“In which place do you buy beer most frequently?”*, in Mexico 65% of participants answered, *“in the supermarket”*, followed by *“convenience stores”* (30%), and *“bar”* (11%). Contrary, in France, 89% of

participants buy beers in the “supermarket”, followed by 48% who buys in “specialized stores/wine stores”, and only 15% in “convenience stores”.

The third question about purchase habits was: “Indicate the extent to which each of the following factors influences your decision when you buy a beer?”. Both in Mexico and France, the factors *price*, *brand*, *familiarity* and *volume* were considered as “important”. A significant difference was found in the factor *flavours* which was considered as ‘extremely important’ by 52% of French participants vs 34% of Mexican participants (Figure 17).

Figure 17. Factors that influence the purchase decision of beer.



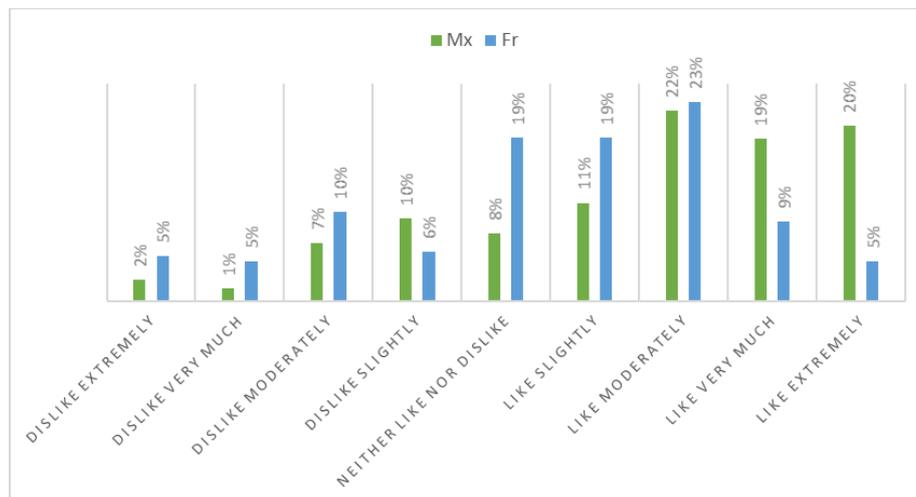
For the evaluation of both emotional and functional concepts, the same questions as the ones in phase I were asked. Participants had to rate for each concept the overall liking, importance, credibility, perceived innovativeness and purchase intention. In addition, one question regarding the provoked emotions after reading each concept was asked.

The overall liking of the concept (question: ‘*What is your appreciation of the concept you have just read?*’) was rated on a 9-point scale (from 1= dislike extremely to 9 = like extremely). Then the questions about the importance (‘*How important for you is the concept you have read?*’), credibility (‘*How do you evaluate the credibility of a beer with the concept that you read?*’), perceived innovativeness (‘*How do you assess the innovative aspect of the concept?*’), and the purchase intention (‘*Based on this concept, how interested would you be in buying this beer if were within your budget?*’) were rated in a 5-point scales (Appendix 3).

### Emotional concept

The emotional concept was significantly more liked by Mexican participants (mean liking of 7 ‘like moderately’) than French (mean liking of 6 ‘like slightly’) (Figure 18).

Figure 18. Overall liking of emotional concept rated by Mexican and French participants.



In addition, results showed significant differences in all the four questions about importance, credibility, innovativeness and purchase intention (Figure 19). For instance, the concept was rated as *very important* by 31% of Mexican participants and 34% of French participants rated it as *not very important*. For the credibility, it was rated as *very believable* by 31% of Mexicans vs 18% of French participants. The perceived innovativeness was rated as *very innovative* by 33% of Mexican vs 19% of French participants. Finally, for the purchase intention participants of both countries are *very interested* (50%-Mx, 54%-Fr) but 16% of Mexican participants rated as *extremely interested* while only 8% of French participants rated it in the same way.

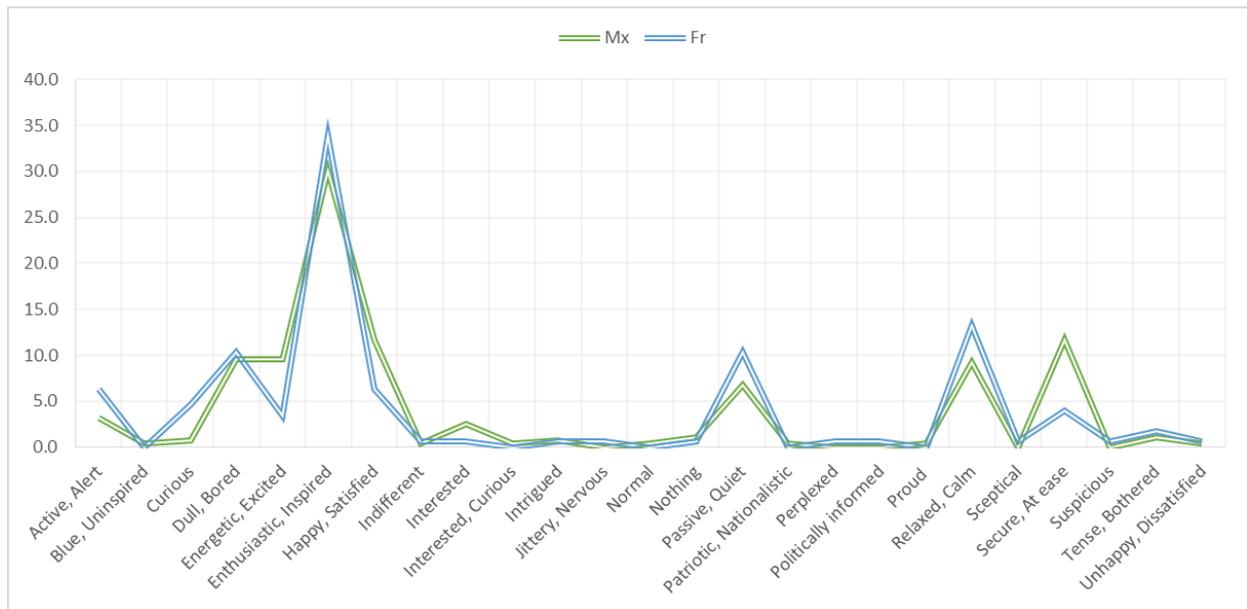
Figure 19. Percentages of responses by Mexican vs French participants for the factors: importance, credibility, perceived innovativeness and purchase intention of the emotional concept.



As it was expected, the emotional concept of the ‘corn beer’ received more acceptance among Mexican participants than the French. However, according to the results, participants from France showed a similar response pattern. This could be attributed to the liking for foreign beers of French participants.

Regarding the questions of the provoked emotions after reading the functional concept (*How do you feel after reading this beer concept?*), 52% of the emotions were positive, 28% negative and 20% neutral. Among the positive emotions, *enthusiastic/inspired*, *happy/satisfied*, and *secure/at ease* were the most frequently mentioned by Mexicans. Also, words as *proud* and *patriotic/nationalistic* were only mentioned by Mexican participants. On the other hand, French participants mentioned more neutral emotions such as *passive/quiet*, *relaxed/calm* and *politically informed*. Among the negative emotions, *dull/bored* was the only one with about 10% of the mentions in both countries (Figure 20).

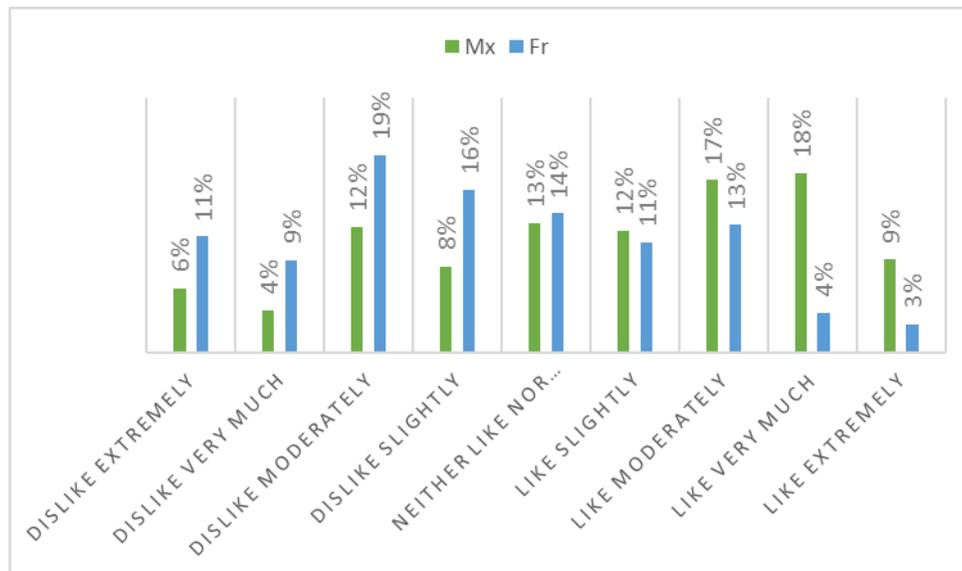
Figure 20. Emotion profiles comparing Mexico and France for the emotional concept.



### Functional concept

Significant differences were found in the overall liking of the functional beer concept between Mexico and France. The concept was rated as *like slightly* by Mexican participants vs French who rated it as *dislike slightly* (Figure 21).

Figure 21. Overall liking of functional concept rated by Mexican and French participants.



As well as in the emotional concept, there were significant differences in the importance, credibility, perceived innovativeness and purchase intention. For the factor *importance*, 40% of Mexicans considered this concept as *important* vs 29% of French participants. About the credibility of the concept, 47% of Mexicans considered it as *extremely believable* while only

26% of French considered it as *believable*. The perceived innovativeness was rated as *innovative* by 46% of Mexicans vs 36% of French participants. For the purchase intention, both 37% of Mexicans and 38% of French are *very interested* in buying this beer, the main difference was that 12% of Mexicans are *extremely interested* and almost 40% of French participants are not interested in buying the beer associated to the functional concept (Figure 22).

Figure 22. Percentages of responses by Mexican vs French participants for the factors: importance, credibility, perceived innovativeness and purchase intention of the functional concept.



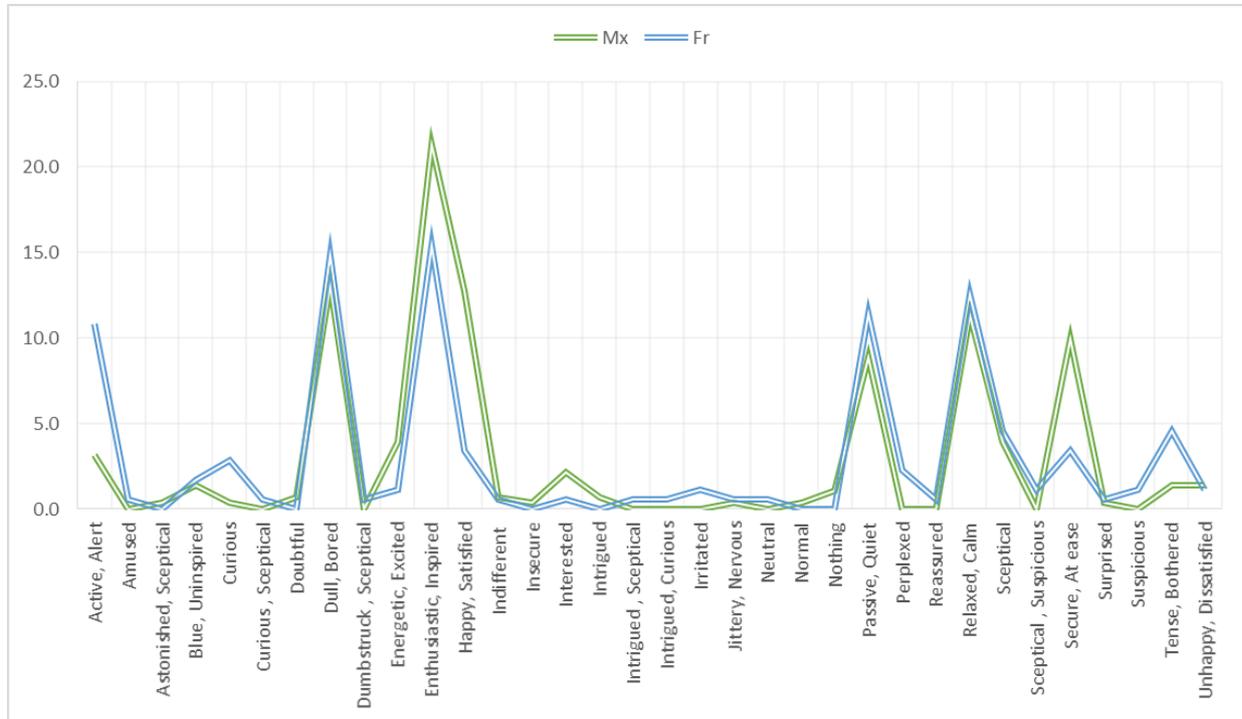
It was interesting that Mexican's respondents rated the factor *credibility* higher than French, and even they considered as more believable than the emotional concept. This could be explained as Mexicans are more aware of the benefits of pigmented corn than French due to the increase of products made with blue corn.

In addition, the cultural point of view takes more relevance. As corn is part of Mexican gastronomy, it is expected that Mexicans feel more attracted by products made with native corn. Although the functional concept was more believable than the emotional concept, it had no impact on purchase intention.

Regarding the questions of the provoked emotions after reading the functional concept (*How do you feel after reading this beer concept?*), 50% of the responses were positive emotions, followed by 32% of negative, and 18% of neutral emotions. Positive emotions were more frequently mentioned by Mexicans than French participants. For example, emotions such as

enthusiastic/inspired and happy/satisfied were mentioned 21% and 12% by Mexicans, and only 15% and 3% by French, respectively. Negative emotions such as *dull/bored* were mentioned 13% and 15% by both Mexican and French participants, respectively (Figure 23).

Figure 23. Emotion profiles comparing Mexico and France for the functional concept.



### Emotional vs Functional concept

In addition, it was performed a comparison between concepts by country. For both countries Mexico and France, significant differences were found in the overall liking between both emotional and functional concepts. The emotional concept obtained higher scores than the functional concept in both countries. In Mexico, the emotional concept was rated as ‘*like moderately*’ (mean: 7.0) while the functional concept was rated as ‘*like slightly*’ (mean: 6.0) (Figure 23). In France, similar behaviour was observed. The emotional concept was rated as ‘*neither like nor dislike*’ (mean: 5.0) while the functional concept was rated as ‘*dislike slightly*’ (mean: 4.0) (Figure 25).

Figure 24. Comparison between the overall liking of the emotional and functional concepts in Mexico.

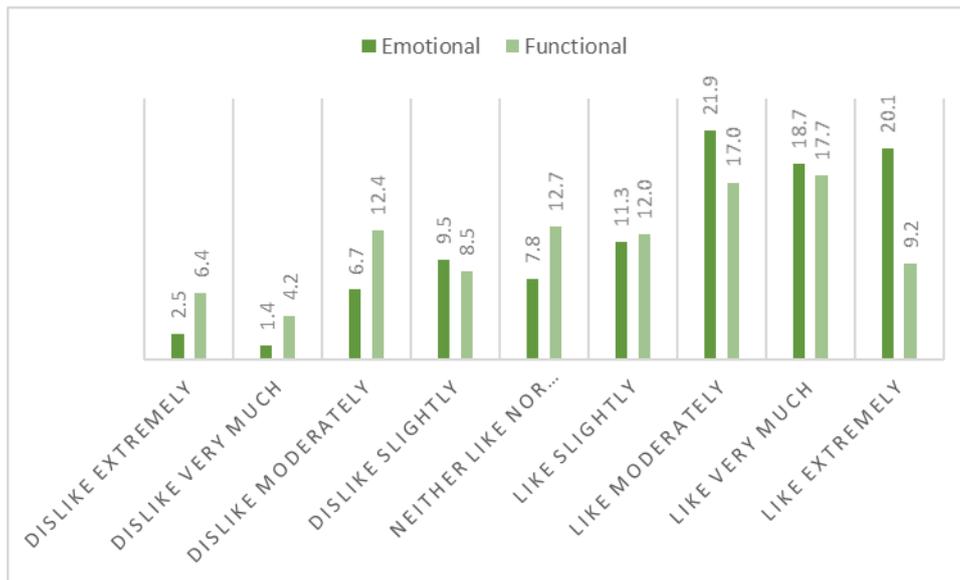
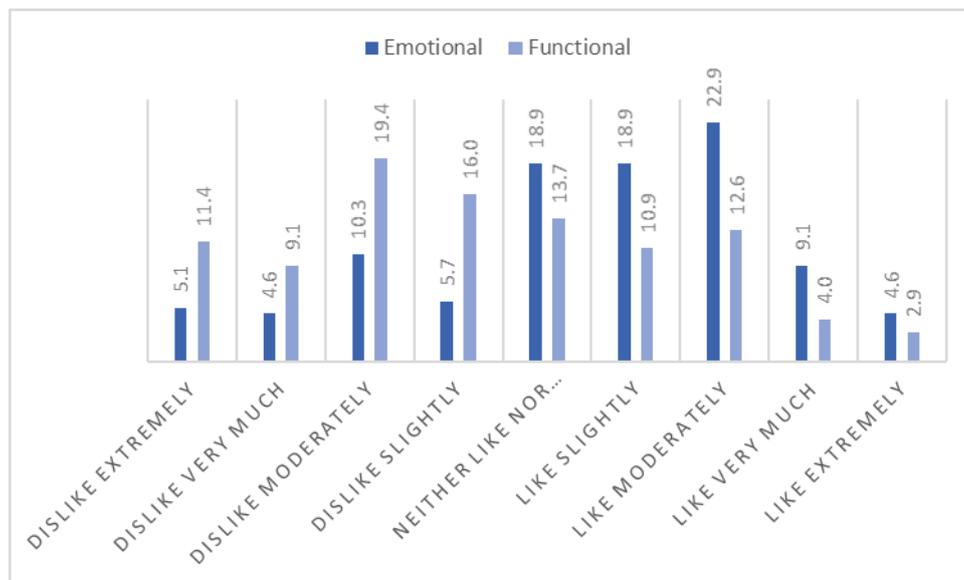


Figure 25. Comparison between the overall liking of the emotional and functional concepts in France.



## 11.4 Conclusions

The appreciation of the concepts was different between Mexican and French participants. As it was expected, Mexicans felt more attracted to the idea of a beer made with pigmented corn than French. Also, for both countries, the emotional concept was more liked than the functional. However, for both countries, the overall liking was not too high (like slightly ~ like moderately), which can be associated with a certain level of neophobia (a reluctance to try new or unfamiliar foods) or an inner fear of novelty (Bernal-Gil et al., 2020). These results are in accordance with Giacalone (2013), who found that when it comes to novel beers, *novelty* is a desirable property as its related to innovation, but too much novelty could reduce beer's

acceptability. Thus, a beer made with pigmented corn could be more appreciated if it does not change the mental concept that consumers have of a typical beer. For instance, the partial replacement of barley with corn could be a better way to reach beer consumers' expectations. Furthermore, according to the results, this type of beer could be accepted among French beer consumers. This opens the possibility to expand the Mexican beer market to an international level.

These results lay the foundation for the formulation of corn beers. Knowing that corn is an ingredient that it is considered innovative, but that in turn, consumers look for a balance between the known and the new, it is proposed to make formulations in which corn (red and/or blue) and barley are combined, starting from beers 100% corn and 100% barley. This is in order to find the(s) formulation(s) that meet the expectations of consumers.

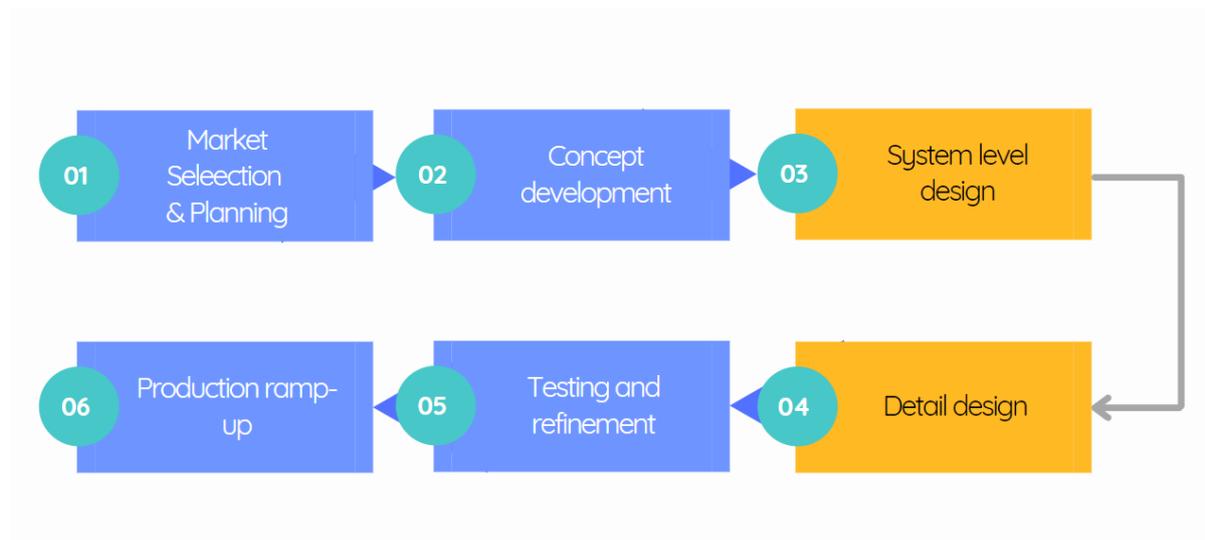


# CHAPTER III

**Product  
implementation:  
Chemical and sensory  
analysis**

*"Malt is the soul,  
hops the spice,  
yeast the spirit,  
and water the body of beer."  
-Dr. Anton Pendl*

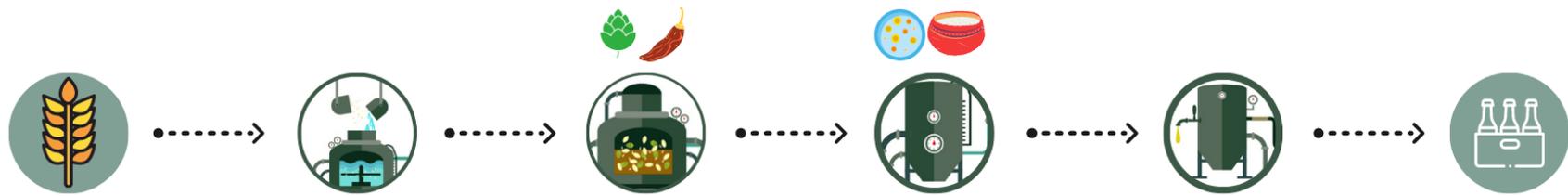
## 12 Chapter III – Product implementation: Chemical and sensory analysis



Study 3 is focused on the 3<sup>rd</sup> and 4<sup>th</sup> phases of the “New Product Development Process”, named System-level design and Detail design. Often, in academic research, these phases are critical as they are the intersection between the laboratory and the commercial industry. These phases are proposed that science plays an assistance role to bridge the gap between laboratory scale to pilot and eventually industry scale. In this part of the project, we develop the prototypes first on a laboratory scale and then we scale up to a pilot plant. Then, we evaluated the prototypes in their chemical and sensory properties.

### 12.1 Introduction

Brewing is an operation consisting of the following steps: malting, milling, mashing, wort extraction, fermentation, and ageing (Figure 26).



▶▶ **Malting**

Controlled germination process where sufficient enzymes are formed, which will convert the grain starches into fermentable sugars.

▶▶ **Mashing**

The enzymes in the malted grains convert the starches into simple sugars. The sugar is extracted from the malt and dissolves into the water and the wort is obtained.

▶▶ **Boiling**

The boil helps to stop the enzymatic reactions and clarify the beer, making any proteins settle out. In this step, hops and adjuncts are added. The alpha acids, compounds in hops that make beer bitter, need to be boiled in order to change shape, dissolve into the wort and impact their bitterness and flavours.

▶▶ **Fermentation**

Yeast is added to the wort to consume the fermentable sugars and produce alcohol and CO<sub>2</sub>, along with other volatile compounds that will impact on a beer's finished flavour.

▶▶ **Maturation / Carbonatation**

This step helps to improve the sensory properties of beer by aging out any rough flavours.

▶▶ **Bottling**

In Kegs, bottles, cans. Maturation also can be done in the bottles.

▶ Sendecho

▶ Beer

Figure 26. Brewing process steps.

The traditional process of brewing has been designed for barley. Thus, some technical implications in malting and brewing pigmented corn have come out. For instance, brewing corn malt takes longer compared to other cereals such as barley, wheat and oats (Diakabana et al., 2013).

Usually, malting and brewing are considered two independent processes. Once the malt is ready for its use, the next step is the mashing of the grains, where the transition of malt to wort occurs. In this step, the malted cereal (corn, barley) is transferred to the mash tun. Then the malt is diluted, filtered and brought to boiling for one or two hours. Mashing causes the natural enzymes in the malt to break down starches and convert them to sugars that eventually will be converted into alcohol. It is also in this step where hops and adjuncts are added. During the mashing process, the temperatures and time can be adjusted, and different combinations can be used. Different temperatures influence the activation of different enzymes affecting the release of proteins and fermentable sugars. The resulting liquid is named wort, and it consists of a combination of fermentable sugars, proteins, suspended fragments of hops. Then, the wort is cooled, aerated and pumped to the fermentation tanks.

The fermentation process consists of the conversion of fermented sugars to alcohol (ethanol) and CO<sub>2</sub> by the activity of yeast enzymes. In addition, other by-products are formed on metabolic sideways. Most of the by-products are volatile compounds that have different odours and flavours, and their interactions and combinations will eventually have an important role in the overall flavour of the beer. Volatile compounds are molecules with a relatively low molecular weight that allow their efficient evaporation in air. When transported by diffusion through the air, these compounds perform diverse functions, from the communication between insects and/or plants to be responsible for the taste and smell of the food and drinks we consume. The structural variety of these compounds is based on a hydrocarbon skeleton with oxygen, nitrogen and sulphur (Herrmann, 2010).

The most important compounds are classified into several classes, including higher alcohols, esters, fatty acids, carbonyl compounds, sulphur compounds, furanic compounds, monoterpenols, C<sub>13</sub>-norisoprenoids and volatile phenols (Olaniran et al., 2017).

Other non-volatile compounds (organic acids such as pyruvic, malic, citric, lactic) are also formed during the fermentation step. These compounds influence the taste and mouthfeel sensations of the beer (Alves et al., 2020).

Once fermentation finishes, the next step is the maturation process. Usually, this step takes place in the same tank, but also the beer could be transferred to another tank or the bottles. During maturation, some chemical and physical mechanisms occur provoking changes in the taste and flavour of the beer. It is also during this step that the second fermentation occurs

provoking the carbonatation of the beer. Thus, maturation directly affects the flavour of the beer and generally, the flavour improves during this step (Masschelein, 1986).

In addition to all the compounds that are formed during the brewing process, there are some others that come from the raw material (malt, hops), which also affect the chemical and sensory properties of the beer. In the case of pigmented corn malts, they contain anthocyanins (cyanidin and pelargonidin). These anthocyanins have an important role in the development of the beer colour but also have beneficial health effects when included in the diet (Cortés et al., 2006). Hops and malt are other sources of volatile and non-volatile compounds such as polyphenols. Polyphenols in beer come from hops (20-30%) and mainly from malt (70-80%). Low molecular weight polyphenols have antioxidative properties that protect the beer from oxidation enhancing taste stability. Polyphenols also have a direct impact on some sensory properties. For example, high molecular weight polyphenols can increase the beer colour and add astringency or dryness to the beer. Moreover, different investigations have found that they act as radical scavengers in the human body (Aron & Shellhammer, 2010). Thus, each stage of the brewing process combined with the raw ingredients contributes to the formation of volatile compounds that generate aromas and flavours in beer, these can interact synergistically or antagonistically with other compounds, causing different aromatic profiles. There are more than 1000 compounds that have been identified in beer, from the raw material as those formed mainly in the fermentation stage (Charry-Parra et al., 2011; Riu-Aumatell et al., 2014).

As mentioned before, all the compounds (volatile and non-volatile) reflect the brewing process and have a strong influence on both the sensory and chemical quality of the beer. Flavour and taste are the most important factors that lead consumers' choices, it is highly relevant to know the sensory profile of the product.

Sensory evaluation plays a key role in product development. Among all the sensory methodologies, descriptive analysis is the most used when it comes to developing a product. Descriptive analysis is a generic term that includes a variety of closely related methods such as the Flavour Profile (Cairncross & Sjostrom, 1950), the Texture Profile (Brandt, Skinner, & Coleman, 1963), Quantitative Descriptive Analysis™ (Stone & Sidel, 2004)), and the Spectrum™ Method (Hootman et al., 2008). Usually, a generic descriptive analysis is used to describe products, which is a combination of QDA and Spectrum.

Quantitative evaluations of the sensory properties of beer are mainly focused on the measure of the appearance, odour, flavour, taste, and mouthfeel sensations, which reflect the sensory profile of the beer(s). Descriptive Analysis is a powerful tool that can provide a complete quantitative description of the sensory characteristics of a product using a trained panel of

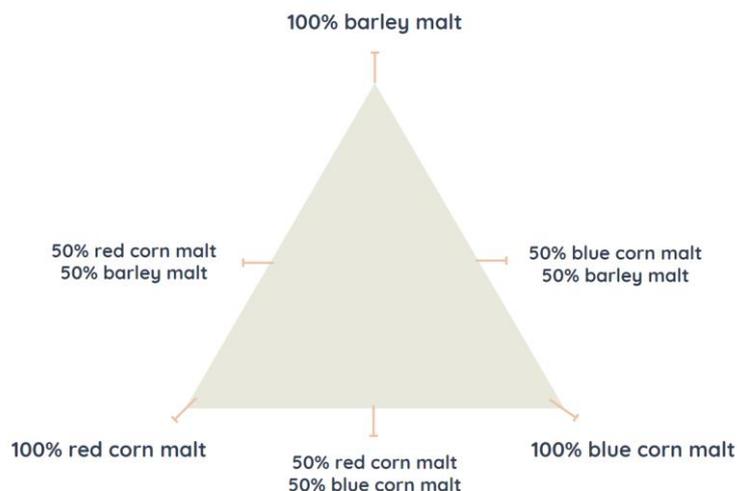
judges (Stone & Sidel, 2004). Such characteristics can then be related to raw materials, process parameters, recipe formulation or other design factors (Giacalone, 2013).

Thus, as all of the sensory properties are a direct reflection of the chemical components (volatiles and non-volatiles) it is interesting to know which specific compounds or groups of compounds directly influence each of the sensory characteristics of the beer (Liu et al., 2012). This could be done through the use of multivariate statistical techniques such as principal component analysis (PCA) and multifactorial analysis (MFA) which allow exploring relationships between the chemical parameters and beer sensory data (Croissant et al., 2011).

According to the results of the concept evaluation (Chapter II), it was proposed 6 formulations based on a mixture design (Figure 27). Also, the selection of the other ingredients and their proportions were proposed aiming to design a beer that matches consumers' expectations.

To this end, six types of beer were developed using different proportions of two varieties of pigmented corn (red and blue) and barley malts (base and caramel).

Figure 27. Mixture design for beer formulations.



All the variables in the brewing process (time, temperature, quantity of additional ingredients) were the same for all six beers. The additional ingredients used for brewing were water, hops (Saaz, 3-5  $\alpha$ -acids and Magnum, 12-15  $\alpha$ -acids, HopUnion LLC, US), guajillo chili (*Capsicum annum*), and dry top-fermenting yeast *Saccharomyces cerevisiae* (Safale US-05, Fermentis, Marcq-en-Baroeul Cedex, France).

The selection of the hops and barley malt was based on the responses of the consumer test in phase I of the concept development (Chapter II). According to the results, consumers expect

the beer to have flavours such as *sweet, bitter, fruity, and spicy*. Thus, the ingredients were selected to achieve these sensory attributes. For instance, Saaz hop provides a delicate bitterness when is added early during the boiling of wort. Magnum hop has a bittering purpose but also adds spice and fruity aromas to the beer.

The addition of guajillo chili as an adjunct during the mashing process has two purposes. First, to preserve the original ingredients of the pre-Hispanic beverage named Sendeché. Second, and to increase the spicy flavour and taste (Figure 5, Chapter I).

This part of the research aims:

**Objective 3:** To build the experimental prototypes of corn beers based on the developed concepts by varying the main ingredient (malt).

**Objective 4:** To understand how the use of pigmented corn malt influences the chemical composition and sensory characteristics of beers

Objective 4.1: Characterise the sensory properties of beers made with pigmented corn malt.

Objective 4.2: Characterise the volatile composition and non-volatile parameters of the beers.

Objective 4.3: Identify sensory attributes that could be influenced by the volatiles and non-volatiles parameters.

Objective 4.4: identify components (sensory, volatiles and non-volatiles) that can be used as indicators of the use of pigmented corn malt.

### **Hypothesis:**

The beers made with a mix of corn malt and barley malt could retain sensory characteristics of traditional corn beverages and traditional beers made with barley malt

### **Outcomes:**

- Poster presentation: “Sensory profile of a gluten-free beer made 100% with corn malt”; 36<sup>th</sup> Congress of the European Brewery Convention”, 14-18 May, Ljubljana, Slovenia.
- Poster presentation: “Characterization of sensory attributes and volatile aroma compounds in Mexican artisan corn beers”; EUROSENSE 2016, Seventh European Conference on Sensory and Consumer Research, 11-14 September, Dijon, Francia.

- Oral presentation: “Influence of corn malt in sensory attributes and volatile aroma compound profiles of Mexican artisan beers”, 18<sup>th</sup> IUFoST World Congress of Food Science and Technology, 21st – 25th August Dublin, Ireland.
- Article 2: Renewing Traditions: A Sensory and Chemical Characterisation of Mexican Pigmented Corn Beers

Figure 28. Poster presented at the 36<sup>th</sup> Congress of the European Brewery Convention, 14-18 May, Ljubljana, Slovenia.

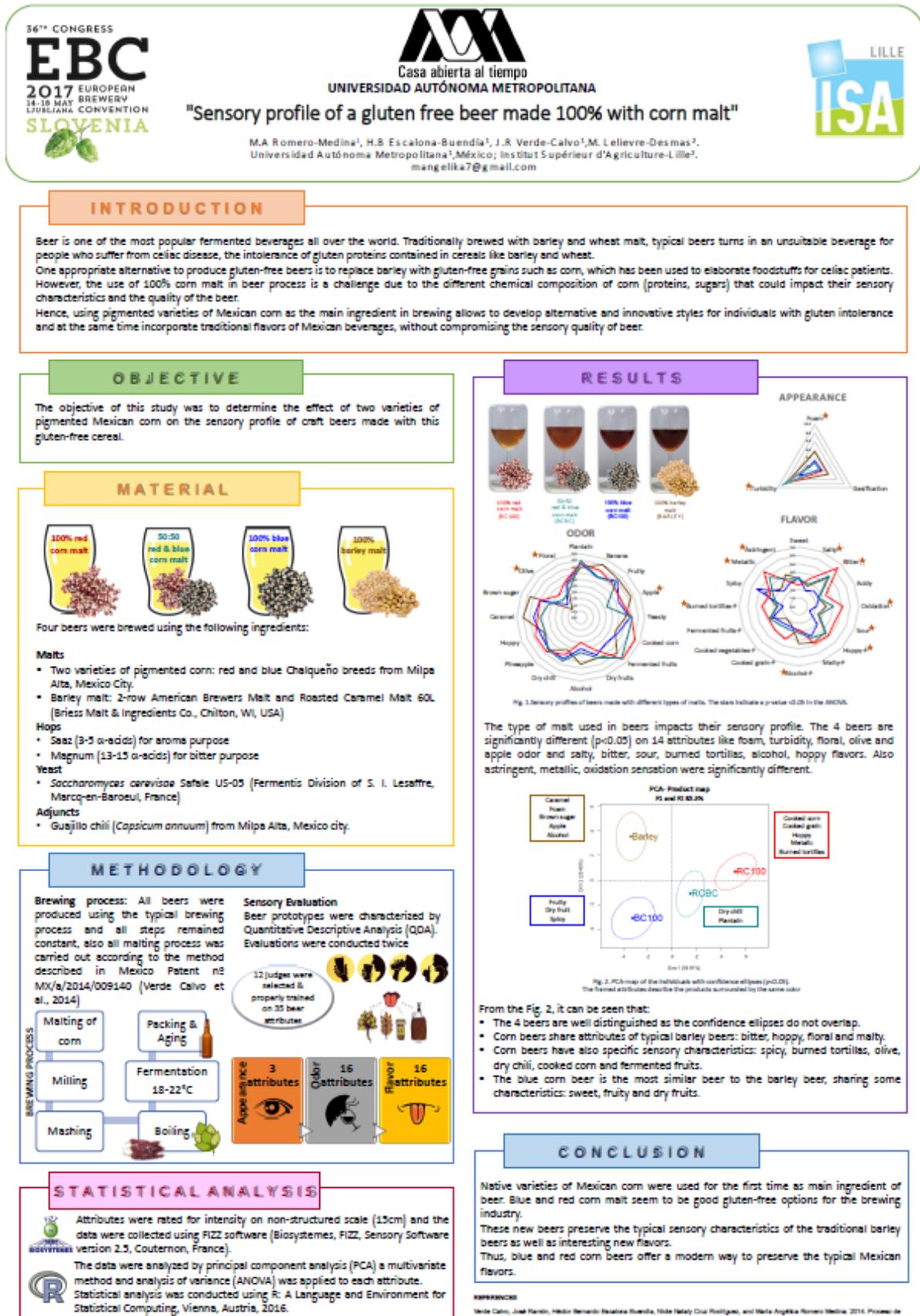


Figure 29. Poster presented at EUROSENSE 2016, Seventh European Conference on Sensory and Consumer Research, 11-14 September, Dijon, Francia.

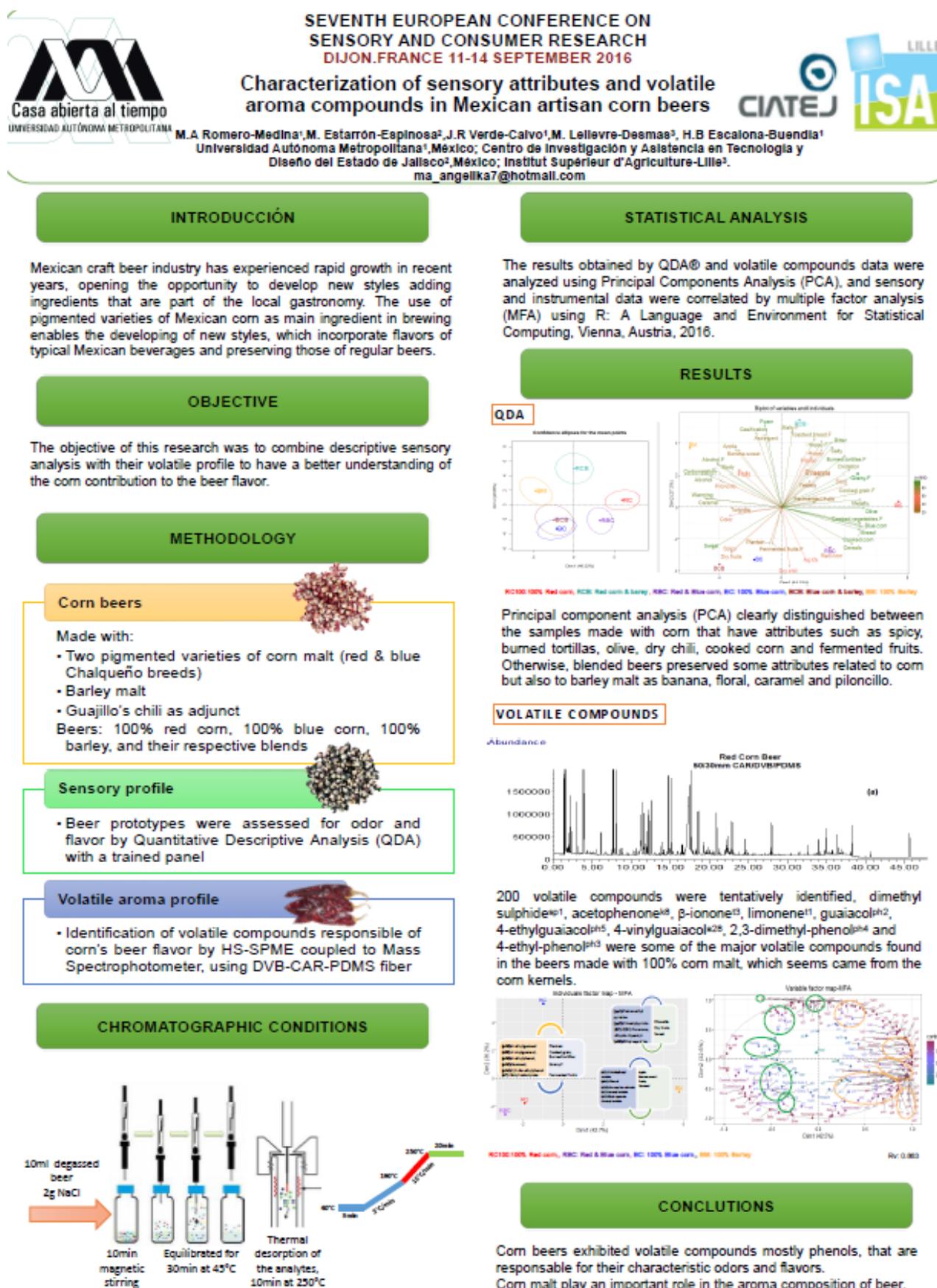


Figure 30. Oral presentation presented at 18th IUFOST World Congress of Food Science and Technology, 21st – 25th August Dublin, Ireland.



Dear María Angélica Romero-Medina ,

Please find enclosed details of your oral presentation

**Speaker Presentations**

<b>Title</b>	Influence of corn malt in sensory attributes and volatile compound profiles of Mexican artisan beers
<b>Paper Status</b>	Accepted
<b>Presentation Type</b>	Oral Presentation
<b>Theme</b>	Innovation in Food Quality and Processing
<b>Session Details</b>	Physical properties of food Aug 24, 2016 2:00 PM - 3:25 PM Dodder Room A
<b>Presenting Author</b>	María Angélica Romero-Medina Affiliations: Universidad Autónoma Metropolitana

### **Influence of corn malt in sensory attributes and volatile aroma compound profiles of Mexican artisan beers**

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Mexican craft beer industry has had a large increase in recent years, opening the opportunity to develop new styles adding ingredients that are part of the local gastronomy. Using pigmented varieties of Mexican corn as main ingredient in beers it allows create styles that incorporate flavors of typical Mexican beverages and preserving those of regular beers.

In this research six prototypes were developed in basis to a mixture experimental design: 100% red corn, 100% blue corn, 100% barley, and their respective blends. The malted grain was the only ingredient varying at defined intervals in order to determine the effect of corn in their sensory attributes and volatile profiles.

Beer prototypes were assessed for odor and flavor by Quantitative Descriptive Analysis (QDA) and analyzed by using headspace solid phase microextraction (HS-SPME) and gas chromatography mass spectrometry (GC-MS).

Principal component analysis (PCA) clearly distinguished between the samples made with corn that have attributes such as spicy, burned tortillas, olive, dry chili, cooked corn and fermented fruits. Otherwise, blended beers preserved some attributes related to corn but also to barley malt as banana, floral, caramel and piloncillo.

180 volatile compounds were tentatively identified, where DMS, acetophenone, guaiacol, 4-vinylguaiacol, 4-ethylguaiacol, 2-undecanol were most closely related to beers made with 100% corn malt. A Multiple Factor Analysis (MFA) revealed that volatile compounds significantly correlated with sensory attributes. Combining descriptive sensory analysis with their volatile profile revealed that corn had a significant influence on the beers flavor and allowed a better understanding to their sensory profile.

*Characterisation of Mexican Pigmented Corn Beers*



Article

## Renewing Traditions: A Sensory and Chemical Characterisation of Mexican Pigmented Corn Beers

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**Abstract:** This study was undertaken to explore how the use of pigmented corn as brewing ingredient influences the sensory profile of craft beers, by using both sensory and chemical analyses. Six pigmented corn and barley beers were brewed and then analysed to obtain their sensory characteristics, volatile composition and non-volatile (alcohol, bitterness, anthocyanins and polyphenol content) composition. ANOVAs, Principal Component Analysis (PCA) and Multiple Factor Analysis (MFA) were used to visualise these data for exploring the differences between beers based on the type of malt and to characterise corn beers considering the relationships between their sensory characteristics and their chemical parameters. The sensory attributes such as fermented fruits, cooked vegetables, tortillas, bread, dried fruits and dried chili characterised beers made 100% with pigmented corn. Over 100 volatiles were identified by head space-solid phase micro-extraction coupled with gas chromatography-mass spectrometry (HS-SPME/GC-MS). Among them, phenols and terpenes were the groups of volatiles that better characterised beers containing corn. The content of anthocyanins in corn beers provide the ‘amber-red-cooper’ colours in beers and may prevent the development of off-aromas and tastes. The use of pigmented corn seems to be a good option to renew the traditional ‘Sendechó’ while preserving some of its sensory attributes.

**Keywords:** *Zea mays*; Sendechó; volatiles; anthocyanins; HS-SPME; GC-MS; sensory profile

### 1. Introduction

Corn (*Zea mays* L.), a cereal native to Mexico, has been the most important cultivated and domesticated crop from ancient times until today [1]. It comes in a great variety of pigmented grains, with colours that range from white and yellow to purple, red, blue and even black [2,3]. In Mexico, ancient civilizations consumed this cereal as the basis of their diet [1]. They developed several fermented beverages based on specific types of pigmented corn, widely referred to as “corn beers” [4–6].

Sendechó is one of these typical fermented beverages made by the Mazahuas population in the Valley of Mexico, whose method of production is very similar to the beer process. It is produced with regional ingredients such as blue pigmented corn that goes through a malting process and Guajillo chili [6], which is a traditional ingredient in Mexican cuisine [7]. But as for most of the traditional beverages, the consumption of Sendechó has gradually declined due to changes in eating habits and urbanisation.

In order to rescue this beverage and preserve some of its sensory properties, we transferred its main ingredients (pigmented corns and guajillo chili) to develop a more modern and consumed beverage, e.g., beer. Therefore, we considered that the use of native varieties of pigmented corn from Mexico for brewing purposes will give added value to both corn grains and beer. Moreover, these types of corn could be used as an alternative cereal in the brewing industry.

Beer is defined as a fermented beverage generally made with four main ingredients: water, malt, hop and yeast [8–10]. Traditionally, barley malt is the most common cereal used in the brewing process [9,10]. Nowadays, as a result of the increased consumption of craft beers, the use of alternative cereals and non-traditional ingredients in the brewing process has increased [11–14]. This allow brewers to create new and different beer styles with a variety of innovative sensory characteristics [9,15].

Several studies of beers have focused on the partial replacement of barley using alternative cereals like wheat [11], rice [12] oats [13] and sorghum [14]. While corn has been considered an economical source of starch [9,16], typically used as an adjunct, authors like Diakabana et al. [17] and Eneje et al. [16] have studied the potential of corn (yellow and white varieties) to produce malt for brewing purposes. Furthermore, in a previous work from our research group, Flores-Calderon et al. [5] developed some beer styles using blue corn malt as the main ingredient. Nevertheless, the use of native varieties of pigmented corn from Mexico has not received similar attention to date.

Since the use of pigmented corn malt as a main ingredient is relatively new to the brewing process [4,5], it is essential to understand the influence of this ingredient on both sensory and chemical composition of these types of beers. Considered as one of the most complex features, beer flavour is generally used in the brewing industry to determine the sensory quality of the beverage. Beer flavour, comprising aromas and tastes, is the result of the combination and interaction of a wide diversity of volatile and non-volatile compounds, originating from the raw ingredients and the brewing process [10,18]. Sensory characteristics of beer are deeply influenced by its chemical profile. Volatile compounds play a key role in the overall aromatic profile of beer. In addition, other non-volatile compounds such as anthocyanins and phenolic components have a significant impact on the sensory attributes such as taste, mouthfeel and colour. Altogether, they serve as a quality indicator and have great importance as they might drive the consumer's acceptance or rejection of this beverage [9]. Although there are many studies regarding sensory and chemical properties of beer [8,11,18,19], little information could be found on beers made with different varieties of corn [4,5]. Moreover, there are no references of the sensory characteristics and volatile compounds of these type of beers.

Thus, in this study we applied both sensory and chemical approaches, combined with an appropriate statistical methodology, to obtain a complete characterisation of beers, and information about those properties that discriminate between samples and explore the associations between the sensory and chemical properties [20,21]. Specifically, the use of multivariate tools like principal component analysis (PCA) and multiple factor analysis (MFA) to analyse sensory and chemical data at the same time can provide a better overview of the sensory characteristics of the 'pigmented corn beers' and chemical components (volatiles and non-volatiles) that can be used as indicators of the use of corn malt.

The main objective of this study was to understand how the use of pigmented corn malt influences the chemical composition and sensory characteristics of beers. To this end, we focused on: (1) characterising the sensory properties of beers made with pigmented corn malt, (2) characterising the volatile composition and non-volatile parameters of the beers (3) identifying sensory attributes that could be influenced by the volatiles and non-volatiles parameters and (4) identifying components (sensory, volatiles and non-volatiles) that can be used as indicators of the use of pigmented corn malt.

## 2. Materials and Methods

In this work, six beers were brewed using different proportions of pigmented corn malt and barley malt (Table 1), hops, water and yeast under an Ale fermentation process. The corn malt was obtained by malting two varieties of pigmented corn (red and blue) and two types of commercial barley malt (base

and caramel) were used. In addition, Guajillo chili (*Capsicum annuum*) was used as an adjunct to preserve the main ingredients of the typical Sendecho beverage. Thereafter, chemical properties of each beer were determined by analysing volatile composition (VoC), alcohol content (ABV), international bitterness units (IBU), total anthocyanins content (TAC) and total polyphenol content (TPC). Moreover, sensory analysis was performed to assess the attributes of the six beers. Finally, a correlation between chemical and sensory data was made to understand the contribution of corn malt to the beer sensory properties.

**Table 1.** Beer formulations.

Prototype	Abbreviation	Beer Formulation
1	BC	100% Blue corn malt
2	RC	100% Red corn malt
3	RBC	50% Red corn malt, 50% blue corn malt
4	Ba	85% Barley base malt, 15 % caramel barley malt
5	BCBa	50% Blue corn malt, 35% base barley malt, 15% caramel barley malt
6	RCBa	50% Red corn malt, 35% base barley malt, 15% caramel barley malt

### 2.1. Corn Malting Procedure

Two Chalqueño varieties of red and blue pigmented corn were purchased locally in Milpa Alta, Mexico City. Each variety of corn was used for the preparation of corn malt. The two varieties of pigmented corn were manually cleaned to remove impurities and then were subjected to a micro-malting procedure as described in Mexico Patent No. 365,910 [22]. The red and blue corn grains were soaked for 12 to 24 h respectively, after which the grains were germinated for three days. Green malt was dried afterwards in a kiln at 50 °C for two days to obtain the base corn malt.

### 2.2. Beer Formulation and Brewing Process

Based on a mixture design, six beers (Table 1) were produced using different proportions of corn and barley malts and brewed under the same conditions. Two batches of each beer (15 L) were produced in a microbrewery pilot plant (30 L) at Universidad Autonoma Metropolitana. For all beers, mashing, brewing, fermentation and maturation procedures were performed according to the procedure described in Mexico Patent No. 365,910 (2014) [22]. Hops (Saaz, 3–5  $\alpha$ -acids and Magnum, 12–15  $\alpha$ -acids, HopUnion LLC, US) were added during boiling of mash to achieve 30 International Bitterness Units (IBU). Guajillo chili (*Capsicum annuum*) was also added during this step. Fermentation of wort by a dry top-fermenting yeast *Saccharomyces cerevisiae* (Safale US-05, Fermentis, Marcq-en-Baroeul Cedex, France) took place in a 20 L fermentation tank at 15 °C for 10 days. The green beer obtained was conditioned by adding sucrose (2 g/L) and immediately packed in amber bottles (355 mL) where maturation was carried out at 5  $\pm$  1 °C for three months.

### 2.3. Analysis of Non-Volatile Components

#### 2.3.1. Alcohol by Volume (ABV)

The volume of alcohol was determined following the ASBC method for Beer-4B, where beer and distillate were measured gravimetrically [23]. Alcohol content was expressed as percentage of alcohol by volume (ABV) and was determined by measuring the specific gravity of the distillate (at 20 °C) and referring to its value in tables.

#### 2.3.2. International Bitterness Units (IBU)

IBU is a standard system used to quantify and express hop bitterness in beer due to the amount of iso-alpha acids. The higher the value, the greater the level of bitterness due to the hops [24,25].

Determination of IBU was estimated following the ASBC method Beer-23A [26]. Aliquots of beer previously degassed were transferred into a 50 mL centrifuge tube and 0.5 mL of 3 M HCl and 10 mL of

2,2,4-trimethylpentane were added. Consequently, samples were shaken and centrifuged at 2500 rpm for 10 min. The absorbance was measured at 275 nm. IBU was obtained by multiplying the absorbance value by a factor of 50.

### 2.3.3. Total Anthocyanin Content (TAC)

The pH differential method was used to quantify total anthocyanins content (TAC) [27]. Results were expressed as mg cyanidin-3-glucoside per liter (C3G/L) for beers based on a molar extinction coefficient ( $\epsilon$ ) of  $26,900 \text{ M}^{-1} \text{ cm}^{-1}$ .

### 2.3.4. Total Polyphenols Content (TPC)

The Folin-Ciocalteu spectrophotometric method developed by Singleton and Rossi [28] was used for the determination of total polyphenols content (TPC) in the beer samples. The measurement was compared with a standard calibration curve of a gallic acid solution over the range 50–1000 mg/L. Results were expressed as mg of gallic acid equivalents per litre (mg GAE/L).

## 2.4. Analysis of Volatile Compounds (VoC)

The volatile composition of beers was analysed by headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography (GC) with mass spectrometry (MS). The extraction and concentration of the volatile compounds were performed using the HS-SPME technique using a 1-cm-long divinylbenzene/carboxen/polidimethylsiloxane (50/30  $\mu\text{m}$  DVB/CAR/PDMS) fibre (Supelco, Mexico). The DVB/CAR/PDMS fibre is the most appropriate for flavour volatile analysis as it covers a wide range of groups of volatile compounds, as has been proved by Dong et al. [29]; Riu-Aumatell et al. [30]. The fibre was heated at 250 °C for 15 min between each analysis to prevent contamination from previous injections.

For the HS-SPME procedure, 10 mL of degassed content from each beer were enclosed in 20-mL glass vials containing 2 g of NaCl. Vials were sealed with a polyethylene and silicone septum cap. The sample was magnetically stirred for 10 min at  $20 \text{ }^\circ\text{C} \pm 1$  for sample/headspace equilibration. After this period, the fibre was exposed to the headspace for 35 min with oscillation at 45 °C; this temperature was maintained throughout the extraction step using a heated circulating bath.

After the extraction of volatile compounds, the fibre was immediately desorbed into GC injection port at 250 °C for 10 min to ensure total desorption. For each sample, the analysis was undertaken in duplicate, taking one sample of each batch, and the results were averaged.

The extracted analytes were analysed in a 7890B/5977A GC-MSD chromatographic system (Agilent Technologies, Palo Alto, CA, USA). Elution and separation of compounds were carried out in a HP-5MS capillary column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$  film thickness, 19091S-433UI). Splitless mode was operated in the injector, and helium was used as the carrier gas at a flow rate of 1.3 mL/min. Oven temperature was set to 40 °C, held for 3 min, raised to 190 °C with a heating rate of 5 °C/min, then raised 15 °C/min to 250 °C and held for 20 min. In the GC-MS system the rate of gas carrier was 1.3 mL/min for 37.5 min, raised 0.5 mL/min to 1.8 mL/min and held until the end of the run.

The 5977A MSD (Agilent Technologies, Palo Alto, CA, USA) detector was at 250 °C and the quadrupole was operated in the electron-impact mode at 70 eV and in the scan range ( $m/z$ ) from 29 to 300, with an ion source temperature of 230 °C.

Data was collected with Mass Hunter GC/MS software (B.07.02.1938). Volatiles were identified by comparing their mass spectrum and their retention times with 36 pure commercial standards. Additionally, all identities were confirmed by comparison of their mass spectra with those of the NIST14 MS library database. In addition, linear retention indices (LRI) were determined with reference to a homologous series of aliphatic hydrocarbons and compared with those reported in literature (Table 2). Since one of the aims of the study was to identify whole volatile compounds that characterise each of the six beer samples, no attempts were made to determine the actual concentration of all

identified compounds. The chromatographic peak area was used as an approach of the abundance of each volatile compound in beers and was expressed as arbitrary units (peak area counts  $\times 10^6$ ) (Table 2).

### 2.5. Sensory Analysis

In this study, we obtained the sensory profile of the six beers using a conventional descriptive method based on the quantitative descriptive analysis [31,32]. Thirteen judges (students from the Autonomous Metropolitan University) were screened and selected based on their sensory acuity to identify and differentiate between the samples, and their potential to describe and communicate sensory perceptions [31].

Panel members were trained in the descriptive language of beer category [33]. First, the panel was familiarised with a wide range of commercial beers. Then, panellists generated a list of attributes pertaining to appearance, odour (nasal), aroma (retronasal), taste and mouthfeel of the beer samples. The panel reached a consensus definition of the terms best describing the attributes of barley and corn beers. Physical references were given in order to develop a common and unified understanding of each attribute. Sensory attributes together with their definitions and physical references used by the panel are shown in Table 3. The attributes were followed by an "Ap", "O", "A", "T", "M", in case these pertained to appearance, odour, aroma, taste or mouthfeel category respectively. Panellist training was accomplished during twelve 1h-working sessions, which involved learning, associating and rating the intensity of the specific beer attributes developed before. Performance of the panel was assessed by measuring its repeatability between sessions, agreement between panellists and consensus, and the discriminative ability of the panel (Supplementary Table S1) [31].

The trained panel subsequently assessed six samples of beers. Three samples of beer were evaluated per session using a balanced sample presentation design. Each beer sample was evaluated in duplicate by each judge. Samples were coded with a randomly selected three-digit number and were presented in monadic form. All samples were kept refrigerated before being served, and 50 mL were presented in a glass at a range of temperature between 5 to 8 °C. A time-out of 5 min between samples was implemented to minimise fatigue, and water and crackers were provided for palate cleansing.

The panellists were instructed to rate the intensity of each attribute using a 15-cm unstructured line scales anchored by "minimum" to "maximum". The evaluation of the colour attribute was done following the instructions of the 'Beer Judge Certification Program (BJCP) Color Guide' [34]. This guide is designed to allow a beer panellist to quickly estimate the colour of a beer sample in Standard Reference Method (SRM) units. The SRM is a numerical scale developed by the American Society of Brewing Chemist (ASBC) to describe beer colour [34]. The scale ranges from 1 SMR (straw) to 40+ SMR (black). All evaluations were performed in an individual sensory evaluation booth equipped with the electronic data-capturing Fizz<sup>®</sup> system (version 2.5; Biosystems, Courtenon, France), and all sessions were conducted in the Spanish language.

Table 2. Volatile compounds identified in all beer samples.

No	Compound Name	LRI <sup>1</sup>	LRI <sup>2</sup>	Peak Area Counts × 10 <sup>6</sup>						ID <sup>3</sup>	Flavour <sup>4</sup>
				BC	RC	RBC	Ba	BCBa	RCBa		
<b>Alcohols</b>											
1	Ethanol	668	527	508.6 ± 132.5	510.5 ± 6.7	354.8 ± 107.4	672.2 ± 14.9	654.2 ± 18.7	548.3 ± 10.8	MS,S	Sweet <sup>6</sup>
2	1-Propanol	536	605	8.8 ± 1.4	2.8 ± 1.6	6.1 ± 4.7	15.0 ± 10.5	6.5 ± 0.2	5.8 ± 0.1	MS,S	Sour <sup>6</sup>
3	2-Methyl-1-propanol	647	644	16.2 ± 11.8	10.4 ± 0.2	16.2 ± 7.4	38.4 ± 28.6	18.1 ± 2.1	26.2 ± 6.7	MS,S	Wine, solvent, bitter <sup>6</sup>
4	3-Methyl-1-butanol	736	750	237.9 ± 118.0	216.4 ± 40.0	171.2 ± 51.3	353.1 ± 22.1	164.7 ± 32.4	287.1 ± 85.8	MS	Whiskey, malt, burnt <sup>6</sup>
5	2-Methyl-1-butanol	755	754	32.9 ± 13.3	32.8 ± 1.2	28.8 ± 9.7	76.6 ± 40.5	34.1 ± 4.4	60.5 ± 4.7	MS	Fermented <sup>6</sup>
6	2,3-Butanediol	769	809	2.0 ± 0.3	2.4 ± 0.2	0.8 ± 0.3	2.32 ± 0.3	0.99 ± 0.2	0.2 ± 0	MS,S	Fruity <sup>6</sup>
7	4-Methyl-1-pentanol	840	835	n.d	n.d	n.d	0.8 ± 0.6	0.3 ± 0.1	0.4 ± 0.1	MS	
8	2-Furanmethanol	851	846	n.d	n.d	n.d	5.7 ± 5.5	2 ± 1.8	1.1 ± 0.3	MS,S	Burnt <sup>6</sup>
9	1-Hexanol	851	854	1.2 ± 1.3	2.4 ± 0.4	6.4 ± 1.8	5.8 ± 4.0	2.7 ± 0.2	3.8 ± 0.8	MS,S	Resin, flower, green <sup>6</sup>
10	3-Methyl-1-hexanol	895	898	n.d	n.d	n.d	1.7 ± 0.8	0.5 ± 0.1	1.2 ± 0.3	MS	
11	1-Heptanol	962	986	1.3 ± 1.3	2.8 ± 1.1	3.2 ± 0.6	2.6 ± 1.7	2.6 ± 0.6	2.3 ± 0.7	MS,S	Chemical, green <sup>6</sup>
12	1-Octen-3-ol	982	1004	n.d	n.d	1.3 ± 0.3	1.2 ± 0.6	1.2 ± 0.2	1.6 ± 0.2	MS,S	Mushroom, earthy <sup>6</sup>
13	2-Ethyl-1-hexanol	1032	1054	3.3 ± 4.3	27.8 ± 3.2	38.7 ± 2.8	52.3 ± 22.0	43.8 ± 14.4	35.7 ± 11.6	MS	Rose, green <sup>6</sup>
14	1-Octanol	1072	1096	5.4 ± 7.1	6.2 ± 2.3	4.7 ± 0.8	6.9 ± 3.6	10.4 ± 3.2	8.7 ± 2	MS,S	Chemical, metal <sup>6</sup>
15	Phenylethyl alcohol	1118	1139	80.1 ± 72.9	36.6 ± 11.7	43.7 ± 5.1	154.4 ± 58.0	112.6 ± 67.7	112.5 ± 32.4	MS	Honey, spice, rose <sup>6</sup>
16	(Z)-3-Nonen-1-ol	1152	1181	n.d	n.d	0.5 ± 0.001	0.8 ± 0.4	7.8 ± 2.1	1.5 ± 0.6	MS	Waxy, green, melon <sup>7</sup>
17	1-Nonanol	1154	1198	n.d	2.0 ± 0.3	n.d	n.d	n.d	4.0 ± 1	MS	Fat, green <sup>6</sup>
18	2-Decanol	1186	1229	14.3 ± 16.8	2.9 ± 2.8	n.d	n.d	n.d	n.d	MS	
19	Citronellol	1233	1255	19.6 ± 22.0	21.1 ± 9.7	3.6 ± 0.1	39.1 ± 20.2	16.8 ± 11.2	9.9 ± 3.1	MS,S	Rose <sup>6</sup>
20	Iso-geraniol	1254	1262	n.d	n.d	n.d	2.0 ± 1.4	1.9 ± 1.7	1.0 ± 0.4	MS	Rose <sup>6</sup>
21	1,9-Nonanediol	-	1292	n.d	1.7 ± 1.3	1.0 ± 0.001	n.d	0.9 ± 0.1	1.0 ± 0.4	MS	
22	1-Decanol	1263	1300	n.d	5.3 ± 3.5	2.2 ± 2.4	13.7 ± 8.6	5 ± 3.7	3.5 ± 1.2	MS	Fat <sup>6</sup>
23	2-Undecanol	1294	1330	8.8 ± 9.1	2.2 ± 1.0	1.0 ± 0.3	5.7 ± 3.8	2.7 ± 1.7	1.5 ± 0.7	MS	
24	Caryophyllenyl alcohol	1568	1608	0.9 ± 0.5	0.3 ± 0.01	n.d	1.2 ± 0.8	n.d	n.d	MS	
<b>Aldehydes</b>											
25	Acetaldehyde	427	503	0.3 ± 0.3	n.d	n.d	0.5 ± 0.2	n.d	n.d	MS,S	Pungent, ether <sup>6</sup>
26	Benzeneacetaldehyde	1044	1068	n.d	n.d	n.d	1.1 ± 0.3	0.5 ± 0.1	0.8 ± 0.2	MS	
27	Nonanal	1104	1130	3.7 ± 4.1	2.9 ± 1.9	1.5 ± 0.001	2.7 ± 1.1	5.4 ± 1.6	2.2 ± 0.8	MS	Fat, citrus, green <sup>6</sup>
28	Decanal	1209	1233	8.2 ± 9.5	1.9 ± 1.7	2.7 ± 0.2	n.d	n.d	2.8 ± 1.6	MS	Soap, orange peel, tallow <sup>6</sup>
<b>Aliphatic hydrocarbons</b>											
29	Tetradecane	1400	1430	2.2 ± 2.2	0.7 ± 0.3	1.2 ± 0.001	3.0 ± 1.4	n.d	n.d	MS	Waxy <sup>5</sup>
30	Pentadecane	1500	1530	2.6 ± 2.9	0.6 ± 0.4	0.8 ± 0.4	3.0 ± 1.5	n.d	n.d	MS	Waxy <sup>5</sup>
<b>Carboxylic acids</b>											
31	Acetic acid	600	619	11.7 ± 0.001	1.5 ± 0.5	n.d	n.d	1.2 ± 1.5	8.5 ± 1.2	MS,S	Sour <sup>6</sup>
32	2-Methyl-propanoic acid	752	779	3.5 ± 0.7	2.4 ± 0.2	7.2 ± 2.3	3.1 ± 0.3	n.d	n.d	MS	Rancid butter <sup>5</sup>
33	3-Methyl-butanoic acid	877	839	1.4 ± 0.001	1.8 ± 1.3	6.9 ± 5.3	3.4 ± 0.001	0.3 ± 0.1	0.8 ± 0.1	MS	Sweat, acid, rancid <sup>5</sup>
34	2-Methyl-hexanoic acid	-	844	n.d	n.d	0.2 ± 0.1	n.d	1 ± 0.8	0.9 ± 0.5	MS	
35	2-Methyl-butanoic acid	896	845	n.d	n.d	0.5 ± 0.1	1.3 ± 0.2	0.7 ± 0.3	n.d	MS	
36	Hexanoic acid	1019	1010	n.d	2.2 ± 0.1	21.4 ± 4.4	17.8 ± 0.2	n.d	13.0 ± 0.6	MS,S	Fatty, sour, sweat, cheese <sup>6</sup>
37	Heptanoic acid	1078	1103	n.d	1.5 ± 1.6	n.d	2.6 ± 2.4	1.0 ± 0.4	1.3 ± 0.7	MS	Cheesy, waxy, sweaty <sup>5</sup>
38	2-Ethyl-hexanoic acid	1116	1167	0.9 ± 0.8	1.0 ± 0.5	n.d	3.8 ± 1.3	n.d	0.6 ± 0.9	MS	

Table 2. Cont.

No	Compound Name	LRI <sup>1</sup>	LRI <sup>2</sup>	Peak Area Counts × 10 <sup>6</sup>						ID <sup>3</sup>	Flavour <sup>4</sup>
				BC	RC	RBC	Ba	BCBa	RCBa		
39	Octanoic acid	1279	1209	608.8 ± 5.3	194.3 ± 4.5	217.3 ± 1.6	411.8 ± 16.4	12 ± 0.4	458.1 ± 30.7	MS,S	Sweat, cheese <sup>6</sup>
40	9-Decenoic acid	1358	1392	35.8 ± 31.8	8.6 ± 6.8	10.9 ± 0.9	n.d.	7.4 ± 0.6	n.d.	MS	
41	Decanoic acid	1373	1399	51.0 ± 45.7	n.d.	23.5 ± 7.0	68.0 ± 54.7	7.8 ± 0.5	10.9 ± 2.9	MS,S	Rancid, fat <sup>6</sup>
42	Hexadecanoic acid	1984	2000	n.d.	3.8 ± 4.0	3.3 ± 3.9	1.6 ± 1.7	n.d.	n.d.	MS,S	Oily <sup>6</sup>
<b>Esters</b>											
43	Ethyl acetate	628	635	25.0 ± 12.7	16.6 ± 9.6	27.1 ± 1.9	57.7 ± 34.8	42.5 ± 6.5	46.0 ± 16.3	MS,S	Pineapple <sup>6</sup>
44	Ethyl propanoate	713	728	n.d.	n.d.	2.1 ± 1.6	2.7 ± 1.4	2 ± 0.1	2.4 ± 0.3	MS	Fruit <sup>6</sup>
45	Propyl acetate	720	731	n.d.	n.d.	n.d.	1.0 ± 0.2	n.d.	0.4 ± 0.1	MS	Sweet, fruity, caramel <sup>7</sup>
46	Ethyl isobutanoate	756	780	n.d.	n.d.	n.d.	1.3 ± 0.4	1.2 ± 0.1	2.8 ± 0.8	MS	Sweet, rubber <sup>6</sup>
47	Isobutyl acetate	776	798	1.8 ± 0.2	1.4 ± 0.3	1.7 ± 1.3	1.4 ± 0.7	2.3 ± 0.2	2.3 ± 0.8	MS	Fruit, apple, banana <sup>6</sup>
48	Ethyl butanoate	804	814	2.2 ± 0.3	2.1 ± 0.5	1.9 ± 0.1	4.5 ± 2.7	9 ± 0.3	5.8 ± 1.8	MS	Apple <sup>6</sup>
49	3-Methylbutyl acetate	877	859	31.7 ± 18.5	38.7 ± 12.7	20.3 ± 7.9	47.9 ± 25.6	113 ± 2.6	66.6 ± 19.7	MS	Fresh, banana, sweet <sup>5</sup>
50	2-Methylbutyl acetate	876	861	2.0 ± 1.2	3.0 ± 1.1	1.2 ± 0.7	2.7 ± 1.5	5.2 ± 0.7	5.6 ± 1.5	MS	Herbal, fermented fruity <sup>5</sup>
51	Ethyl pentanoate	900	875	n.d.	n.d.	n.d.	1.1 ± 0.6	1.5 ± 0.1	2.2 ± 0.5	MS	Yeast, fruit <sup>7</sup>
52	Ethyl iso-hexanoate	-	974	n.d.	n.d.	n.d.	0.8 ± 0.4	1.3 ± 0.7	1.4 ± 0.5	MS,T	Sweet, fruity, tropical, green, apple <sup>7</sup>
53	Methylbutyl propanoate	-	992	0.7 ± 0.5	1.2 ± 0.8	n.d.	n.d.	0.9 ± 0.4	n.d.	MS,T	
54	Ethyl hexanoate	1002	1025	8.9 ± 7.5	14.8 ± 8.8	10.0 ± 2.2	27.7 ± 13.9	151.2 ± 89.3	139.8 ± 41.5	MS,S	Apple peel, fruit <sup>6</sup>
55	Hexyl acetate	1014	1039	2.1 ± 1.4	3.6 ± 2.3	1.8 ± 0.6	1.8 ± 0.4	3.3 ± 1.2	1.3 ± 0.4	MS	Fruity, spicy, herbal, sweet wine, rubbery <sup>7</sup>
56	2-Methylbutyl isobutanoate	1014	1042	5.0 ± 2.9	15.7 ± 10.2	n.d.	n.d.	1.1 ± 0.4	n.d.	MS	Fruity, ethereal <sup>7</sup>
57	Ethyl 5-methylhexanoate	-	1088	n.d.	1.1 ± 0.5	n.d.	0.9 ± 0.3	6.7 ± 3.5	4.3 ± 1.2	MS,T	
58	Ethyl benzoate	1185	1197	n.d.	n.d.	n.d.	4.0 ± 2.2	2.7 ± 1.7	n.d.	MS	Chamomile, flower <sup>6</sup>
59	Ethyl octanoate	1198	1225	67.1 ± 59.9	63.5 ± 36.1	51.8 ± 6.2	n.d.	724.7 ± 45.0	3.9 ± 1.3	MS,S	Fruit, fat <sup>6</sup>
60	Ethyl phenylacetate	1252	1273	n.d.	n.d.	0.8 ± 0.2	2.8 ± 1.3	2.2 ± 1.3	1.4 ± 0.7	MS	Fruit, sweet <sup>7</sup>
61	Phenethyl acetate	1265	1285	14.5 ± 13.9	5.4 ± 2.4	5.8 ± 0.8	20.6 ± 9.3	15.2 ± 8.3	8.5 ± 2.8	MS	Rose, floral <sup>7</sup>
62	Ethyl nonanoate	1295	1326	1.8 ± 1.5	n.d.	0.7 ± 0.00	2.0 ± 1.1	3.2 ± 1.9	1.9 ± 0.9	MS	Fruity, rose <sup>6</sup>
63	Methyl geranoate	1323	1354	6.6 ± 6.1	3.0 ± 1.6	3.3 ± 0.7	3.4 ± 1.6	2.2 ± 1.5	0.7 ± 0.3	MS	Floral <sup>6</sup>
64	Ethyl benzenepropanoate	1390	1379	n.d.	n.d.	n.d.	1.7 ± 1.0	1.5 ± 1	n.d.	MS	
65	Ethyl (E)-4-decenoate	-	1408	4.3 ± 3.0	1.7 ± 0.8	n.d.	6.9 ± 1.0	3.6 ± 1.2	3.7 ± 1.1	MS,T	
66	Ethyl 9-decenoate	1387	1417	15.2 ± 1.2	13.2 ± 7.0	12.7 ± 3.2	15.5 ± 6.3	59.6 ± 18.7	22.7 ± 6.4	MS	
67	Ethyl decanoate	1397	1426	20.3 ± 14.0	12.5 ± 5.9	13.5 ± 4.6	50.1 ± 17.1	24.3 ± 5.9	31.6 ± 10.6	MS,S	Grape, fruit <sup>6</sup>
68	Isoamyl octanoate	-	1478	0.7 ± 0.6	n.d.	0.3 ± 0.1	1.5 ± 0.5	0.7 ± 0.3	1.1 ± 0.4	MS,T	
69	Ethyl dodecanoate	1494	1628	9.9 ± 4.3	3.1 ± 1.3	1.6 ± 0.7	8.7 ± 4.4	n.d.	n.d.	MS	Leaf <sup>6</sup>
70	Dibutyl maleate	-	1571	1.6 ± 1.1	n.d.	0.4 ± 0.001	0.8 ± 0.6	n.d.	n.d.	MS,T	
71	Ethyl <i>cis</i> -9-pentadecenoate	-	1622	6.4 ± 4.3	0.9 ± 0.5	0.4 ± 0.1	n.d.	n.d.	n.d.	MS,T	
72	Ethyl tetradecanoate	1793	1832	1.5 ± 0.6	0.7 ± 0.1	0.5 ± 0.1	1.7 ± 1.0	4.8 ± 1.6	n.d.	MS,S	Oily, violet <sup>6</sup>
73	2-Ethylhexyl salicylate	1816	1847	1.3 ± 0.7	2.9 ± 3.7	1.6 ± 0.5	5.2 ± 6.4	0.9 ± 0.5	1.2 ± 0	MS	
74	Ethyl 9-hexadecenoate	-	2015	n.d.	0.9 ± 0.5	1.1 ± 1.0	0.9 ± 0.6	0.3 ± 0.1	n.d.	MS,T	
75	Ethyl hexadecanoate	1991	2038	1.1 ± 0.2	1.1 ± 0.4	1.9 ± 0.9	2.1 ± 1.5	0.3 ± 0.2	n.d.	MS	Waxy <sup>6</sup>
76	Isopropyl palmitate	-	2070	n.d.	n.d.	2.5 ± 2.5	2.2 ± 2.6	n.d.	n.d.	MS,T	
77	1-Propylpentyl dodecanoate	-	2152	0.6 ± 0.2	3.5 ± 3.9	2.8 ± 2.5	4.5 ± 5.2	n.d.	n.d.	MS,T	

Table 2. Cont.

No	Compound Name	LRI <sup>1</sup>	LRI <sup>2</sup>	Peak Area Counts × 10 <sup>6</sup>						ID <sup>3</sup>	Flavour <sup>4</sup>
				BC	RC	RBC	Ba	BCBa	RCBa		
<b>Furans</b>											
78	Acetylfuran	893	881	n.d	n.d	n.d	1.8 ± 1.5	0.3 ± 0.1	n.d	MS,S	Balsamic <sup>6</sup>
79	3-Methyl-2,3-dihydro-1-benzofuran	-	1178	n.d	n.d	0.5 ± 0.1	n.d	1.3 ± 1.0	n.d	MS,T	
80	2,3-Dihydro-benzofuran	-	1246	2.5 ± 2.7	3.9 ± 0.5	n.d	4.3 ± 2.0	n.d	3.9 ± 1.1	MS,T	
81	Dihydro-5-pentyl-2(3H)-furanone	-	1392	n.d	n.d	n.d	16.7 ± 7.7	5.6 ± 1.6	7.8 ± 2.8	MS,T	
<b>Aromatic hydrocarbons</b>											
82	Styrene	893	867	33.2 ± 18.7	32.3 ± 19.7	25.7 ± 0.4	56.8 ± 25.3	52.9 ± 13.1	78.0 ± 27.4	MS	Balsamic, gasoline <sup>6</sup> Mothball-like <sup>5</sup>
83	1,4-Dichloro-benzene	1015	1035	29.0 ± 28.7	13.0 ± 0.5	23.5 ± 3.3	29.7 ± 11.4	18.0 ± 1.1	15.7 ± 2.7	MS	
84	Squalene	2833	2881	2.6 ± 0.3	16.4 ± 16.2	18.7 ± 21.0	12.9 ± 14.8	n.d	n.d	MS	
<b>Ketones</b>											
85	2-Pentanone	636	708	n.d	n.d	n.d	0.6 ± 0.4	0.6 ± 0	n.d	MS	Ether <sup>6</sup>
86	3-Methyl-2-pentanone	759	777	n.d	1.1 ± 1.2	n.d	n.d	n.d	n.d	MS	
87	Acetophenone	1041	1091	n.d	n.d	0.4 ± 0.1	0.7 ± 0.2	n.d	n.d	MS,S	Must, flower, almond <sup>6</sup> Fruity, sweet, waxy, soapy, herbaceous, coconut <sup>5</sup>
88	2-Nonanone	1091	1118	n.d	1.3 ± 0.8	n.d	n.d	0.9 ± 0.3	n.d	MS	
89	β-Damascenone	1386	1386	n.d	n.d	n.d	n.d	7.8 ± 2.7	6.7 ± 1.6	MS	
90	β-Ionone	1493	1526	1.2 ± 0.1	0.8 ± 0.2	n.d	n.d	0.6 ± 0.3	0.4 ± 0.2	MS,S	
<b>Miscellaneous</b>											
91	Methoxy-phenyl-oxime	-	883	9.1 ± 6.9	4.1 ± 1.3	3.5 ± 0.4	8.2 ± 7.4	n.d	0.5 ± 0.3	MS,T	
92	Geranyl vinyl ether	-	1259	3.0 ± 3.0	1.3 ± 1.0	n.d	n.d	n.d	n.d	MS,T	
93	9-Decen-1-ol methyl ether	-	1312	7.5 ± 7.8	n.d	0.7 ± 0.2	n.d	1.6 ± 0.01	n.d	MS,T	
<b>Phenols</b>											
94	Phenol	980	1007	n.d	n.d	5.7 ± 0.001	4.6 ± 1.5	n.d	1.0 ± 0.5	MS	Phenolic, medicinal <sup>6</sup> Smoke, sweet, medicine <sup>6</sup> Spice, clove <sup>6</sup> Spice, smoke, clove, medicinal <sup>5</sup> Spicy, cooling, thymol-like, herbal and camphoreous <sup>5</sup> Smoky, bacon <sup>5</sup> Phenolic <sup>5</sup>
95	2-Methoxy-phenol	1089	1115	3.3 ± 3.6	n.d	2.3 ± 0.3	n.d	2.4 ± 1.9	0.4 ± 0.1	MS,S	
96	4-Ethyl-phenol	1287	1193	4.4 ± 4.9	0.8 ± 0.1	41.4 ± 4.0	1.5 ± 0.1	0.7 ± 0.3	1.0 ± 0.4	MS,S	
97	4-Ethyl-2-methoxy-phenol	-	1308	8.0 ± 7.9	1.7 ± 0.8	22.2 ± 4.0	n.d	2.3 ± 2	2.0 ± 0.8	MS,T	
98	2-Methyl-5-(1-methylethyl)-phenol	1307	1323	4.4 ± 4.9	0.7 ± 0.5	0.5 ± 0.2	2.0 ± 1.8	n.d	n.d	MS	
99	2-Methoxy-4-vinylphenol	1315	1344	1.8 ± 1.6	21.2 ± 4.7	23.0 ± 4.2	15.4 ± 7.8	2.5 ± 1.5	2.6 ± 0.6	MS	
100	2,6-Di-tert-butylphenol	1444	1502	2.0 ± 2.0	n.d	2.8 ± 0.001	n.d	n.d	n.d	MS,T	
<b>Pyrrrole and pyrazine</b>											
101	2-Acetylpyrrole	1045	1086	n.d	n.d	n.d	1.3 ± 0.5	0.5 ± 0.2	n.d	MS	Nut, walnut, bread <sup>6</sup> Nutty <sup>7</sup>
102	Tetramethyl-pyrazine	-	1122	n.d	n.d	n.d	1.2 ± 0.3	1.6 ± 0.6	0.7 ± 0.1	MS,T	
<b>Sulphur compounds</b>											
103	Dimethyl sulfide	505	569	3.8 ± 2.6	3.2 ± 1.6	3.8 ± 0.4	4.7 ± 1.2	2.7 ± 0.4	2.4 ± 0	MS	Cabbage, sulphur, gasoline <sup>6</sup>
<b>Terpenes</b>											
104	β-Myrcene	992	1016	10.7 ± 11.9	21.9 ± 17.8	n.d	n.d	4.7 ± 1.7	9.6 ± 4.7	MS,S	Balsamic, must, spice <sup>6</sup> Lemon, orange <sup>6</sup> Flower, lavender <sup>6</sup>
105	Limonene	1033	1056	42.6 ± 57.6	11.3 ± 5.8	1.2 ± 0.5	0.7 ± 0.5	n.d	n.d	MS,S	
106	Linalool	1100	1126	40.7 ± 46.9	37.0 ± 14.1	8.7 ± 0.4	22.3 ± 9.9	28.3 ± 11.9	18.5 ± 4.8	MS,S	

Table 2. Cont.

No	Compound Name	LRI <sup>1</sup>	LRI <sup>2</sup>	Peak Area Counts × 10 <sup>6</sup>						ID <sup>3</sup>	Flavour <sup>4</sup>
				BC	RC	RBC	Ba	BCBa	RCBa		
107	Camphor	1139	1171	n.d.	n.d.	0.6 ± 0.1	1.3 ± 0.8	1 ± 0.9	n.d.	MS,S	Camphor <sup>6</sup>
108	Geraniol	1276	1283	12.0 ± 13.1	3.8 ± 2.1	2.2 ± 0.2	13.8 ± 7.9	3.6 ± 2.2	1.9 ± 0.8	MS,S	Rose, geranium <sup>6</sup>
109	Caryophyllene	1467	1454	2.4 ± 1.8	1.5 ± 0.4	n.d.	1.5 ± 0.4	0.8 ± 0.2	2.7 ± 1	MS,S	Wood, spice <sup>6</sup>
110	Humulene	1467	1489	11.3 ± 5.1	7.4 ± 2.5	0.7 ± 0.1	6.5 ± 2.2	1 ± 0.4	11.6 ± 4.5	MS,S	Wood <sup>6</sup>
111	3-Methoxy-2-naphthalenol	-	1518	n.d.	n.d.	n.d.	2.7 ± 1.3	0.3 ± 0.2	0.4 ± 0.2	MS,T	
112	δ-Cadinene	1519	1559	0.7 ± 0.3	n.d.	n.d.	1.2 ± 0.3	n.d.	0.5 ± 0.2	MS	Thyme, medicine, wood <sup>6</sup>
113	E-Nerolidol	1539	1597	2.7 ± 1.8	n.d.	0.6 ± 0.1	3.6 ± 1.8	1.2 ± 0.6	0.5 ± 0.2	MS	Wood, flower, wax <sup>6</sup>
114	Caryophyllene oxide	1573	1612	1.4 ± 1.3	n.d.	n.d.	1.7 ± 1.1	0.8 ± 0.4	0.7 ± 0.1	MS,T	Herb, sweet, spice <sup>6</sup>
115	Humulene oxide	1642	1641	1.3 ± 1.0	0.9 ± 0.3	0.3 ± 0.1	9.8 ± 5.3	0.4 ± 0.2	n.d.	MS,S	Herb <sup>6</sup>
116	Cubenol	1645	1666	1.2 ± 0.7	0.9 ± 0.5	0.4 ± 0.2	1.2 ± 0.4	0.4 ± 0.2	n.d.	MS	Spice, herb, green tea <sup>6</sup>
117	Di-epi-1,10-cubenol	1613	1669	2.0 ± 1.2	n.d.	n.d.	3.3 ± 1.7	0.3 ± 0.1	n.d.	MS	
118	Calarene epoxide	-	1672	7.9 ± 4.9	n.d.	n.d.	n.d.	1.1 ± 0.6	0.7 ± 0.1	MS,T	Woody <sup>5</sup>
119	τ-Cadinol	1635	1679	4.3 ± 2.8	n.d.	n.d.	4.5 ± 3.0	n.d.	n.d.	MS	Herb, weak spice <sup>6</sup>
120	δ-Cadinol	1674	1689	0.5 ± 0.6	n.d.	n.d.	1.1 ± 0.1	0.7 ± 0.3	n.d.	MS	Herb <sup>6</sup>
121	α-Cadinol	1676	1695	2.8 ± 1.8	n.d.	n.d.	1.9 ± 1.9	0.3 ± 0.1	n.d.	MS	Herb, wood <sup>6</sup>

Chromatographic peak area (peak area counts × 10<sup>6</sup>) of the flavour volatile compounds. Results are expressed as mean ± standard deviation (n = 2); BC = 100% blue corn, RC = 100% red corn, RBC = 50:50 red and blue corn, Ba = 100% barley, BCBa = 50:50 blue corn and barley, RCBa = 50:50 red corn and barley. <sup>1</sup> LRI = Linear retention index (NIST values (<http://webbook.nist.gov/chemistry/name-ser.html>)). <sup>2</sup> LRI = Linear retention index on HP-5MS column (Agilent Technologies), calculated via duplicated averaged alkanes, and found to be comparable with NIST values (<http://webbook.nist.gov/chemistry/name-ser.html>). <sup>3</sup> ID = Identification used as confirmation of compounds per: MS = library match; S = standards; T = tentative. <sup>4</sup> Flavour descriptors according to <sup>5</sup> The Good Scents Company (<http://www.thegoodscentscompany.com/>), <sup>6</sup> Flavornet (<http://www.flavornet.org/flavornet.html>) and <sup>7</sup> Pherobase (<http://www.pherobase.com/>). n.d. no detected.

**Table 3.** Sensory attributes, description and physical references used in this study (attributes pertaining to appearance, odour, aroma, taste and mouthfeel are designated by an “Ap”, “O”, “A”, “T”, “M” respectively after the attribute).

Attributes	Abbreviation	Description	Reference
Colour	Colour-Ap	Refers to the colour of the beer	Standard Reference Method (SRM) scale
Turbidity	Turbidity-Ap	Refers to the haziness of the beer	Range of different beer samples
Banana	Banana-O	Sweet gum flavoured banana	Isoamyl acetate (Siebel® kit)
Fruity	Fruity-O	A mix of fruits as pear, strawberry and grapefruit	Linalool (Siebel® kit)
Apple	Apple-O	Green apple	Acetaldehyde (Siebel® kit)
Cooked corn	Cook.corn-O	“Esquites odour”	Dimethyl sulfide (Siebel® kit)
Fermented fruits	Ferm fruits-O	Traditional fermented beverage made of a mix of fruits as pineapple, guava and apple	“Tepache”
Dried fruits	Dried fruits-O	Raisins, prunes, plum	Firmenich® reference
Dried chili	Dried chili-O	Odour of the Guajillo chili	Guajillo chili (6 g/L)
Pineapple	Pineapple-O	Ripe pineapple	Firmenich® reference
Hoppy	Hoppy-O	Pine -herbaceous odour	Tea made of Saaz and Magnum hops (0.5 g/L)
Bread	Bread-O	Fresh bread recently cooked	Firmenich® reference
Caramel	Caramel-O	Associated to caramel	Firmenich® reference
Brown sugar	Brown sugar-O	Product elaborated from raw brown sugar	“Piloncillo”
Olive	Olive-O	Vinegar-like	Acetic acid (Siebel® kit)
Floral	Floral-O	Flowers-like, roses	Geraniol (Siebel® kit)
Hoppy	Hoppy.A	Pine -herbaceous aroma	Tea made of Saaz and Magnum hops (0.5 g/L)
Malty	Malty.A	Malty-like	Firmenich® reference
Alcohol	Alcohol-A	A warming sensation in the mouth and throat	Firmenich® reference
Cooked vegetables	Cook.veg-A	Mix of cooked vegetables	Dimethyl sulfide (Siebel® kit)
Burnt tortillas	Tortillas-A	Aroma related to tortillas after being heated	Burnt tortillas
Sweet	Sweet-T	Associate with sugar taste	Sucrose 7.5 g/L
Bitter	Bitter-T	Associate with bitter taste	Isolone (Siebel® kit)
Sour	Sour-T	Associate with acid taste	Lactic acid (Siebel® kit)
Oxidised	Oxidised-M	Papery, cardboard	trans-2-nonenal (Siebel® kit)
Spicy	Spicy-M	Pungent sensation in the tongue caused by chili	Guajillo chili (6 g/L)
Metallic	Metallic-M	Metal-like	Ferrous sulfate (Siebel® kit)
Astringent	Astringent-M	Sensation of dryness in the tongue and mouth	Tannic acid (0.6 g/L)
Carbonatation	Carbonatation-M	Sensation tingle in the tongue related to CO <sub>2</sub>	Peñafiel mineral water
Fullness	Fullness-M	Refers to the perceived density while it is being consumed	Range of different beer samples

## 2.6. Statistical Analysis

Data relative to the peak chromatographic areas of the identified volatile compounds were reported as the average of the two independent replicates  $\pm$  standard deviation (six beer samples, each one by duplicate).

Analysis of variance (ANOVA) was performed on the sensory and non-volatile data (ABV, IBU, TAC, TPC) to ascertain significant differences among all six beer samples. A post-hoc Tukey's test was carried out when a significant difference ( $p < 0.05$ ) was detected among samples.

To explore the sensory differences among the beer samples a Principal Component Analysis (PCA) with Pearson correlation coefficients was performed on the table beers  $\times$  attributes (6 rows  $\times$  30 columns) containing the mean intensity scores obtained by each beer for each sensory attribute (calculated over the panellist and the repetitions). No rotation option was applied.

Multiple factor analysis (MFA) is a useful statistical method to analyse the similarities and discrepancies between a set of observations explained by data tables of different groups of variables. It can also be used to show correlation between those sets of variables [21,35].

In this study, MFA was conducted on the data matrices of sensory and chemical (volatile and non-volatiles) variables. More specifically the sensory matrix was divided into two matrices of respectively 19 'odour-aroma' variables (14 odour attributes and 5 aroma attributes) and 7 'taste-mouthfeel' variables (3 taste attributes and 4 mouthfeel attributes). The goal of this separation was to provide a better representation of the chemical data contributions on the odour-aroma and taste-mouthfeel attributes.

Therefore, the MFA was computed on four data tables consisting of: 19 odour-aroma attributes, 7 taste-mouthfeel attributes, 121 volatiles and 4 non-volatile parameters. Additionally, attributes namely colour, turbidity, carbonation and fullness, which are important for beer characterisation but are not directly influenced by volatile components, were used as supplementary (non-active) variables in the analysis.

All statistical analyses were performed using XLSTAT (version 2018.7, XLSTAT-Sensory package, Addinsoft, Paris, France).

## 3. Results and Discussion

### 3.1. Analysis of Non-volatile Parameters

Results for the non-volatile analyses of beers are shown in Table 4. We can see significant differences ( $p < 0.05$ ) among all samples on every parameter analysed (ABV, IBU, TAC and TPC).

**Table 4.** Mean score of beer samples for each non-volatile parameter.

Parameter		Beer					
		BC	RC	RBC	Ba	BCBa	RCBa
Alcohol (% v/v)	ABV	3.71 c	2.98 cd	1.93 d	7.01 ab	5.45 b	7.21 a
International bitterness units	IBU	14.57 b	19.05 a	19.62 a	18.45 a	18.92 a	15.72 b
Anthocyanins (mg/L)	TAC	14.45 a	8.84 b	14.60 a	0.00 e	3.90 d	6.17 c
Polyphenols (mg GAE/L)	TPC	750.0 ab	331.0 c	367.5 c	398.5 c	849.5 a	721 b

Values with different letters across a row are significantly different ( $p < 0.05$ ) according to the Tukey post-hoc test. BC = 100% blue corn, RC = 100% red corn, RBC = 50:50 red and blue corn, Ba = 100% barley, BCBa = 50:50 blue corn and barley, RCBa = 50:50 red corn and barley.

The content of alcohol (ABV) was significantly higher in beers that contained barley malt than in those made only with corn malt. This might be explained as corn has shown a low diastatic power compared to barley [5,9], which leads to wort contained less fermentable sugars and thus, less alcohol content. As the brewing process remained under the same conditions for all beers, it was surprising to find that the bitterness unit (IBU) in beers were significantly different only for the blended beer made of red corn and barley malt (15.72 IBU) and the blue corn beer (14.57 IBU). These beers showed

lowest IBU than the rest of beers (ranged between 15.7 to 19.6). The IBU is a measurement of how much iso- $\alpha$ -acids (1 IBU = 1 ppm iso-humulone) is in the final product, but it does not always really tell if a beer is bitter or not [24]. The amount of iso- $\alpha$ -acids in the beer depends on the time and temperature the hops spend in the boiling step [25]. Thus, minor changes in temperature or time the hops are added to the wort could change the amount of iso- $\alpha$ -acids in beer. Additionally, some authors have reported the susceptibility of this method to the interference from other compounds present in beer, such as polyphenols, that absorb light at the wavelength of measurement (275 nm). Therefore, minor contributions from compounds unrelated to bitterness can be detected (oxidised fatty acids), whereas others contributing to bitterness are not detected [36]. Moreover, coloured beers absorb light which directly decrease the emission intensity and result in lower IBU values [37]. Despite limitations, the IBU method is widely used as an indicator of bitterness in quality control [24,25].

Beers containing only corn malt showed a higher content of anthocyanins (TAC) than those blended beer made of barley and corn malt. The anthocyanins value for beers made of blue corn and red corn malt varied from 14.6 to 8.84 mg C3G/L respectively. These results are in agreement with Flores-Calderón et al. [5] who assessed different styles of blue corn beer and reported values that ranged from 13.2 to 18.7 mg C3G/L. A significantly higher difference between beers made of blue corn malt than the one made of red corn malt is expected as a greater amount of anthocyanins has been reported in varieties of blue corn than in the red corn variety [38]. Also, as was expected, the beer made of 100% barley malt did not show presence of anthocyanins. Red and blue corn contain anthocyanins, such as pelargonidin-3-glucoside and cyanidin-3-glucoside, which are responsible of the colour of the grains. Additionally, these anthocyanins have been reported to have various biological activities, such as antioxidant, antimicrobial, antimutagenic and anticancer effects [3,38]. Regarding sensory profile, presence of anthocyanins in beer not only has an effect on colour (ranging from amber-red-cooper) but also on taste and mouthfeel as these compounds could contribute with bitterness and astringency attributes. Thus, the presence of anthocyanins in pigmented corn beers could improve the quality of these beverages.

Finally, all the beers showed considerable amounts of total phenolic content (TPC). The main polyphenols present in a typical barley beer are hydroxybenzoic, cinnamic and ferulic acids. Malt is the main source of polyphenol compounds, providing 70 to 80% of them. Also, a small proportion is originated from hops (20–30%), such as  $\alpha$ - and  $\beta$ - acids and their isomeric forms [36,38,39]. In beers made of pigmented corn malt, the presence of polyphenols is also expected. Blue and red corn also have shown the presence of phenolic compounds such as cyanidin-3-glucoside and pelargonidin-3-glucoside, respectively. In addition, ferulic acid and *p*-coumaric acid could be found in these varieties of corn [5]. The results showed significant difference between beers. Higher quantities of TPC were found in those beers made of blue corn malt (BC) and the blended beers made of red and blue corn and barley (RCBa, BCBa). The value of polyphenols ranged between 398.5 to 750 mg GAE/L. Other studies have shown similar results for beers made of blue corn (342 to 560 mg GAE/L) [5,16] and traditional beers made of barley malt (152.0 to 339.12 mg GAE/L) [40]. The differences of the total content of polyphenols may be explained by the variation in the quantity and quality of raw material, the brewing process and the storage conditions during ageing. Polyphenols provide beer with bitterness and astringency but also improve its functionality in terms of foamability, oxidative stability and heat stability which help to preserve the beverage during storage and ageing [39,41].

### 3.2. Volatile Composition

One hundred and twenty-one volatile compounds were identified in beer samples by HS-SPME/GC-MS. The chromatographic data of the volatile compounds of each beer is summarised in Table 2. Compounds were classified into 12 groups of which, the most abundant include esters, representing ~29% of the volatiles, followed by alcohols (~20%), terpenes (~15%) and phenols (~6%). These compounds, particularly alcohols and esters have been the most reported volatiles in

barley beers [42]. The major volatiles detected in this study were consistent with those of previously published studies [11,18,42,43].

As mentioned before, esters were the largest group found in all beer samples. Esters are the most common compounds in the majority of beers and these volatiles are considered desirable as they act in synergy with other compounds and contribute with most of the pleasant fruity-floral aromas in beer [44,45]. According to our results, it seems that beers made with barley malt contain higher number of esters than the beers made with corn malt (Table 2). For instance, ethyl propanoate, ethyl isobutanoate, ethyl pentanoate, ethyl isohexanoate, ethyl benzoate and isopropyl palmitate were only found in beers made with barley (Ba, BCBa, RCBa). It is well known that the presence of alcohols leads the production of esters [44]. Thus, the presence of a greater number of esters in barley beers could be attributed to their content of alcohols, which are precursors of these compounds.

Esters such as ethyl acetate, 3-methylbutyl acetate, ethyl hexanoate, phenethyl acetate, ethyl 9-decenoate and ethyl decanoate were found in all samples in higher abundance than the rest of the esters.

Ethyl octanoate, a product of fermentation by *Saccharomyces* yeast, was detected in all five beers that contain corn malt except in the one made of 100% barley malt. Conversely, octanoic acid was more abundant in the barley beer than in the corn beers, which is consistent with Saerens et al. [45] who found that higher levels of unsaturated fatty acids in beers, like in the corn beer samples, result in a decrease in ethyl ester production. The contrary effect can be seen for ethyl hexanoate and hexanoic acid, where in samples that exhibited a higher peak area of the ester, the presence of the acid seems to be reduced (BCBa and RCBa).

Alcohols were the second largest group of volatiles found in beers. We identified 24 alcohols and some of them were found in all six beer samples such as ethanol, 2-methyl-1-propanol, 3-methyl-1-butanol, 2-methyl-1-butanol, 2-ethyl-1-hexanol, phenylethyl alcohol and citronellol. These alcohols come mainly from alcoholic fermentation while others such as citronellol and phenylethyl alcohol come from the essential oils of hops. According to Lyu et al. [12] and Dong et al. [29] aromas like sweet alcohol, rough, whiskey, fruity and rose could be attributed to these compounds.

In addition, some alcohols such as 2-furanmethanol, 4-methyl-1-pentanol, 3-methyl-1-hexanol and iso-geraniol were only found in those beers that contain barley malt (Ba, BCBa and RCBa). Of them, 2-furanmethanol is a product of Maillard reactions that occur during the roasting process of malt, especially in the production of 'dark' and 'caramel' malts; hence the caramel malt used in Ba, RCBa and BCBa beers could be the source of this volatile [30]. Interestingly, to our knowledge, there are no reports of iso-geraniol in beers. This compound is the result of the partial oxidation of geraniol. It was previously identified in some flowers, fruits (grapes) and the essential oil of lemon, imparting a pleasant rose odour [46].

In beers, terpenic compounds are generally derived from the hop essential oils, which are added to the wort during the boiling process. These compounds have been related to pleasant aromas like citrus, flowery and lilac [29,30]. We identified 18 terpenes in the beer samples, most of them have previously been reported in barley beers [30,47]. Only linalool, geraniol and humulene, associated with flower, geranium and wood aromas respectively, were detected in all six samples of beer. In turn, limonene and  $\beta$ -myrcene were found in beers made 100% with corn malt (RC, BC). In addition, these beers (RC, BC) showed more abundance of limonene and linalool than the other samples of beer. Interestingly,  $\delta$ -cadinol and  $\alpha$ -cadinol were found in those beers made with blue corn malt but (BC and BCBa) and 3-methoxy-2-naphthalenol was found only in those that contain barley malt (Ba, BCBa, RCBa). Among these terpenes, limonene have been previously reported in corn starch and corn products [48,49].

Seven phenol volatile compounds were identified among the beer samples. These compounds contribute to clove and spice aromas in beers, which are desirable in some Belgian styles (amber and Trappist beers) and wheat beers [50]. For instance, 4-ethyl-2-methoxy-phenol was detected in all beers containing red and/or blue corn malt, but not in barley beer. Buttery and Ling [49] reported that

4-ethyl-2-methoxy-phenol is one of the major components in products like corn tortillas and tortilla chips. Furthermore, 2-methoxy-phenol was found only in beers containing blue corn malt. Even though 4-ethyl-phenol and 2-methoxy-4-vinylphenol were found in all beers, these compounds exhibited a higher peak area in those beers that contain both red and blue corn malt (RBC) than in the other beers. Of those, 4-ethyl-phenol is usually found in beers made of wheat malt. This molecule is formed from the biodegradation of hydroxycinnamic acids, such as ferulic and coumaric acid, during wort boiling. In high concentrations it imparts unpleasant aromas like medicinal, phenolic, clove-like, or smoky. However, in some beer styles such as Belgian wheat and German Weizen these aromas are appreciated [51,52]. 2-methoxy-4-vinylphenol and 4-vinylphenol (the precursor of 4-ethyl-phenol) have been reported as major components of sweet corn products such as tortillas [53].

Styrene was the most abundant hydrocarbon found in all beer samples. This compound usually comes from the malt and it derives from the metabolism of cinnamic acid in barley malt by top-fermenting yeast [18]. Its presence in the corn beers is explained as its formation occurs in parallel to the formation of 2-methoxy-4-vinylphenol and 4-vinylphenol. Styrene has been described as a “sweet-smelling colourless fluid” [54].

Interestingly, dimethyl sulfide (DMS) exhibited a higher peak area in the beer made 100% with barley malt (Ba), followed by those made with corn malt (BC, RC, RBC). DMS is usually lost during the kilning of malt and the boiling of the wort, however its presence in the beer depends on the type of malt used. This sulphur compound has been reported in barley beers. Its presence is desirable in some styles of beers, like some lagers, while in others is not desirable as it adds sweet corn aroma to the beer [10]. In addition, DMS has been identified as an important contributor to the aroma of corn products [48,49].

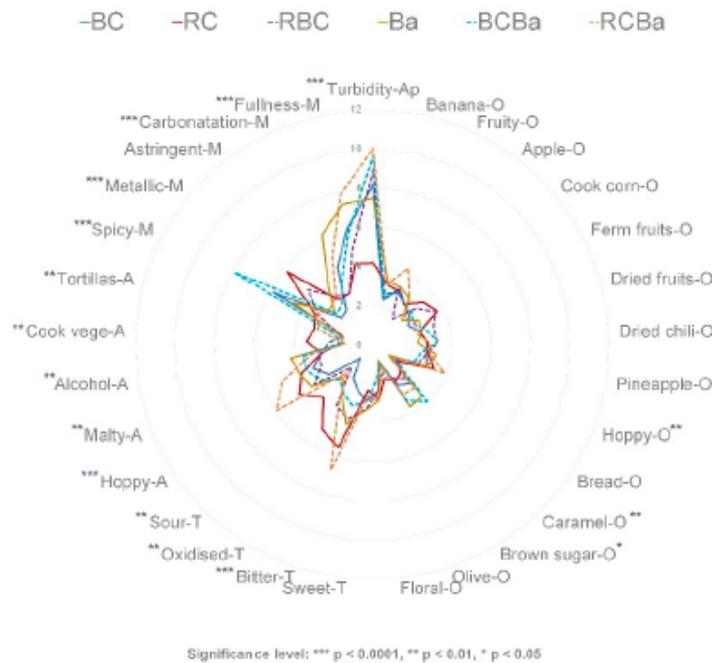
Additionally,  $\beta$ -ionone was only found in beers made with blue and red corn malt (BC, RC, BCBa, RCBa) with the exception of RBC. This ketone has been previously reported as potential contributor of hop aroma. It has been identified in tortillas and corn dough [49], in late-hopped and dry-hopped beers [55] and in samples of whiskey made with corn [56].

### 3.3. Descriptive Sensory Analysis

The sensory panel developed a list of 30 attributes to describe the appearance, odour, taste, aroma and mouthfeel characteristics perceived in all beer samples (Table 3). The panel was asked to be as specific as possible in identifying attributes. Some terms and references were similar to those defined in the “beer flavour wheel”, developed by Meilgaard [33], but others were unique attributes related to the presence of pigmented corn and chili.

The mean scores of the attributes were plotted in a radial diagram (except for the colour attribute) (Figure 1). Significant differences ( $p < 0.05$ ) were found in 17 out of the 30 attributes across the samples (Supplementary Table S1). In order to have a complete description of all sensory characteristics of the beers, all attributes were kept and used in the subsequent analysis. We can see that the non-significant attributes were mainly those pertaining to the odour category. These odour characteristics are common to most of the commercial beers and some of them are the result of the volatile compounds developed during the fermentation process (e.g., banana, apple, floral, fruity). Thus, as all steps in the brewing process remained the same, we can expect some similarities between beers.

All beers in this study exhibited a range of sensory characteristics commonly found in most of the commercial beer samples, however some characteristics such as ‘dried fruits-O’, ‘dried-chili-O’, ‘brown sugar-O’, ‘tortillas-A’ and ‘spicy-M’ are not in the common lexicon of beers [33]. Thus, the pigmented corn malt and the chili used in these beers appear to contribute to the development of these attributes. Despite the fact that cooked vegetable-A and cooked corn-O are usually associated with off-aromas in barley beers, we could expect that the pigmented corn beers develop these characteristics as they are sensory attributes found in the ‘Sendechó’ beverage [4] and in many corn-derived products [48,49].



**Figure 1.** Sensory profile of the six beers. BC = 100% blue corn, RC = 100% red corn, RBC = 50:50 red and blue corn, Ba = 100% barley, BCBa = 50:50 blue corn and barley, RCBa = 50:50 red corn and barley.

The beer made 100% with barley malt (Ba) had a significantly higher intensity of brown sugar and caramel attributes than the other beers, which was expected as the caramel malt used in this beer contributes with the development of these aromas. Furthermore, alcohol aroma was higher in barley beer (Ba) than in the others, this is reasonable as barley malt contributes more to the formation of fermentable sugars than corn and therefore barley beers had higher alcohol content than the beers made with corn malt (see Table 4).

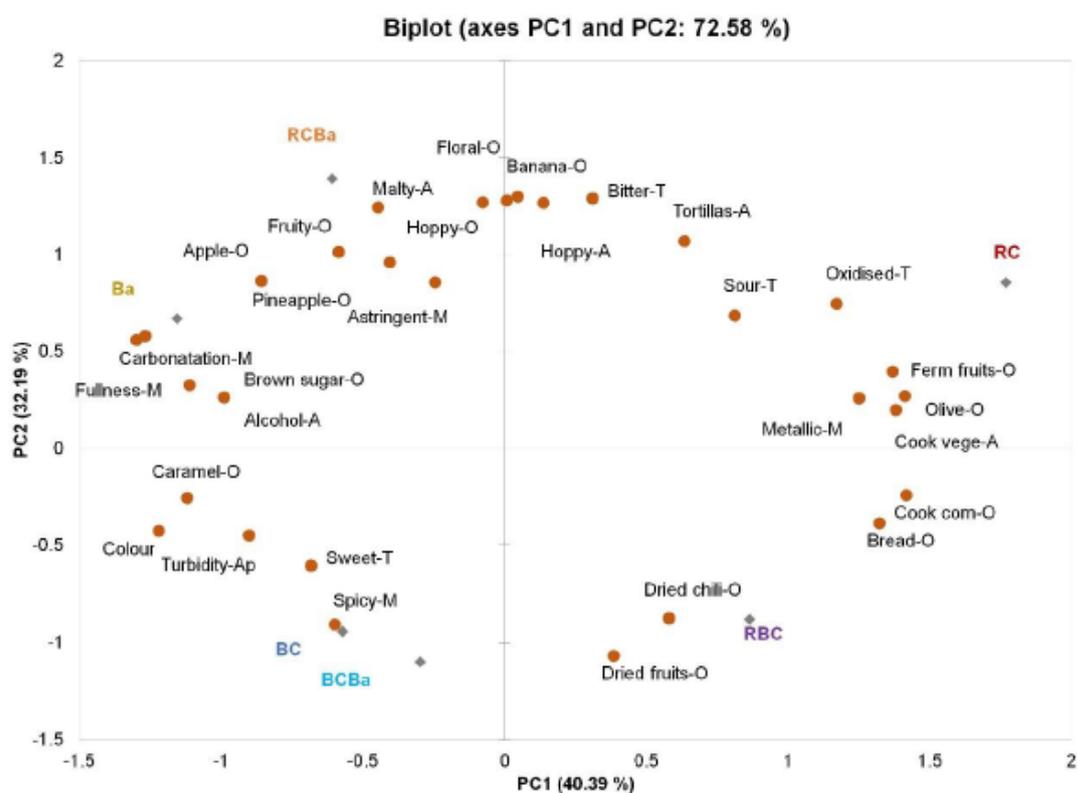
RC and RCBa, both containing red corn malt, were rated higher in bitter taste, as compared to the other beers. In general, those beers containing red corn malt (RC and RCBa) were characterised by higher intensity of aroma attributes such as cooked vegetables and tortillas, related to the type of corn used. In addition, sour taste, oxidised and metallic sensations were scored high in the RC beer. The latter attributes are usually associated to an ageing effect [32].

Despite the fact that Guajillo chili was added to all the beers in the same proportion and conditions during the brewing process, the perception of spicy attribute was different in all the beers. For instance, the beer made of blue corn and barley (BCBa) was rated significantly higher in spicy mouthfeel than the rest of beers, followed by blue corn beer (BC). The perception of the 'spicy' or 'pungent' sensation elicited by the capsaicin (the active ingredient of the Guajillo chili) may be influenced by factors such as the temperature, acidity and carbonatation of the beverage [57]. In addition, phenolic compounds that evoke an oral irritation [39,41] might increase the perception of this sensation. Thus, the content of polyphenols in BC and BCBa might contribute to the increase perception of the attribute spicy. Beers made with barley (Ba, BCBA, RCBa) had a higher carbonatation sensation than those beers made with pigmented corn malt (BC, RC, RBC). The perception of the fullness, which is associated with the body of the beer, was higher in the beers that contain blue corn and/or barley malts (BC, Ba, RCBa and BCBa) than in the ones made with red corn malt (RC and RCBa). The fullness palate sensation is related to the unfermentable sugars namely dextrans, developed during the mashing process. These compounds contribute to the body of the beer without imparting sweetness [10].

The assessment of a beer's appearance includes its colour, which according to the SMR colour chart it can range from straw to black. All beer samples analysed are in the range of the colours that goes from 10 SMR to 15 SMR units. Significant difference can be observed (Supplementary Table S1)

between the RC beer with a ‘medium amber’ colour (9 SMR), the BC beer with a ‘light brown-reddish’ colour (15 SMR) and the rest of the beers with a ‘cooper-red’ colour (12–13 SMR). It is well known that malt has the greatest impact on beer colour because of its content of melanoidins and Maillard compounds, which add colours that range from yellow, orange to red and brown [58]. In this case, the anthocyanins in the pigmented corn beers contribute to develop of these ‘amber–red-cooper’ colours, especially in those beers made 100% with red and blue corn malt. In acidic solutions such as beer, anthocyanins are chemically stable and turns their colours to reddish tones [3].

With the aim of illustrating the differences among beers produced by different types of malt (red corn, blue corn and barley), a PCA was applied on the total data set of 30 attributes. The biplot obtained is shown in Figure 2. The first two components (PC) explained 72.58% of the total variation in the samples with contributions of 40.39% by PC1 and 32.19% by PC2, where most of the attributes contributed considerably to samples discrimination.



**Figure 2.** Principal Component Analysis (PCA) bi-plot of variables and individuals of descriptive sensory data. BC = 100% blue corn, RC = 100% red corn, RBC = 50:50 red and blue corn, Ba = 100% barley, BCBa = 50:50 blue corn and barley, RCBa = 50:50 red corn and barley.

PCA permitted a clear-cut separation of the samples based on the type of malt used.

PC1 opposed the beers made with barley malt like Ba, RCBa and BCBa (on the left) to the RC and RBC beer (on the right). On the other hand, PC2 opposed beers made of red corn malt (positive side) to beers made of blue corn malt (negative side). The RC beer was characterised by attributes such as fermented fruits-O, olive-O, tortillas-A, cooked vegetables-A, metallic-M and oxidised-M. On the contrary, BC and BCBa were characterised by spicy-M, sweet-T, Turbidity-Ap.

The beer made of 100% barley malt (Ba) was discriminated along PC1 (at the negative side) and was characterised by brown sugar-O, apple-O, alcohol-A, carbonatation-M and fullness-M.

Blended beer made of both type of corn malt (RBC) was placed in between red corn beer (RC) and blue corn beer (BC), sharing attributes of both malts used such as bread-O, cooked corn-O and dried chili-O and dried fruits-O. This behaviour was also shown in blended beer made of red corn

and barley malt (RCBa), preserving the sensory characteristics of both 100% barley (Ba) and 100% red corn (RC) beers such as apple-A, fruity-A, banana-A, malty-A and floral-A, attributes that are more common in typical barley beers.

These sensory data showed that by adding corn malt to the beer formulation, the sensory profile of the typical barley beer can be reached easily, while preserving at the same time odours and aromas of corn products, especially those of the Sendecho beverage such as corn and spicy and dried chili [4].

### 3.4. MFA of Sensory Attributes and Chemical Data

In this study, MFA was used to explore the differences and similarities between beers due to the type of malt used in brewing. In addition, MFA helped to identify associations between sensory and chemical datasets that brought us to know those components (sensory and chemical) that can be used as markers of beers made with pigmented corn malt.

The first two dimensions (Dim 1 and Dim 2) in Figure 3 accounted for 56.31% of the total variation with contributions of 31.19% by Dim 1 and 25.12% by Dim 2.

First, the variable plot (Figure 3b) shows that Dim 1 separates samples based on the sensory 'odour-aroma' attributes (in green; 34.05% of the variance) and the 'non-volatile' components (in pink; 34.53% of the variance). For Dim 2, the groups of variables 'volatiles' (in orange) and 'taste-mouthfeel' (in blue) are those that contribute the most to the dimension with 22.41% and 44.91% of variance respectively. The plot of the individuals (Figure 3a) allows us to visualise the global resemblance between beers by considering the information of all variables (sensory and chemical). It clearly showed that Dim 2 separated the samples based on the type of malt used, with beers made with pigmented corn (red and blue) on the top of the plot, and the beers that contain barley malt plotted on the bottom (Figure 3a).

Second, the RV coefficients (Table 5) show the relationship between the data matrices, the closer the RV coefficient to 1, the more similar the matrices [21,35]. According to the RV, a good correlation can be observed between the 'odour-aroma' and 'non-volatile' variables (0.740). Moreover, a better correlation between 'volatiles' and 'taste-mouthfeel' variables (0.649) than for 'odour-aroma' and 'volatiles' data matrices (0.509).

**Table 5.** RV coefficients between odour-aroma, taste-mouthfeel, volatiles, non-volatiles and supplementary data matrices of the MFA.

	Odour-Aroma	Taste-Mouthfeel	Volatiles	Non-Volatiles	Supplementary	MFA
Odour-Aroma	1.000	0.403	0.509	0.740	0.741	0.846
Taste-mouthfeel	0.403	1.000	0.649	0.374	0.307	0.755
Volatiles	0.509	0.649	1.000	0.364	0.428	0.793
Non-volatiles	0.740	0.374	0.364	1.000	0.321	0.779
Supplementary	0.741	0.307	0.428	0.321	1.000	0.579
MFA	0.846	0.755	0.793	0.779	0.579	1.000

A deeper analysis of Figure 3 allows detailing these relations between the different types of variables that strengthen the characterisation of the beers. On the negative side of Dim 1 of the variable plot (Figure 3b), it can be observed that the sensory attributes floral-O, hoppy-O and pineapple-O are positively correlated mainly with esters (i.e., ethyl butanoate (48), phenylethyl acetate (61), ethyl (E)-4-decenoate (65), ethyl decanoate (67), isoamyl octanoate (68), terpenes (i.e., geraniol (108),  $\delta$ -cadinene (112), humulene oxide (115),  $\delta$ -cadinol (120), and alcohols (i.e., phenylethyl alcohol (15), citronellol (19) and 1-decanol (22)). Numbers correspond to those on Table 2. Esters and alcohols are well known for their floral and fruity contribution to the beers, and terpenes are more likely associated with herb and green odours-aromas, which are consistent with the description of the hoppy odour. These correlations between the sensory attributes and the volatiles compounds strengthen the aromatic profile of the barley beer (Ba). Also compounds such as 2-nonanone (88), heptanoic acid (37), 2-ethylhexanoic acid (38) and acetaldehyde (25) were also correlated with the sensory attributes

mentioned before. The positive correlation of these fruity and floral sensory attributes with carboxylic acid compounds could suggest that the presence of esters, even in low levels, might reduce the perception of off-aromas like sweat and rancid, caused by octanoic acid [59].

On the positive side of Dim 1, RBC (Figure 3a) can be separated from the other beers mainly by the presence of phenol volatile compounds. Among them, phenol (94), 2-methoxyphenol (95), 4-ethylphenol (96) and 4-ethyl-methoxy-phenol (97) showed association with the sensory attributes related to the presence of pigmented corn malt such as cooked vegetables-A, cooked corn-A, olive-O and fermented fruits-O (Figure 3b). These compounds and the sensory attributes allow us to differentiate between the beers made 100% with corn malt, suggesting that these phenol compounds could be used as indicators of the use of pigmented corn in the brewing process.

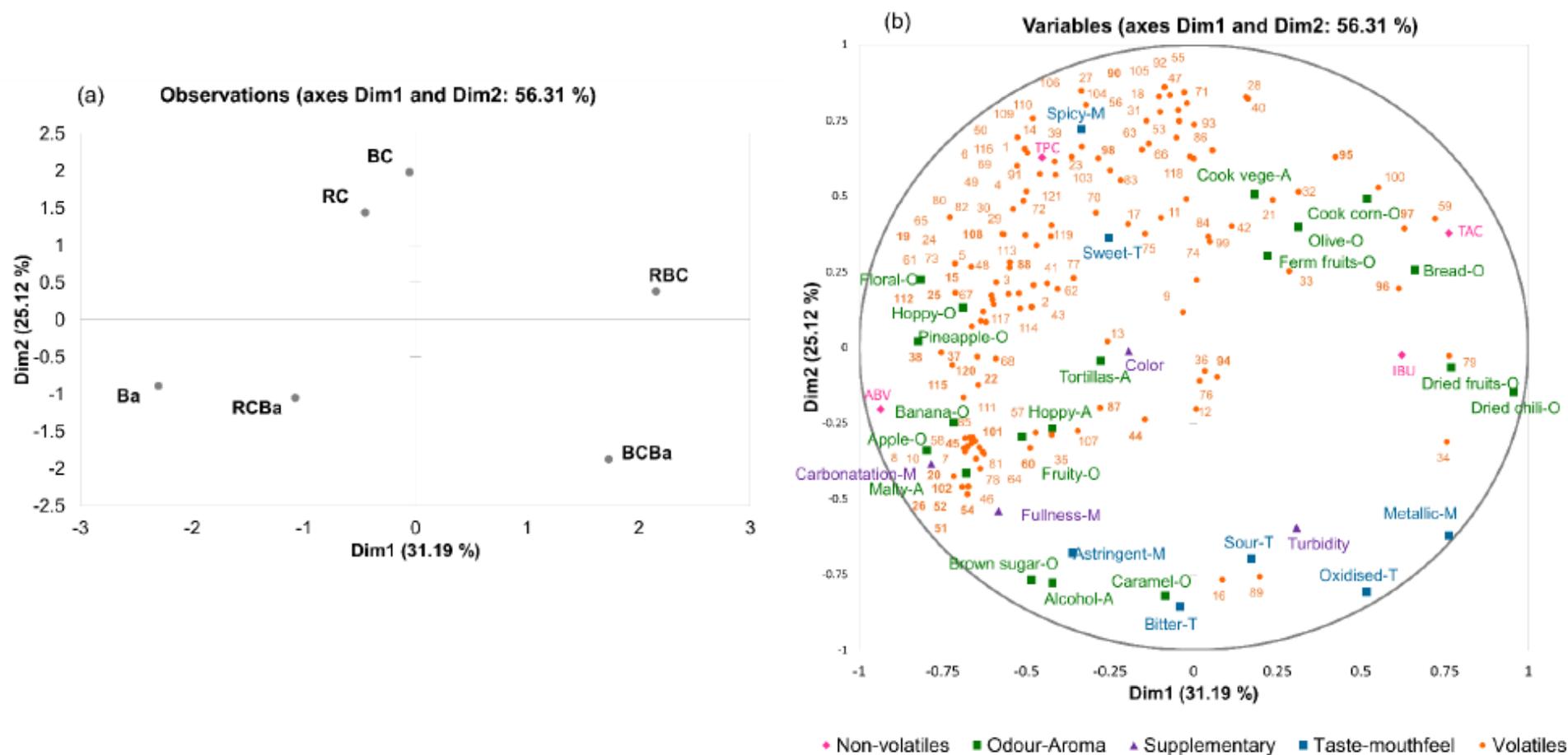
On the negative side of Dim 2, we found positive correlations between attributes such as malty-A banana-O, brown sugar-O, tortillas-A and fruity-O and the compounds 2-furanmethanol (8), ethyl propanoate (44), propyl acetate (45), ethyl pentanoate (51), ethyl isohexanoate (52), ethyl hexanoate (54), iso-geraniol (20), acetophenone (87), 2-acetylpyrrol (101) and tetramethyl-pyrazine (102). The presence of these compounds, characterised by fruity, bread, brown sugar and caramel aromas [45,60], is consistent with the use of roasted malts (caramel malt) in the beers associated to these compounds (RCBa and Ba). Furthermore, on Dim2 (negative side), a weak correlation was also found for benzeneacetaldehyde (26) with astringent, which is consistent with the results obtained by Owusu et al. [61], where the presence of this compound has been associated with the astringent mouthfeel in products as cocoa and dark chocolates.

The positive side of Dim 2 is positively correlated with beers made from red corn malt (RC) and blue corn malt (BC) (Figure 3a, top side). These beers are well characterised by compounds such as linalool (106), limonene (105),  $\beta$ -ionone (90) and 4-ethyl-2-methoxy-phenol (90). These volatile compounds have been found in other corn products such as tortillas and pop-corn [49,60] and especially limonene and  $\beta$ -ionone have also been reported in samples of whiskey made with corn [56]. Thus, these compounds could also be used as markers of the presence of corn in beers.

In addition, the spicy attribute was strongly correlated with 2-methyl-5-(1-methylethyl)-phenol (98) well known as carvacrol—a key aroma compound in oregano spice—that is concordant with the pungent mouthfeel associated with this compound [60]. Unexpectedly, dimethyl sulfide (103) which usually imparts cooked vegetable off-odour also showed a positive correlation with the spicy attribute. This behaviour could be attributed to the high abundance of phenylethyl alcohol (15) that could suppress the perception of this compound [8].

Correlations between the non-volatiles variables (ABV, IBU, TPC, TAC) and the sensory and volatile data were also studied. For instance, a positive correlation was found between alcohol sensory attribute and alcohol content (ABV). Regarding the total polyphenol content (TPC), a negative correlation was observed between TPC and metallic and oxidised sensory attributes, confirming that polyphenols help to retard the development of these attributes in beer [54]. Moreover, TPC showed a positive correlation with carvacrol volatile (98). According to Lee et al. [62] carvacrol is a volatile compound that has exhibited potent antioxidant activity.

It is well known that anthocyanins do not impart aromas, but sometimes these compounds have been related to an astringent or bitter taste [41]. Even though, no obvious correlations were found between TAC and bitter or astringent attributes. The results showed a positive correlation between TAC and phenol compounds such as phenol (94), 2-methoxy-phenol (95), 4-ethyl-phenol (96) and 4-ethyl-2-methoxy-phenol. This could suggest an interaction between the anthocyanins that comes from corn malt and those phenol volatile compounds. According to Dufour and Sauvaitre [63] and Ruta and Farcasanu [64], interactions between anthocyanins and some aroma compounds such as phenol and 2-methoxy-phenol, lead the formation of copigments, which improve the stability of the anthocyanins and hence the colour stability of the beverage.



**Figure 3.** Multiple Factor Analysis (MFA) of descriptive sensory and chemical data of the six beer samples. (a) Observations plot of MFA, (b) Variables plot of MFA. Numbers correspond to the compounds listed in Table 2. BC = 100% blue corn, RC = 100% red corn, RBC = 50:50 red and blue corn, Ba = 100% barley, BCBa = 50:50 blue corn and barley, RCBa = 50:50 red corn and barley.

Apparently, no positive correlation was found between IBU parameter and bitter sensory attribute. However, there are other components that could contribute to the perception of bitterness such as the Maillard products formed during the kilning and roasting process of caramel and dark malts [57,65]. In addition, bitterness can be masked by sweetness due to sugars (residual sugar) that remain after the fermentation process. As has been mentioned before, IBU measures a beer's bitterness due to the  $\alpha$ -acids of the hops, which gives an approximate idea of beer bitterness but there are other compounds that could impart or mask the bitter taste. Thus, it is not possible to directly correlate IBU to the perceived sensory bitterness [41].

Finally, the different groups of variables (sensory and chemical) had different influences in each beer. The major difference was found for the BCBa and RCB which were mainly described based on taste-mouthfeel attributes and non-volatile parameters respectively. Beers Ba and RCBa were mainly described based on the odour-aroma attributes and volatile compounds. For beers made 100% with pigmented corn (RC and BC) the group of volatiles had more influence in their characterisation. Overall, the volatile composition also separates beers depending on the presence of corn, supporting the fact that the use of corn as an ingredient clearly alters the sensory profile of beers.

#### 4. Conclusions

It is well known that sensory evaluation plays an important role when new products need to be characterised, but it is also an important quality factor used to control the brewing process. In this study, sensory evaluation enabled the complete description of the corn beers.

Beers made with these specific types of pigmented corn (red and blue) are mainly characterised by fermented fruits, cooked vegetables odours, tortillas, bread, dried fruits and dried chili.

We evidenced for the first time that among the groups of volatile compounds, ketone ( $\beta$ -ionone), terpenes (limonene, linalool) and phenol volatiles (2-methoxy-phenol, 4-ethyl-phenol and 2-methoxy-4-vinylphenol, 4-ethyl-2-methoxy-phenol, 4-ethyl-2-methoxy-phenol), as well as the presence of anthocyanins appear as relevant criteria for corn beers differentiation. The latter can also be used as indicators to determine whether a beer is made with pigmented corn malt or not and therefore be used as a quality parameter in further studies. Moreover, the study of the relationship between the sensory attributes and the chemical parameters by MFA allowed to elucidate the effect of each type of malt (red corn, blue corn and barley malt) on the chemical parameters (VOC, ABV, IBU, TAC, TPC) and the association with the sensory attributes.

Both varieties of corn malt showed a clear influence in all parameters measured, especially in their sensory profiles. However, the blended beers (RCBa and BCBa) show the closest resemblance to a typical barley beer, while preserving those traditional aromas and tastes of the 'Sendechó' beverage. Additionally, the use of pigmented corn malt could help to prevent the development of off-aromas (e.g., oxidised), which could extend the shelf life of the beer.

This study will enable the Mexican brewing industry to gain an insight into the use of alternative and native cereals, which could renew and preserve autochthonal beverages in a modern way. Whether the sensory characteristics of these beers may carry the acceptance or rejection of consumers needs to be further investigated.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2304-8158/9/7/886/s1>, Table S1: Sensory attribute score means of beer samples.

**Author Contributions:** A.R.-M., H.B.E.-B., J.R.V.-C. and M.L.-D., conceptualization and design; A.R.-M., H.B.E.-B., J.R.V.-C. and M.E.-E., methodology, acquisition, formal analysis; A.R.-M., H.B.E.-B., data interpretation and writing-original draft; A.R.-M., H.B.E.-B., J.R.V.-C., M.E.-E. and M.L.-D., review and editing. All authors have read and agreed to the published version of the manuscript.

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26 **13.2 Conclusion**

27 The results of this study confirm the potential of pigmented corn grains for the production of  
28 malt and beer, thus pigmented corn malts are feasible for brewing purposes. However, some  
29 differences can be highlighted:

- 30 • Volatile profile: Beers can be differentiated according to their volatile composition  
31 that is strongly influenced by the type of malt used (red corn, blue corn, and barley  
32 malts). It was possible to identify those volatiles that come from corn malts. Thus,  
33 these findings could be used as fingerprints for beers made with corn.
- 34 • Sensory profile: The volatile composition had a large influence on the sensory  
35 properties of beers. Those beers made with corn are mainly characterised by  
36 attributes that seem more to the pre-Hispanic beverage named Sendechô. The  
37 beers made with a combination of barley and corn malts managed to have sensory  
38 characteristics of both beverages. Hence, these beers made with corn and barley  
39 could better meet consumers' expectations.
- 40 • Chemical properties: Beers made with corn had similar chemical properties to barley  
41 beers. However, corn beers showed the presence of anthocyanins, improving the  
42 antioxidant capacity of beers. Despite consumers did not have a predilection for the  
43 concept of 'healthy' beer (Chapter II), this could be used for marketing to reach those  
44 consumers who seek healthy tendencies in the food system.

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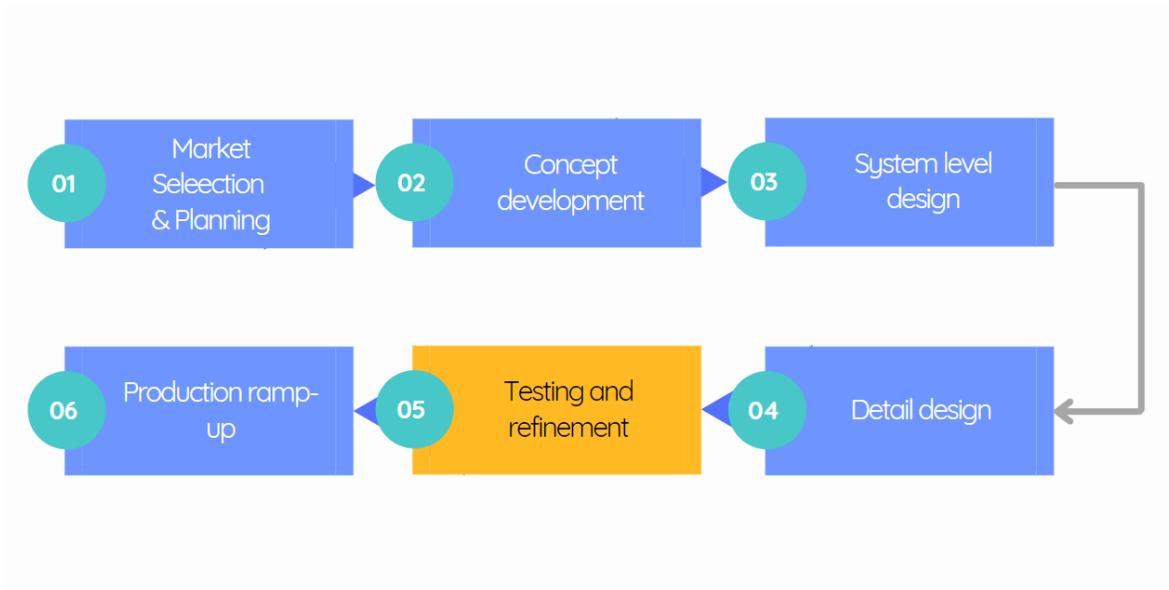


# CHAPTER IV

**Product performance:  
consumer test**

*Beer is people"*  
- Garrett Oliver

49 **14 Chapter IV. Product performance: consumer test**



50

51 The last part of the project (Study 4) takes part of the 5<sup>th</sup> phase of the “New Product  
52 Development Process” named “Testing and refinement”. In this phase, science just takes  
53 an assisting role. Is at this moment where the real product could be tested with consumers  
54 to evaluate its performance and reliability. We made use of the sensory evaluation to test  
55 consumers’ expectations of the 6 prototypes developed before.

56 **14.1 Introduction**

57 The named “craft beer renaissance” (originated in the United States) (Gianluca Donadini &  
58 Porretta, 2017) has elicited the emergence of independent breweries in Mexico, increasing  
59 the popularity of craft beers across the country. These microbreweries are characterised for  
60 producing beers that are considered out of the ordinary. Innovation and creativity are the  
61 hallmarks of the craft movement. Thus, producers are in constant search of new ingredients  
62 and new technologies to create beers that enthrall consumers. In this search of innovation,  
63 brewers have turned to see local and native grains such (e.g., pigmented corn, amaranth)  
64 as well as local fruits, herbs and chili. All of this with the purpose to create beers with a  
65 diversity of flavours but also to reinterpret and renew local gastronomic traditions.

66 Beer preference and choice is highly influenced by sensory attributes (taste, flavour, texture,  
67 colours) and factors related to the purchasing process (price, brand, distribution,  
68 differentiation and packaging) (Aquilani et al., 2015). In addition, consumers’ expectations

69 play an important role in a new product acceptance. Expectations are part of the top-down  
70 processing, which consist in interpreting perceptions by using our experience and prior  
71 knowledge of certain product (Köster, 2009). When it comes to a new product, consumers  
72 have the inherent tendency to approach (neophilia) and avoid (neophobia) it at the same  
73 time (van Trijp & van Kleef, 2008). In the craft beer category, some studies revealed that  
74 consumers tend to prefer beers that deliver a moderate level of novelty (Gianluca Donadini  
75 & Porretta, 2017). In contrast, craft beer mainstream drinkers are more interested in  
76 exploring new beer tastes and experiences, especially if these products make them diff from  
77 the industrial beer consumers (Gómez-Corona, Escalona-Buendía, et al., 2016).

78 Thus, beer made with native pigmented corn grains such as red and blue Chaqueño  
79 varieties, are novel and innovative products that could create high expectations in beer  
80 consumers. Among the factors that influence consumers' expectations, the information  
81 about the ingredients used in creating these beers could have a deep impact on the overall  
82 liking of these products.

83 This part of the research focused on how information about the use of pigmented corn  
84 influences consumers' expectations and lead to the acceptance or rejection of these beers.

85 **Objective 5:** to determine whether the information about the use of local and traditional  
86 Mexican gastronomy ingredients (red and blue pigmented corns and Guajillo chili) to brew  
87 a beer affects consumers' expectations on the level of liking

- 88 • Objective 5.1: Determine the effect of the information of the beers in three different  
89 conditions (blind, expected and informed) on the liking of consumers.

#### 90 **Hypothesis:**

91 Consumers liking, and expectations will be higher in beers made with pigmented corn than  
92 beers made with barley malt.

#### 93 **Outcomes:**

- 94 • Poster presentation: "Influence of product information in consumer's liking of artisan  
95 corn beers"; 8th Food Science, Biotechnology & Safety Congress, Latin Food, 14-  
96 16th November, Puerto Vallarta, Mexico.
- 97 • Article: "Use of native pigmented corn in the brewing process and its influence on  
98 consumers expectations".

99



103 *14.2 Article 3 – Use of native pigmented corn in the brewing process and its*  
104 *influence on consumers expectations.*

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114 Abstract

115 The increasing market of craft beer in Mexico has opened the opportunity to explore different  
116 styles of beer. The use of local ingredients such as pigmented corn malts, allow the creation  
117 of beers with novel and different flavours. Information presented to the consumer influences  
118 consumers' expectations and overall liking of the product. This study aims to investigate how  
119 information about the ingredients of the beer influences consumers' liking. Beer consumers  
120 (n=100) evaluated six craft beers made with a combination of three malts (red corn, blue  
121 corn, and barley) under three information conditions (blind, expected and informed). Results  
122 show a significant effect caused by information of malt on consumers' liking of beers.  
123 Information about pigmented corn malt created high expectations however beers made  
124 100% of corn malt showed a lower liking than the beers containing a combination of barley  
125 and pigmented corn malt. This pattern was the same in both blind and informed conditions.  
126 It was possible to identify three clusters of consumers with different liking patterns. This  
127 study revealed that there is a segment of consumers who appreciate more the novelty of  
128 these beers.

129 1. Introduction

130 In the last decade, the emergence of independent breweries has grown massively in Mexico.  
131 With an average annual growth rate of 53%, craft beers' popularity boomed across the  
132 country, bringing attention to brewers and consumers who seek innovation, pleasure and a

133 sense of identity and belonging (*BeerectorioMX: Estadísticas*, n.d.; Calvillo, 2017; Gianluca  
134 Donadini & Porretta, 2017). This new trend of craft beer production has led to the  
135 development of a great variety of beers brewed with local cereals such as corn (Flores-  
136 Calderón et al., 2017; Romero-Medina et al., 2020) and amaranth (Pérez Gerrero, 2017),  
137 as well as unconventional ingredients such as chili, chocolate, star anise, mezcal, and  
138 different local fruits and spices.

139 However, the use of indigenous and local products to produce fermented beverages is not  
140 new. Countries, where it is difficult to access imported barley due to the high prices, have  
141 used alternative cereals such as sorghum, millet, corn, rice, wheat or even non-grain  
142 sources such as plantains, local fruits, peas and soybeans (Agu, 1995; K. Shale, J.  
143 Mukamugema, R. J. Lues, P. Venter, 2013; Rubio-Flores & Serna-Saldivar, 2016). Using  
144 local ingredients in brewing serves to preserve traditional beverages and cultural heritage  
145 while adding economic value to those products. Although the use of local ingredients in  
146 brewing is innovative, producing a well-accepted beer by consumers is the real challenge  
147 (Ceccaroni et al., 2019; Taylor et al., 2013).

148 The brewing process's versatility allows producers to incorporate a wide variety of  
149 ingredients and thus develop beer styles with different flavours (Taylor et al., 2013), thereby  
150 showing their originality, which is the hallmark of every brand. However, consumers have  
151 become more demanding since they have more options and more tools to make informed  
152 purchasing decisions (Gianluca Donadini & Porretta, 2017).

153 Although craft beer consumers are more open to exploring new experiences (in terms of  
154 flavours) when it comes to a well-known product such as beer, consumers tend to penalize  
155 those beers they consider being "out of the ordinary" or "too innovative". Besides, consumers  
156 who are new in the 'craft movement' and are more used to consuming industrial beers, are  
157 usually less attracted by the craft beers' flavours. Some studies have demonstrated that  
158 consumers have a significant deviation from their sensory expectations, particularly in the  
159 craft beer category. They tend to prefer products that do not vary much from the original  
160 product (Gianluca Donadini & Porretta, 2017).

161 Many factors determine the decision-making process when it comes to choosing or buying  
162 food products. It is well known that consumers are influenced by all the information  
163 surrounding the product of interest (Varela et al., 2010). For instance, many studies on the  
164 influence of the extrinsic factors such as price (Guinard et al., 2001; Schnettler et al., 2009),  
165 brand (Guinard et al., 2001; Varela et al., 2010), label and packaging (Goerlitz & Delwiche,

166 2004; Torres-Moreno et al., 2012) have shown the effect on consumer acceptance and  
167 purchase intent. However, intrinsic factors related to the product itself also play an important  
168 role in consumer preferences, liking and expectations (Caporale & Monteleone, 2001).  
169 Among them, sensory characteristics (Johansen et al., 2010; Meillon et al., 2010), health  
170 and functional properties (Ares et al., 2008; Johansen et al., 2010), country/region of origin  
171 (Caporale & Monteleone, 2001; Iaccarino et al., 2006; Nacef et al., 2019a), the origin of raw  
172 material (Di Monaco et al., 2007), process production (Caporale & Monteleone, 2004;  
173 Iaccarino et al., 2006) and ingredient name (Ares et al., 2009; Concha-Meyer et al., 2019)  
174 are some factors that have demonstrated to influence consumers' assessment.

175 Additionally, the consumers' values and attitudes, along with the product familiarity are other  
176 factors that affect consumers' acceptability and decisions. When an unknown product is  
177 chosen, all the prior knowledge and experiences stored in consumers' memory are essential  
178 as they use it to make their judgments and create their own pre-trial beliefs (García-Barrón  
179 et al., 2020; Olson & Dover, 1979). Before the product is tasted, consumers retrieve  
180 information from previous experiences with a similar one, and the expectations generated  
181 previously will lead to buying or rejection of the product (Legendre et al., 2019; Lucia et al.,  
182 2014; Nacef et al., 2019a).

183 Then, when the product is tasted, the expected sensory attributes are compared with the  
184 real ones. It is at this point where all the intrinsic and extrinsic factors may change consumer  
185 expectations and like (Deliza & Macfie, 1996; García-Barrón et al., 2020; Nacef et al.,  
186 2019a). When those expectations meet the real hedonic experience, confirmation occurs.  
187 Otherwise, disconfirmation occurs if the expectations do not meet the real hedonic  
188 experience. This discrepancy could be either positive or negative. According to the theory  
189 of assimilation/contrast proposed by Anderson (1973) (Anderson, 1973), both assimilation  
190 and contrast can occur depending on the magnitude of the disconfirmation. When the  
191 expectations are lower than the real hedonic experience, the disconfirmation is positive,  
192 meaning that the product is better than expected. On the contrary when expectations are  
193 higher than the real hedonic experience negative disconfirmation occurs, which means that  
194 the product is worse than expected (Anderson, 1973; Patricia Silva et al., 2017).

195 In general, if confirmation or positive disconfirmation occurs, there is a higher probability that  
196 consumers purchase the product than when a negative disconfirmation occurs, which  
197 usually leads to a product rejection (Caporale & Monteleone, 2001; Vidal et al., 2013). This

198 is where all the additional information could be positive as consumers create an idea of what  
199 to expect of the product and could adjust their expectations.

200 Regarding a new beer, consumers are usually unable to try it before purchase. Thus,  
201 brewers have had to create different strategies to appeal to consumers' interests. Providing  
202 additional information about the ingredients that were used to brew the beer could be  
203 essential to create expectations about its sensory characteristics (Chaya et al., 2010).

204 Even though beer has been widely studied, only a few studies related to the influence of  
205 information about the colour of the beer (Van Doorn et al., 2019), name (Patricia Silva et al.,  
206 2017), brand (Jaeger et al., 2021; Lucia et al., 2014), packaging (Chaya et al., 2010) and  
207 manufacturing process (Caporale & Monteleone, 2004) on consumers' liking and  
208 expectations have been done. Among all the factors mentioned before, the effect of the  
209 information on consumers' expectations about using local and traditional ingredients of  
210 certain regions/countries to use in the brewing process has not yet been explored.

211 This study aims to determine whether the information about the use of local and traditional  
212 Mexican gastronomy ingredients (red and blue pigmented corns and Guajillo chili) to brew  
213 a beer affects consumers' expectations on the level of liking and to what extent consumers'  
214 demographic characteristics influence consumer's acceptance.

215 Assuming that corn and chili are the main ingredients of Mexican gastronomy and Mexicans  
216 are used to its taste and flavours, we hypothesize that those beers brewed with local corn  
217 will lead consumers to have higher expectations than those beers brewed with barley.

## 218 2. Material and Methods

### 219 2.1. Beer Samples

220 Six beers were produced using different proportions of two varieties of pigmented corn malt  
221 (blue and red corn of Chalqueño race) and two types of barley malt (base and caramel)  
222 based on a mixture design. Table 1 shows the ingredients of the six beer formulations.

223  
224

*Table 1. Information about the ingredients of the six beer samples (presented to consumers in the informed condition)*

<b>Beer</b>	<b>Beer formulation</b>
<b>BC100</b>	100% Blue corn malt, hop, Guajillo chili, and water
<b>RC100</b>	100% Red corn malt, hop, Guajillo chili, and water
<b>RBC50</b>	50% Red corn malt, 50% blue corn malt, hop, Guajillo chili, and water
<b>BAR100</b>	100% Barley malt, hop, Guajillo chili, and water
<b>BCBAR50</b>	50% Blue corn malt, 50% barley malt, hop, Guajillo chili, and water
<b>RCBAR50</b>	50% Red corn malt, 50% barley, hop, Guajillo chili, and water

225

226 The beers in this study were brewed in a microbrewery pilot plant (30L) at Universidad  
227 Autonoma Metropolitana, according to the method described in Mexico Patent No. 365910  
228 (Verde Calvo et al., 2019). All the samples were stored at constant temperature ( $11^{\circ}\text{C} \pm 1$ )  
229 for 3 months before the test. Then, 12h before serving, the samples were placed in a  
230 refrigerator at  $4^{\circ}\text{C}$ . The bottles were opened immediately before serving and recapped right  
231 after pouring to maintain carbonatation. 50 mL of the samples were poured into a 177 mL  
232 crystal-clear plastic cup coded with a 3-digit number and served at 5 to  $8^{\circ}\text{C}$ .

## 233 2.2. Consumer test

234 One hundred mainstream beer consumers (52 females and 48 males) with an average age  
235 of 32 were selected to participate in this study. The consumers were chosen if they used to  
236 drink either industrial or craft beers at least once a year.

237 The experiment was carried out in two sessions (45 min) one week apart. The sessions were  
238 held at the sensory laboratories of the ITESO (Jesuit University of Guadalajara) and CIATEJ  
239 (Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco), both  
240 located in the city of Guadalajara, Jalisco, the second biggest town in Mexico. Evaluations  
241 were performed in individual sensory booths under white light and air circulation, both  
242 laboratories designed according to ISO 8589 (ISO International Organization for  
243 Standardization, 2007). Data were collected using Fizz software program (version 2.5;  
244 Biosystems, Courtenon, France).

245 The test consisted of a hedonic test and a questionnaire to obtain information about  
246 consumers' beer consumption habits and socio-demographic information. In the hedonic  
247 test, consumers were asked to evaluate the liking of the samples under three conditions:  
248 blind (B), expected (E) and informed (I). In the two tasting conditions (blind and informed),  
249 the six samples of beer were presented in balanced order, monadically with a 30-sec interval  
250 between evaluations. Water was provided to consumers to rinse their mouth after tasting  
251 each sample. In the three conditions (blind, expected and informed), the liking of each beer  
252 was rated using a 10-cm unstructured linear scale anchored at the extremes with the terms  
253 "extremely dislike" (left side) and "extremely liked" (right side).

254 Both blind and expected test conditions took place in the first session. For blind condition,  
255 consumers were asked to taste and rate the liking of the beers without any information about  
256 the products. The only information provided to the consumers was that they were about to  
257 taste different beer samples. The expected condition test took place after a 5-min break. For  
258 this condition, the following question was shown on a screen: "*How much do you expect to*  
259 *like a beer made with the following ingredients?*". Then, the information about the ingredients  
260 of each beer (Table 1) along with an image of the type of malt(s) used in each beer (barley,  
261 red corn and/or blue corn) was shown to consumers. For all the beers that contain corn on  
262 its formulation, the following statement was given: "*The blue/red corn is a Mexican variety*  
263 *of corn that comes from Milpa Alta, Mexico*". Consumers were asked to read the information  
264 carefully and rate each beer without tasting any sample.

265 The informed condition test took place in the second session, one week apart. In this  
266 session, consumers were provided with additional samples of each beer along with the  
267 information presented in the expected condition (described before). Then, consumers were  
268 asked to read the information before tasting and rate the liking of each beer. After the tasting,  
269 consumers were asked to answer a non-mandatory open-ended question stating separately  
270 what they liked and disliked about each product. Finally, a questionnaire was provided to  
271 each consumer to obtain their sociodemographic information and their beer and corn  
272 consumption habits.

## 273 2.5. Data analysis

274 Analysis of variance (ANOVA) was performed on blind, expected and informed liking scores  
275 to evaluate differences among beer samples for each condition. Significances of differences  
276 between samples were determined by the post-hoc Tukey test ( $p < 0.05$ ). To evaluate the  
277 effect of information on consumers liking, paired Student's t-test ( $p < 0.05$ ) were performed

278 on the differences between expected and blind (E-B), informed and blind (I-B) as well as the  
279 differences between informed and expected (I-E) conditions for each of the six beers. If the  
280 difference (E-B) is significantly different from zero, a disconfirmation of expectation occurs.  
281 If the difference between (I-B) is different from zero, the disconfirmation can be associated  
282 with an assimilation or contrast effect. An assimilation effect occurs if the ratio between (I-  
283 B)/(E-B) is higher than zero. Otherwise, if the ratio is lower than zero, a contrast effect  
284 occurs. In the case of assimilation, if the difference between (I-E) is significantly different  
285 from zero, consumers do not completely assimilate towards their expectation and the  
286 assimilation is incomplete. If not, the assimilation is complete (García-Barrón et al., 2020;  
287 Nacef et al., 2019b; Vitale et al., 2020).

288 In addition, a Hierarchical Cluster Analysis (unstandardized scores, Euclidian distance,  
289 Ward's method) was carried out on the blind condition scores across the six samples to  
290 identify possible consumer segments based on product liking/disliking (Jaeger et al., 2020)  
291 retaining three-cluster solution. Then, the analyses of expectations (mentioned before) were  
292 applied in the same way for each cluster. All the statistical analyses were performed using  
293 XLSTAT (version 2018.7, Addinsoft, Paris, France).

### 294 3.0. Results and Discussion

#### 295 *Questionnaire: Consumption habits toward beer*

296 The questionnaire was provided to have an overview of consumers' frequency consumption  
297 of craft and industrial beers and to know which are the main factors for buying beer.  
298 Consumers were asked about their frequency of beer consumption (craft and industrial), the  
299 situations in which they consume either, craft or industrial beer (e.g., home, restaurant, bar,  
300 party), and the aspects for buying beer (e.g., price, origin, brand, new product, flavours,  
301 ingredients, label, awards, familiarity and volume). Participants were more likely to consume  
302 industrial beer more often, with 29% of participants consuming industrial beer 2-3 times per  
303 week vs 10% consuming craft beer. On the other hand, 35% of participants declared to  
304 consume craft beer occasionally in a year and only 24% consume this type of beer once per  
305 month. When asked about the situation of beer consumption, participants use to consume  
306 more industrial beer at parties (78%), at the bar (73%), and at home (56%). On the other  
307 hand, 56% of participants consume craft beer in restaurants.

308 About the factors for buying beers, 43% of the participants said that the flavour is "extremely  
309 important" when buying either industrial or craft beer. In addition, participants consider as  
310 "important" the price (47%), brand (43%), volume (42%), and ingredients (37%). It was

311 interesting to find out that aspects as the origin of the beer, label design, awards and  
312 familiarity are considered 'very important' only for a little percentage of the participants  
313 (respectively, 10%, 12%, 10%, and 27%).

314 *Overall results*

315 Mean liking scores of beer samples in the three different conditions (blind, expected and  
316 informed) are shown in Table 2.

317 Table 2. Mean liking scores for the six beer samples were evaluated under the three different conditions: Blind (B), Expected (E) and Informed (I)  
 318 and expectation effect.

Sample	Blind (B)	Expected (E)	Informed (I)	E-B			I-B			I-E		
				Mean	Effect	p-value	Mean	Effect	p-value	Mean	Effect	p-value
BC100	2.4 <sup>c,C</sup>	5.9 <sup>a,AB</sup>	3.1 <sup>b,C</sup>	3.5	Negative disconfirmation	0.0001	0.7	Assimilation	0.022	-2.8	Incomplete	0.0001
RC100	1.8 <sup>c,C</sup>	5.3 <sup>a,B</sup>	2.4 <sup>b,C</sup>	3.5	Negative disconfirmation	0.0001	0.6	Assimilation	0.035	-2.8	Incomplete	0.0001
RBC50	2.4 <sup>b,C</sup>	5.4 <sup>a,B</sup>	3.1 <sup>b,C</sup>	3.0	Negative disconfirmation	0.0001	0.7	Assimilation	0.03	-2.3	Incomplete	0.0001
BAR100	6.2 <sup>b,A</sup>	6.5 <sup>ab,A</sup>	7.2 <sup>a,A</sup>	0.3	Confirmation	ns	1.0	--	--	0.6	--	--
BCBAR50	6.3 <sup>a,A</sup>	6.3 <sup>a,A</sup>	6.8 <sup>a,AB</sup>	-0.1	Confirmation	ns	0.5	--	--	0.6	--	--
RCBAR50	5.0 <sup>b,B</sup>	6.1 <sup>a,AB</sup>	6.0 <sup>a,B</sup>	1.0	Negative disconfirmation	0.001	0.9	Assimilation	0.008	-0.1	Complete	ns

319 Different lowercase superscripts within a column indicate significant differences according to Tukey's test ( $p < 0.05$ ).  
 320 Different capital superscripts within a row indicate significant differences according to Tukey's test ( $p < 0.05$ ).  
 321 ns, not significant

322 Considering the blind condition, all the beers showed significant differences. Major  
323 differences were observed between beers made only with corn and beers containing barley.  
324 The beers made with barley (BAR100) and the blended beers made with red and blue corn  
325 (BCBAR50, RCBAR50) showed an acceptance overall liking from 5.0 to 6.3. Contrary, beers  
326 made only with corn (BC100, RC100 and RBC50) showed a lower liking score ranging from  
327 1.8 to 2.4; indicating that sensory characteristics did not please the consumers. Thus, the  
328 replacement of barley malt with corn malt produces a significant decrease in liking scores.  
329 This pattern was similar in the other conditions (expected and informed).

330 It is well known that malt is one of the major contributors to beer flavour. Thus, partial or total  
331 substitutes of barley malt in beer have been found to have an impact on its sensory  
332 characteristics (Atnafu Yemata, 2015). For instance, the use of pigmented corn malt in  
333 brewing develops flavours such as fermented fruits, cooked vegetables, tortillas, bread, and  
334 dried fruits (Romero-Medina et al., 2020), which could not be appreciated by consumers,  
335 especially by those who used to drink industrial beers.

336 Under the expected condition, all the beers were rated in the acceptance zone, with liking  
337 scores ranging from 5.3 to 6.5, higher scores than in the blind condition. Significant  
338 differences were observed in all the beer samples. The beers showed the same pattern as  
339 in the blind condition, where the beers containing only corn (red and/or blue) were rated  
340 lower than the ones containing barley in their formulation. However, the samples that  
341 showed a major difference in overall liking score compared with the blind condition were  
342 BC100, RC100 and RBC50, which were those containing only corn. Beers RC100 and  
343 RBC50 presented a significantly lower liking score than the rest of the samples. The latter  
344 could be associated with the type of corn (red corn) used to brew these beers. In Mexico,  
345 the red variety of corn is less well-known than the blue variety as is cultivated in limited areas  
346 of the country (Graillet Juárez et al., 2019). These results suggest that the expectations  
347 raised by the information about the use of Mexican corn in the beers. However, consumers  
348 still have a major acceptance for those beers where barley is one of the main ingredients.  
349 This also highlights the fact that even when craft beer is a product known for its versatility in  
350 terms of the cereals used for its elaboration (e.g., rice, wheat, oat, corn), consumers still  
351 have a greater preference for the 'traditional' ingredients such as barley.

352 Under the informed condition, significant differences were found among the beers. The  
353 same pattern as in blind and expected conditions was found, where the beers made only  
354 with corn were scored lower (from 2.4 to 3.1) than the beers that contain barley in their

355 formulation (from 6.0 to 7.2). However, beers made with corn were scored higher in the  
356 informed condition than in blind condition. These results suggest that the information about  
357 corn helped to increase the acceptance of these kinds of beers. Also, there were significant  
358 differences from blind to the informed condition for BAR100 and RCBAR50 increasing their  
359 overall liking score. For these last beers, there was no significant difference between the  
360 scores in the expected and informed conditions.

361 The analysis of expectations was done to evaluate the effect of information on consumers'  
362 liking. To know if the expectations generated by the ingredients and origin of corn are  
363 confirmed or disconfirmed, we considered the differences between expected and blind liking  
364 scores (E-B) (Table 2). Negative disconfirmation occurred for all the 'corn beers' (BC100,  
365 RC100 and RBC50) and for the blended beer sample RCBAR50 as significant differences  
366 were found between (E-B). Thus, the sensory characteristics did not meet the expectations  
367 created by the ingredients and the beers were worse than expected. This negative  
368 disconfirmation was associated with an assimilation effect as the difference between  
369 informed and blind scores (I-B) was significantly different from zero. When assimilation  
370 happens, it indicates that the information provided about the ingredients and the origin of  
371 corn has influenced the liking of these beers, and the liking in informed condition moved  
372 towards consumer's expectations. In addition, the differences between informed and  
373 expected scores (I-E) were calculated to know if for all the beer samples made only with  
374 corn (BC100, RC100 and RBC50) assimilation was incomplete (I-E) meaning that the  
375 sensory characteristics did not compensate the expectation created by the information in  
376 the informed condition. Contrary, for the beer sample made with red corn and barley  
377 (RCBAR50), the assimilation was complete. In this case, the information had a high  
378 influence on the liking in the informed condition compensating for the decrease in liking.

379 On the other hand, for beers BAR100 and BCBAR50, no significant differences were found  
380 between expected and blind liking scores (E-B), indicating a confirmation of expectation.  
381 Regarding these results, the fact that the beers that include barley in their formulation meet  
382 consumers' expectations indicates that consumers are more accustomed to the sensory  
383 flavours related to barley malt. Also, in the case of the beer BCBAR50, where a partial  
384 replacement of barley malt was done with blue corn malt, the confirmation could be  
385 explained as 'blue corn' is one of the most common pigmented corn used in a wide variety  
386 of traditional dishes in Mexico. Therefore, consumers may be familiar with the type of  
387 flavours that this corn could impart.

388 In general, providing information about ingredients and the origin of corn had a great  
 389 influence on consumers expectations, specifically in those beers made 100% with corn malt.  
 390 This suggests that beer consumers are willing to explore new products made with local or  
 391 native ingredients (such as pigmented corn), which is a good sign for the craft beer industry.  
 392 However, the sensory profile of these products must be improved, trying to obtain a similar  
 393 flavour profile to those found in beers made with barley malt.

394 To our knowledge, there are no studies on the effect of information on consumers' liking  
 395 about the origin of a certain ingredient in a product. However, some studies have been  
 396 proved that the origin of the product plays an important role in consumers acceptance and  
 397 choice of products, generating higher expectations and positive evaluations (García-Barrón  
 398 et al., 2020; Iaccarino et al., 2006). Also, consumers tend to prefer local products and those  
 399 that match their culture and traditions to keep their identity and eating habits (Ricci et al.,  
 400 2019). Thus, knowing that corn is a local and traditional grain in Mexican culture and heritage  
 401 could explain why consumers created high expectations for the 'corn beers'. Therefore, the  
 402 use of pigmented corn malts in brewing could be an interesting option to create a beer that  
 403 shares Mexican consumers' identity and might in time appeal to new beer consumers.

#### 404 **Analysis of expectations on consumer segments**

405 Using an HCA, three consumer clusters were distinguished based on their product  
 406 liking/disliking scores. Table 3 shows the demographic characteristics and consumption  
 407 habits of consumer segments with statistical differences obtained through Chi-square  
 408 analysis within each cluster. Table 4 shows the mean hedonic scores provided by each  
 409 group of consumers under the three evaluated conditions (blind, expected and informed).

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414 *Table 3. Demographic information and habits of consumption of consumers of the identified clusters presented*  
 415 *as percentages.*

Categories	Total (n=100)	Cluster 1 (n=42)	Cluster 2 (n=34)	Cluster 3 (n=24)
------------	------------------	------------------------	------------------------	------------------------

<b>Age</b>				
18-24 years	25	26.2b	26.5ab	20.8a
25-35 years	39	45.2ab	38.2b	29.2a
36-45 years	25	16.7b	26.5ab	37.5a
46+ years	11	11.9b	8.8a	12.5a
<b>Gender</b>				
Male	48	40.5a	58.8a	45.8a
Female	52	59.5a	41.2a	54.2a
<b>Education level</b>				
High school	10	11.9a	8.8a	8.3a
University	90	88.1b	91.2b	91.7b
<b>Occupation</b>				
Student	45	50.0b	47.1b	33.3ab
Professional	27	26.2b	32.4b	20.8ab
Employee	27	23.8b	20.6b	41.7b
Retired	1	0.0a	0.0a	4.2a
<b>Frequency of consumption-Industrial</b>				
2-3 times per week	29	28.6b	29.4a	29.2a
Once per week	25	28.6b	20.6a	25.0a
2-3 times per month	25	23.8b	23.5a	29.2a
Once per month	12	14.3ab	14.7a	4.2a
Occasionally in a year	8	4.8ab	8.8a	12.5a
Never	1	0.0a	2.9a	0.0a
<b>Frequency of consumption-Craft</b>				
2-3 times per week	10	11.9ab	5.9a	12.5a
Once per week	10	14.3ab	5.9a	8.3a
2-3 times per month	17	16.7ab	17.6ab	16.7a
Once per month	24	26.2b	17.6ab	29.2a
Occasionally in a year	35	31.0b	50.0b	20.8a
Never	4	0.0a	2.9a	12.5a
<b>Corn consumption</b>				
Yes	97	97.6b	97.1b	95.8b
No	3	2.4a	2.9a	4.2a
<b>Corn beverages consumption</b>				
Yes	42	45.2a	50.0a	25.0a
No	58	54.8a	50.0a	75.0b

416 Different lowercase letters within the same cluster indicate significant differences between demographic  
417 characteristics and habits of consumption according to the Chi-square test ( $P < 0.05$ ) of K proportion with the  
418 Marascuilo procedure.

419 Table 3 (Continued).

	Categories	Total (n=100)	Cluster 1 (n=42)	Cluster 2 (n=34)	Cluster 3 (n=24)
Situ atic	Home				
	Craft	29	35.7ab	11.8a	41.7b

Buying factors	Industrial	56	50.0b	67.6b	50.0b	
	Neither	15	14.3a	20.6a	8.3a	
	<b>Restaurant</b>					
	Craft	51	54.8b	44.1b	54.2b	
	Industrial	43	35.7b	50.0b	45.8b	
	Neither	6	9.5a	5.9a	0.0a	
	<b>Bar</b>					
	Craft	22	23.8b	23.5c	16.7a	
	Industrial	73	71.4c	76.5b	70.8b	
	Neither	5	4.8a	0.0a	12.5a	
	<b>Party</b>					
	Craft	19	19.0b	17.6a	20.8a	
	Industrial	78	78.6c	79.4b	75.0b	
	Neither	3	2.4a	2.9a	4.2a	
	<b>Price</b>					
	Not important at all	6	0.0a	5.9ab	16.7ab	
	Less important	27	31.0bc	11.8ab	41.7a	
	Important	47	47.6c	58.8c	29.2a	
	Very important	18	16.7ab	23.5b	12.5ab	
	Extremely important	2	4.8a	0.0a	0.0a	
	<b>Origin</b>					
	Not important at all	23	21.4ab	26.5ab	20.8ab	
	Less important	35	23.8ab	44.1b	41.7b	
	Important	32	40.5b	20.6ab	33.3ab	
	Very important	10	14.3a	8.8a	4.2a	
	<b>Brand</b>					
	Not important at all	4	4.8a	2.9a	4.2a	
	Less important	29	26.2ab	38.2b	20.8ab	
Important	43	45.2b	29.4b	58.3b		
Very important	21	23.8a	20.6ab	16.7a		
Extremely important	3	0.0a	8.8ab	0.0a		
<b>New product</b>						
Not important at all	11	11.9ab	8.8ab	12.5a		
Less important	50	47.6c	50.0c	54.2b		
Important	26	26.2bc	26.5bc	25.0ab		
Very important	12	11.9ab	14.7ab	8.3a		
Extremely important	1	2.4a	0.0a	0.0a		

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*Different lowercase letters within the same cluster indicate significant differences between demographic characteristics and habits of consumption according to the Chi-square test ( $P < 0.05$ ) of K proportion with the Marascuilo procedure.*

423

Table 3 (Continued).

Categories	Total (n=100)	Cluster 1 (n=42)	Cluster 2 (n=34)	Cluster 3 (n=24)
------------	------------------	------------------------	------------------------	------------------------

<b>Buying factors</b>	<b>Flavours</b>				
	Not important at all	1	0.0a	0.0a	4.2a
	Less important	2	2.4ab	2.9a	0.0a
	Important	17	23.8bc	5.9a	20.8ab
	Very important	37	31.0c	44.1b	37.5b
	Extremely important	43	42.9c	47.1b	37.5b
	<b>Ingredients</b>				
	Not important at all	5	4.8a	8.8a	0.0a
	Less important	31	35.7b	26.5ab	29.2b
	Important	37	38.1b	41.2b	29.2b
	Very important	19	14.3ab	14.7ab	33.3b
	Extremely important	8	7.1a	8.8a	8.3ab
	<b>Label design</b>				
	Not important at all	12	14.3abc	2.9a	20.8ab
	Less important	39	40.5c	44.1c	29.2b
	Important	34	33.3bc	35.3bc	33.3b
	Very important	12	9.5ab	11.8ab	16.7ab
	Extremely important	3	2.4a	5.9a	0.0a
	<b>Awards</b>				
	Not important at all	27	33.3c	20.6ab	25.0abc
	Less important	32	28.6bc	29.4b	41.7c
Important	30	28.6bc	32.4b	29.2bc	
Very important	10	7.1ab	17.6ab	4.2ab	
Extremely important	1	2.4a	0.0a	0.0a	
<b>Familiarity</b>					
Not important at all	4	4.8a	0.0a	8.3a	
Less important	26	26.2abc	29.4b	20.8a	
Important	33	35.7bc	29.4b	33.3a	
Very important	27	21.4abc	29.4b	33.3a	
Extremely important	10	11.9ab	11.8a	4.2a	
<b>Volume</b>					
Not important at all	9	2.4a	11.8ab	16.7ab	
Less important	28	31.0bc	26.5b	25.0ab	
Important	42	54.8c	35.3b	29.2b	
Very important	20	9.5ab	26.5b	29.2b	
Extremely important	1	2.4a	0.0a	0.0a	

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*Different lowercase letters within the same cluster indicate significant differences between demographic characteristics and habits of consumption according to the Chi-square test ( $P < 0.05$ ) of K proportion with the Marascuilo procedure.*

427 Table 4. Mean liking scores for the six beer samples of each consumer cluster evaluated under three conditions (blind, expected and informed)

Cluster	Sample	Blind (B)	Expected (E)	Informed (I)	E-B			I-B			I-E		
					Mean	Effect	p-value	Mean	Effect	p-value	Mean	Effect	p-value
1	BC100	4.1b	6.6a	4.3b	2.5	Negative Disconfirmation	0.000	0.2	ns	0.674	-2.3	--	
	RC100	3.2b	5.7a	2.9c	2.5	Negative Disconfirmation	0.000	-0.3	ns	0.516	-2.8	--	
	RBC50	3.9b	6.0a	4.2bc	2.1	Negative Disconfirmation	0.000	0.3	ns	0.557	-1.8	--	
	Barley 100	7.0a	6.6a	7.3a	-0.4	ns Confirmation	0.304	0.3	--		0.7	--	
	BCBAR50	6.9a	6.8a	7.1a	-0.1	ns Confirmation	0.842	0.2	--		0.2	--	
	RCBAR50	6.3a	6.4a	6.7a	0.1	ns Confirmation	0.768	0.4	--		0.3	--	
2	BC100	1.2d	5.9a	2.3b	4.7	Negative Disconfirmation	0.000	1.1	Assimilation	0.009	-3.6	Incomplete	0.000
	RC100	0.7d	5.6a	2. b	4.8	Negative Disconfirmation	0.000	1.7	Assimilation	0.000	-3.1	Incomplete	0.000
	RBC50	1.6d	5.5a	2.6b	3.9	Negative Disconfirmation	0.000	1.0	Assimilation	0.029	-2.9	Incomplete	0.000
	Barley 100	6.2b	6.4a	7.5a	0.1	ns Confirmation	0.841	1.3	--		1.2	--	
	BCBAR50	7.8a	5.8a	7.5a	-2.0	Positive Disconfirmation	0.000	-0.3	--	0.386	1.7	--	
	RCBAR50	4.5c	6.0a	6.1a	1.5	Negative Disconfirmation	0.002	1.6	Assimilation	0.005	0.1	Complete	0.855
3	BC100	1.1c	4.8bc	2.1c	3.7	Negative Disconfirmation	0.000	1.1	Assimilation	0.021	-2.6	Incomplete	0.000
	RC100	0.9c	4.1c	1.5c	3.2	Negative Disconfirmation	0.000	0.7	ns	0.120	-2.5	--	
	RBC50	0.7c	4.0c	1.8c	3.3	Negative Disconfirmation	0.000	1.0	Assimilation	0.020	-2.2	Incomplete	0.000
	Barley 100	4.8a	6.7a	6.3a	1.9	Negative Disconfirmation	0.005	1.5	Assimilation	0.027	-0.4	Complete	0.476
	BCBAR50	3.2b	5.8ab	5.3ab	2.7	Negative Disconfirmation	0.000	2.2	Assimilation	0.002	-0.5	Complete	0.481
	RCBAR50	3.5ab	5.5abc	4.4b	2.0	Negative Disconfirmation	0.002	0.9	ns	0.193	-1.1	--	

428 Different letters in the same column within each cluster show significant differences according to Tukey's test ( $p < 0.05$ ). ns, not significant

As shown in Table 4, significant differences in liking scores were found via ANOVA among beers in each condition and within clusters, except for the expected condition in clusters 1 and 2. These differences in the mean scores for the three segments of consumers show their different liking patterns. There was a tendency in the three clusters for blind condition (B), where beers made only with corn malts (either blue or red corn) were rated with lower scores than the beers containing barley malt. However, consumers of cluster 1 rated higher (scores from 3.2 to 4.1) the three samples of 'corn beers' (BC100, RC100 and RBC50) than consumers of clusters 2 and 3 (scores from 0.8 to 1.6). Beers containing barley malt obtained higher scores in clusters 1 and 2 (from 4.5 to 6.9) than in cluster 3 (3.1 to 4.7).

In expected condition (E), there were no significant differences in liking scores between beers in clusters 1 and 2. The major differences were found in cluster 3, where consumers show higher liking scores for those beers made with barley malt or a combination with blue corn malt (BAR100 and BCBAR50) than those beers containing red corn malt show lower liking scores. Thus, the information provided to consumers had a different effect on consumers depending on their sociodemographic characteristics and habits of beer consumption.

The analysis of expectations revealed a negative disconfirmation for all the corn beers (BC100, RC100 and RBC50) in the three clusters. These results show that even when consumers like the idea of having a beer made with local and traditional ingredients, the sensory properties play a relevant role in the acceptance of beer. To analyze the influence of information on beer samples' liking, I-B were calculated in each cluster. For cluster 1, no significant differences were detected, indicating that the information provided about the beers did not influence the liking of either beer. On the contrary, these 'corn beers' showed a significant difference (except for RC100, cluster 3) relating the disconfirmation to an assimilation effect in clusters 2 and 3. However, the assimilation was incomplete, suggesting that the information provided moved in the direction of expectations, but the sensory properties did not please consumers liking.

On the other hand, the largest difference in liking scores among clusters corresponds to beers containing barley (BAR100, BCBAR50 and RCBAR50). Consumers in clusters 1 and 2 showed significantly higher liking scores (in the liking/acceptance zone) than consumers in cluster 3. This pattern was consistent under the three evaluation conditions (blind, expected and informed). For these beers, the analysis of expectations showed a large difference among clusters. For cluster 1, a confirmation of expectations was observed. Thus, their expectations met the real experience. Cluster 2 showed three effects namely confirmation, positive and negative disconfirmation). Beer made only with barley (BAR100) showed a confirmation of expectations (acceptable as expected). Then, for beers BCBAR50 and RCBAR a positive and

negative disconfirmation respectively was observed. The negative disconfirmation was associated with a complete assimilation. When a positive disconfirmation occurs, consumers are more influenced by information and the product is more acceptable than the expectations created. Regarding beer made with blue and barley malts (BCBAR50), the information about the use of blue corn had a great influence on the acceptability of this beer. The fact that RCBAR50 beer was less acceptable than BCBAR50 could be associated with a lack of knowledge and familiarity that consumers have with the red corn. In this sense, consumers of cluster 2 are more likely to accept beers made with a mixture of barley and pigmented corn only if information about the ingredients is provided.

In contrast, consumers in cluster 3 showed negative disconfirmation for beers BAR100, BCBAR50 and RCBAR50, meaning that the beers were less acceptable than expected. However, for both BAR100 and BCBAR50 the disconfirmation was associated with a complete assimilation effect. As explained before, the information provided influences consumers' liking. Moreover, no significant difference was detected for RCBAR50 when analysis of disconfirmation was done (I-B). This indicates that for consumers of cluster 3, the information provided does not influence the acceptability of this beer.

Although for the three clusters, the 'corn beers' were considered as unliked or in the unacceptable zone, it was possible to distinguish a segment of consumers (cluster 2) that could accept this kind of beer more easily if the sensory characteristics are improved. Thus, a reformulation of the beers made 100% with either blue or red corn malt is needed. The fact that consumers from clusters 1 and 2 appreciated more beers containing barley and corn malt may be related to the fact that these consumers are more familiar with the flavours of beverages made with corn than those consumers from cluster 3 (Table 3). Also, consumers from clusters 1 and 2 used to consume craft beers more frequently than those from cluster 3 (Table 3). Therefore, they could expect these beers to taste different from industrial beers, as they are more accustomed to the different flavours of craft beers than consumers from cluster 3. Previous studies have shown that 'craft beer consumers are willing to try novel products and relates craft beers with different and selected ingredients as well as with new tasting experiences (Gómez-Corona, Escalona-Buendía, et al., 2016).

In addition, the results obtained by consumers from cluster 3 could indicate that these consumers are not used to consume craft beers and they could perceive these novel ingredients as a negative quality. These results are in accordance with Giacalone D (2013) (Giacalone, 2013), who found that for some beer consumers 'novelty' is linked to a negative quality.

Based on the results, it seems that there are some demographic characteristics and consumption habits within each cluster that makes possible to differentiate consumers based on their liking/disliking patterns. However, there was not possible to identify specific variables among clusters that help us to understand the differences between clusters. Along with these variables (demographic characteristics and consumption habits), there are other factors, such as the sensory characteristics that need to be modified and improve to assure consumers' beer acceptance. Therefore, it is essential to identify consumers' drivers of liking that predict consumer acceptance and/or preferences to deliver a beer(s) with the characteristics that consumers want.

Finally, when it comes to reinterpreting the traditional beer styles, changing or replacing ingredients and generating an entirely new flavour is not well accepted. Maybe for consumers who do not usually drink craft beers, the introduction of new ingredients that end up adding new flavours has to be gradual. The partial replacement of barley malt with corn malt could help to maintain traditional beer flavours while adding some new ones, making the beer's flavour slightly different.

## **Conclusions**

This study was the first to explore the effect of replacing barley malt (partial or total) with a native cereal such as pigmented corn (information provided) on consumers' liking for craft beers. The results showed that consumers expectations rise when information about the ingredients and the origin of corn is provided. Moreover, when this information is given and is followed by a tasting evaluation (informed condition), consumers significantly increase their liking for those beers made with pigmented corn malts compared to blind tasting. These results show that information is highly influential in affecting consumers' expectations and liking when it comes to a novel craft beer made with native or local ingredients. The partial replacement of barley malt with corn malt (BCBAR50 and RCBAR50) showed more acceptance than beers made 100% with corn malt (BC100, RC100 and RBC50). This highlights the fact that even when consumers are willing to try new styles of beer and new flavours, they also tend to appreciate more a beer that reminds them of the typical flavours of a barley beer. However, since their expectations and liking increased for those beers containing both barley and corn malt (either blue or red corn malt), we can assume that there is a certain degree of nostalgia and emotional connections that provoke the acceptability in these kinds of beers. Nevertheless, to assure the success of either of these beers in the beer marketplace, a reformulation is needed to improve their sensory characteristics as food choice is mainly dominated by sensory preferences.

In addition, it was possible to identify three segments of consumers with different liking/disliking patterns. This revealed that there is a potential niche market for these novel craft beers. However, it was not possible to identify key variables among clusters that affect the expectations/liking and that could help us to understand the differences between them. Future research should include a more detailed questionnaire about sociodemographic data, consumption habits and attitudes towards craft and industrial beers to establish a profile of the potential consumer of pigmented corn beers.

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## *15 General discussion*

The main objective of this thesis was to contribute to a more complete understanding of the use of pigmented corn varieties in the brewing of their chemical and sensory aspects to provide the craft brewing industry with the knowledge of the feasible use of native cereals as an alternative to the production of innovative beers which in turn, could help to revalue traditional drinks like Sendechó.

This work was comprised by four studies in which four hypotheses were tested resulting in four chapters.

In addition, to develop the studies of this thesis we make use of the phases of the new product development process. This process served us as a tool in the study of the corn beer(s) with a view of bridging the gap between the laboratory and the commercialization in the craft beer market.

The first step in the new product development process mainly comprise the research made in the laboratory to demonstrate the feasibility of the new technology proposed, in this case, the use of pigmented corn for brewing purposes. As previous research had mainly focused on the characterisation of the malting process of pigmented corn grains and the chemical characterisation of the corn malts obtained, this project was centred on the chemical aspects that have not been investigated before, such as the volatile composition.

Beer flavour is mainly influenced by the ingredients used in its process. Also, beer flavour largely determines the product acceptance that will end up with the success or rejection of the product. Therefore, the study of the volatile composition of raw material helped us to understand the extent of the influence of pigmented corn in the finished beers.

Volatile compounds in corn grains and malts play an important role in the flavour composition of the beer. The results of this work (Chapter I, Study 1) showed that both varieties of corn, blue and red, have a similar volatile profile in their unmalted and malted form.

Results demonstrated that the volatile profile of brewing malts from different cereals, corn and barley, are quite similar. However, it is possible to distinguish them by certain volatiles of the group of phenols and terpenes. We found that among these compounds, some compounds are inherent in the corn grain and remain throughout the malting process, while others were developed during the malted process. For instance, blue corn malt is better characterised by aldehydes and furans, which usually are responsible for the green, fresh and grass aroma of the malt. Red corn malt is represented by phenols and terpenes such as 2-methoxy-4-vinyl

phenol, phenol, 4-ethyl-phenol, limonene and geranyl acetone. The group of volatiles that serves to distinguish corn malts from barley malts have been reported in other corn-based products such as tortilla, tortilla chips and pop-corn (Buttery & Ling, 1995,1998) and have been associated to be the major contributors of the typical flavour of corn. Therefore, we can expect that the pigmented corn malts contribute with both, flavours of a typical brewing malt and characteristic flavours of corn-based products.

This part of the research brings new knowledge to the understanding of the volatile composition of corn grains and their malts under certain conditions. Such knowledge can be used in future research to monitor the evolution of volatile compounds by modifying certain parameters during malting and profiling corn malts with different volatile composition that will eventually influence the flavour of beer.

At this point in the project, we knew that pigmented corn is viable for beer production and that beer will have an aromatic profile similar to corn-based products. However, the consumer perspective on the idea of a pigmented corn-based beer had not been studied. Therefore, the second phase of the project was based on the development of concepts, which belongs to the second phase of the product development process (Chapter II, Study II).

This stage was composed of two phases, in which the development of two concepts was proposed from two different perspectives, one appealing to the emotional side and the other appealing to the healthy side of the incorporation of pigmented corn grains. The first phase of the study was conducted with Mexican consumers, and served to improve the concept of corn beer, as well as to obtain those key characteristics that consumers expect to find in this type of beer. During the second phase of concept development, it was decided to incorporate a cross-cultural approach in order to know if this drink could succeed in a market other than Mexican. The results of the 1st phase showed that in general the potential consumers of this beer expect a product that is distinguished by the aroma and/or flavour of corn, and its derivatives, such as tortilla; but that continues to preserve the sensory properties of a barley beer, such as bitterness.

The cross-cultural study between Mexican and French consumers showed that Mexican consumers are more attracted to the idea of a beer made with corn, which is understandable since corn is part of the daily life of Mexicans.

In addition, both consumers showed similar behaviour towards emotional and functional concepts. While the emotional concept was evaluated better and with higher credibility, the functional concept obtained a significantly lower rating, especially in the credibility of the same. A similar feature for both countries was that overall taste ratings were not as high as expected, which may be associated with a certain level of neophobia or an inner fear of novelty.

Once this analysis was carried out, we were in the prototyping stage, which includes phase 4 and 5 of the development of new products (Chapter III, Study 3). In this phase, we made use of the results of the previous stage to formulate the prototypes. Since consumers expect a beer that contains typical sensory characteristics of corn products but preserves the main ones of a barley beer, 6 prototypes were developed using 100% each of the malts (blue corn, red corn and barley) as well as mixtures of such 50% malts. Once the samples were obtained, they were evaluated under two perspectives, chemistry and sensory, in order to understand the effect that these malts had on the sensory properties of the beers. The results showed that beers made with these specific types of pigmented corn (red and blue) show a sensory profile mainly characterized by fermented fruits, cooked vegetable odours, tortillas, bread, dried fruits and dried chilli. According to the results, it is possible to brew a beer with pigmented corn (blue and red), and even when the produced beers have similar properties to a barley beer, there are some specific properties (volatile, non-volatile compounds, sensory attributes) that are inherent of the pigmented corn malts (See Chapter III). Also, it was possible the correlation between both the sensory and the chemical parameters, allowed us to elucidate the effect of each type of malt (red corn, blue corn and barley malt) on the chemical parameters and the association with the sensory attributes. For instance, we can highlight the positive correlation between beers made with corn malt (red and blue) with volatile compounds such as linalool, limonene,  $\beta$ -ionone, and 4-ethyl-2-methoxy-phenol. Also, a positive correlation between the content of anthocyanins (characteristic of blue and red corn beers) and the phenol compounds such as phenol, 2-methoxy-phenol, 4-ethyl-phenol, and 4-ethyl-2-methoxy-phenol. During the development of new products, the evaluation of the sensory characteristics and with it of the compounds that impact on these characteristics is important to know if our product meets the characteristics that were proposed in the design of the product and to lay the foundations for chemical and sensory parameters that could be used as quality parameters in subsequent steps as well as in future research.

Finally, once the beers were developed and evaluated, we moved to the stage of development of new products in which tests are carried out with consumers in order to know the taste of consumers on these products (Chapter IV, Study 4). In addition, we went a step further to know if the beers met the expectations of consumers. This step is paramount, as even at this stage we will be able to return few steps and make the appropriate modifications to the product if required, to meet consumers needs and thus ensure success in the market.

The results of this phase demonstrated that beer consumers are willing to try different beer experiences in terms of flavour. Also, the information provided to consumers greatly influences the expectations by increasing the liking of corn beers.

Unfortunately, we noticed that expectations over pigmented corn beers were higher than the real experience. Thus, is still something that is missing in terms of its sensory properties, so it is highly recommended to reformulate the beers, especially the ones made 100% with corn. The study about expectations also shows us that even when beer consumers are more open to new styles of beer, they tend to accept more easily beers that retain sensory characteristics from barley beers.

## *16 General conclusions*

The results of this thesis add knowledge about the use of pigmented corn in the brewing industry. It was possible to obtain for the first time the characterization of corn malts and to know the key compounds that can serve as fingerprints of corn malts. We could observe that the volatile and non-volatile composition of these corn malts directly influence the sensory properties of beer. In addition, by mixing barley with corn we obtain a volatile and sensory profile that share properties of both cereals, and with this we can ensure a better acceptance among beer consumers who are looking for innovative sensory profiles, but which do not differ so much from the beer concept they already know.

In this sense, the information presented to the consumer is essential both to generate expectations and to increase the acceptance of the product. Therefore, the development of a new concept that returns to the most outstanding of corn beer will be key to the proper promotion of the product. We also saw that as a concept, corn beer could have a niche market in France, which could lead to the revaluation of native corn outside the country.

Future research is needed to improve the sensory properties by reformulating beers made with pigmented corn. But, to deliver a beer that meets consumers' expectations, it is necessary to investigate the consumers' drivers of liking towards these beers.

In addition, the use of native varieties of corn in brewing is an innovative resource to renovate traditional beverages such as Sendecho, while preserving Mexican traditions in a modern way. Also, using pigmented corn as the main raw ingredient in brewing adds value to this grain, which in turn could help to promote the cropping and consumption of these varieties of corn.

Finally, the use of the "New Product Development Process" helped us to develop a product that is almost ready for its commercialisation and served as a tool to bridge the gap between science and industry. Despite the improvements in its flavour that must be done, this research proves that it is feasible to use native and local grains such as pigmented corn for brewing purposes.

## 17 References

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Casa abierta al tiempo

UNIVERSIDAD AUTÓNOMA METROPOLITANA

# ACTA DE DISERTACIÓN PÚBLICA

Nº 00252

Matrícula: 2141800586

Elaborando cerveza con malta de maíz pigmentado:  
Propiedades químicas, sensoriales y expectativas del consumidor



*MARIA ANSELICA ROMERO MEDINA*  
MARIA ANSELICA ROMERO MEDINA  
ALUMNA

REVISÓ  
*Rosalía Serrano*  
MTRA. ROSALÍA SERRANO DE LA PAZ  
DIRECTORA DE SISTEMAS ESCOLARES

Con base en la Legislación de la Universidad Autónoma Metropolitana, en la Ciudad de México se presentaron a las 8:00 horas del día 26 del mes de noviembre del año 2021 POR VÍA REMOTA ELECTRÓNICA, los suscritos miembros del jurado designado por la Comisión del Posgrado:

DRA. SOCORRO JOSEFINA VILLANUEVA RODRIGUEZ  
DRA. MIRNA ESTARRON ESPINOSA  
DRA. SYLVIE CHOLLET  
DR. JOSE RAMON VERDE CALVO

Bajo la Presidencia de la primera y con carácter de Secretario el último, se reunieron a la presentación de la Disertación Pública cuya denominación aparece al margen, para la obtención del grado de:

DOCTORA EN BIOTECNOLOGIA  
DE: MARIA ANGELICA ROMERO MEDINA

y de acuerdo con el artículo 78 fracción IV del Reglamento de Estudios Superiores de la Universidad Autónoma Metropolitana, los miembros del jurado resolvieron:

Aprobar

Acto continuo, la presidenta del jurado comunicó a la interesada el resultado de la evaluación y, en caso aprobatorio, le fue tomada la protesta.

DIRECTORA DE LA DIVISIÓN DE CBS

*Sara Lucía Camargo Ricalde*  
DRA. SARA LUCIA CAMARGO RICALDE

PRESIDENTA

*Socorro Villanueva Rodríguez*  
DRA. SOCORRO JOSEFINA VILLANUEVA  
RODRIGUEZ

VOCAL

*Mirna Estarrón Espinosa*  
DRA. MIRNA ESTARRON ESPINOSA

VOCAL

*Sylvie Chollet*  
DRA. SYLVIE CHOLLET

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