Desarrollo tecnológico de un destilado de agave adicionado con arándano y manzana como mezcladores

Technological development of an agave distil-late added with blueberry and apple as mixers

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Resumen

El tequila es la bebida "representativa de México por excelencia", proveniente del Agave tequilana Weber variedad Azul. ¿Es benéfico para la salud el uso de mezclado-res naturales en una bebida? El agave es la materia prima principal utilizada en este proyecto, el objetivo primordial fue el desarrollo de un destilado de agave, adicionado con frutos de arándano y manzana para degustar la bebida sin el uso de mezcladores comunes ofreciendo al consumidor una bebida agradable al paladar. El producto obte-nido fue un destilado de agave adicionado con ambos frutos macerados. La levadura utilizada fue de panificación fresca a pH 4.7. Las condiciones de cocción fueron 15 psi, 121 °C durante 8 h en un autoclave. La molienda se realizó en un molino mecánico obteniendo un rendimiento del 30%. La fermentación se llevó a cabo en recipientes de plástico. Al término de las destilaciones se adicionó agua y los frutos macerados hasta una concentración final de 35°GL que es lo establecido por la NOM-006-SCFI-2012. El costo por litro del producto final fue de $326.00 MX, se considera este producto como una alternativa en la disminución de la cantidad de azúcares y por ende benéfico en la salud del ser humano.

Palabras clave: tequila, frutos, destilación, fermentación, bebida.

Abstract

Tequila is a “representative of Mexico par excellence” drink, coming from the Agave tequilana Weber variety Azul. ¿Is the use of natural mixers in a drink beneficial for health? Agave is the main raw material used in this project, the main objective was the development of an agave distillate, added with blueberry and apple fruits to taste the drink without the use of common mixers, offering the consumer a pleasant drink on the palate. The product obtained was an agave distillate added with both macerated fruits. The yeast used was fresh baking at pH 4.7. Cooking conditions were 15 psi, 121°C for 8 h in an autoclave. The grinding was carried out in a mechanical mill obtaining a yield of 30%. Fermentation was carried out in plastic containers. At the end of the dis-tillations, water and the macerated fruits were added to a final concentration of 35°GL, which is established by NOM-006-SCFI-2012. The cost per liter of the final product was $ 326.00 MX, this product is considered as an alternative in reducing the amount of sugars and therefore beneficial to human health.

Keywords: tequila, fruits, distillation, fermentation, drink.

1. INTRODUCTION

Tequila (Mexican product) is obtained from the fermented of sugars and the distillation of Agave juice (Agave tequilana Weber blue variety) [16]. Agave tequilana Weber is a xerophilous plant with blue-greenish, thin, and almost flat leaves; it is approximately 125 cm long and 10 cm wide and has a 2 cm dark red terminal spine [18]. To be called Tequila, this beverage must be produced in a specific area well established, when it is not produced in that territory, the beverage is not considered as Tequila.

Since 1619 there is a history of small wineries in Western Mexico, where the tradition of making agave distillates arises [7]. In particular “mezcal de tequila” quickly gained a reputation as it emerged in a period marked by capital investments and the prosper-ous growth of the local industry. Thus, at the end of the XIX century the mezcal of the area was already called by the inhabitants and visitors as “tequila” [14, 15]. Currently, tequila can be consumed mixed with high sugar beverages known as mixers, increasing the sugar consumption, which can lead to health issues as overweight and obesity. These health problems are some of the most important widely, being the fifth risk fac-tor into the death causes. It has been reported that two-thirds of the world’s population lives in places where obesity is related to other diseases that cause death, and it has been reported that 44% of mortality from diabetes is associated with obesity, 23% with ischemic heart disease, while 41% is related to different types of cancer [33].

Fruits juices have 70 – 95% of humidity, sugars, vitamins, and some enzymes, the recent interest in fruit juices consumption derives from the high content of natural vegetables antioxidants [4]. Recently, red fruits and berries are consumed due to their high content of polyphenols compounds, mainly flavonoids which show antioxidant properties, and are capable of prevent some degenerative diseases inci-dence [24].

In the present work, two fruits were used, Cranberry and Red Delicious Apple. Cran-berry is a fruit that is widely recognized for its potential health benefits, the protective effect is largely associated with the antioxidant capacity of different photo chemicals capable of preventing or slowing down the oxidative processes involved in numerous pathologies [1]. Chihuahua is the main apple (Malus domestica Borkh) producer in Mexico, with 70% of the national production, the two varieties most produced are “Golden Delicious” and “Red Delicious”, with 54 and 37% respectively. Apple quality characteristics includes sensory attributes as size, color, freshness, taste, and texture [29].

The aim of the present work was the development of a pilot plant level technology to obtain an agave distillate incorporated with blueberry and apple juice to reduce the use of mixers.

2. MATERIALS AND METHODS

2.1. Plant material

Agave tequilana Weber var. Azul Agave hearts were purchased with producers from El Arenal, Jalisco and transferred to the Research Laboratory I and to the Pilot Plant of Fruits and Vegetables both at the National School of Biological Sciences of the Na-tional Polytechnic Institute. Agave distillate was obtained from three Agave tequilana Weber var. Azul hearts with average weight of 11.66 kg.

Red Delicious apple and blueberry (Vaccinium macrocarpon).

2.2. Chemical reagents

Fresh yeast (Saccharomyces cerevisiae), distilled water, HCl concentrated, Fehling A (Cu2SO4•5H2O y MgSO4•7H2O) and Fehling B (NaOH y KNaC4O6•4H2O), NaOH 1N, blue methylene, phenolphthalein, (NH4) SO4, (NH4)2HPO4, MgSO4•7H2O.

2.3. pH and acidity determination

Both were measured using a digital pH meter (OAKTON, Acorn model, Series pH 6), according with the AOAC (1996) method [2].

2.4. Soluble solids determination

Was measured with a digital refractometer (HI 96801, Hanna Instruments) calibrated with water before each measure according with NMX-F-527-1992 [9].

2.5 Reducing sugars determination

Reducing sugars were measure following the Fehling method according with NMX-F-495-SCFI-2012 [10].

2.6 Alcoholometer method (% Alc. Vol.).

Breathalyzer method. This measurement is since the density (or specific weight) of a hydroalcoholic solution decreases inversely proportional to the amount of alcohol it contains [10].

2.7 Agave pineapple cooking

Cooking was carried out in an autoclave (POLINOX S.A.) at a pressure of 15 psi (lb/in2) and 121 °C of temperature during 8 h.

2.8 Agave pineapple grind

The agave hearts were milling in a mechanical roller mill, collecting the must obtained for its subsequent fermentation and the fiber and skin obtained was discarded [11].

2.9 Fermentation

The wort obtain after milling was adjusted to a pH between 4.5–5. Some nutrients as (NH4) SO4, (NH4)2HPO4, and MgSO4•7H2O were added to unbalance the medium. Two grams of fresh yeast were added to 1 L of wort, sterile aeration was maintained for 1 h. After this time this wort with yeast was mixed with the rest of the wort. A kinetic fer-mentation was performed taking samples every 24 h for 7 days, samples were taking by triplicate and kept refrigerated.

2.10 Distillation

A double distillation was carried out on the volume obtained in the fermentation stage. The distillation process was carried out in two stages. The first distillation is called destruction, which is done at 45 or 50% alcohol, in this first stage a part of the conden-sate (heads) was removed, and the second distillation is called rectification, which is done at 75% alcohol. According to NOM006SCF2012, the sample it should not contain less than 35% alcohol by volume [10].

2.11 Juice extraction method

Apple and blueberry were scalded before the juice extraction. The juice was obtained with a juice extractor, juice was sieved through 2 mm mesh and after through 1 mm mesh to eliminate pulp residues. Soluble solids were quantified as mentioned in section 2.4 [23].

2.12 Sensory evaluation

Three formulations of distilled from

agave added with blueberry and apple juice were prepared. Sensory evaluation test was designed to evaluate consumer acceptance to select the sample with highest ac-ceptance. The evaluated sensory characteristics were color, smell, flavor, and consist-ence. To this purpose hedonic scale of five points was used, each point in the hedonic scale had a numerical value as follow: 2 = Like very much, 1= Like moderately, 0= neither like or dislike, -1= Dislike moderately, and -2= Dislike very much. The sensory evaluation was performed with 32 untrained judges. Samples were presented to un-trained judges in aleatory order, each sample was codified with 3 aleatoric numbers [22].

2.13 Statistical analysis

All the results were expressed as the mean values of tree replicates +- the standard devi-ation. The analysis of the results among samples were analyzed with an ANOVA test, significant differences were determined at 95% confidence. Sigma Plot V.12 software to this purpose was used.

3. RESULTS Y DISCUSSION

3.1 Agave hearts cooking

At this stage it is very important to control the variables of temperature and acidity. If the temperature is lower than necessary, consequently, inulin is not degraded and therefore ethanol is not produced. At high temperatures Maillard reactions or carameli-zation can occurs, lowering the reducing sugars concentration, inducing formation of degradation compounds such as enols, furfural, and hydroxymethylfurfural, which can affect the fermentation efficiency. During cooking, in addition to hydrolyzing the inulin, some sugars are caramelized, and compounds that contribute to the characteristic tequila aroma and flavor are developed [34].

The agave process was made in triplicate, the average weight of the agave hearts be-fore cooking was 11.66 kg. Few works report the dimensions and weights of the Agave tequilana Weber; however, these characteristics allowed us to compare, corroborate or contrast differences or similarities [30]. The agave hearts cooking was carried out as it mentioned in section 2.7. Cooking is a critical step in the tequila production process because, in addition to carrying out the hydrolysis of the inulin present in the agave, a series of chemical reactions is carried out that give rise to the formation of a great va-riety of organoleptic compounds that influence in the aroma and taste of tequila [21]. Agave hearts contain an acidic juice, rich in carbohydrates, which through cooking becomes a juice with a high concentration of fermentable sugars. The residue that re-mains at the end is known as “bitter honey” and is used for farmland [19, 28].

Grinding is a critical point in the mezcal production process, due to its influence on the finished product yield [12]. Grinding is a complicated process due to grinding condi-tions and equipment must be selected according with material characteristics such as size, shape, hardness, moisture, fracture strength, compression strength, modulus of elasticity, and others [11]. The grinding of the cooked agave hearts was carried out with a mechanical roller mill. The juice obtained is called “wort”. The quantity of wort obtained was 3.5 kg, which corresponds to a yield of 30%.

3.2 Fermentation process standardization

The yeast used in a fermentation process is very relevant.

Yeast production has several products, such as yeast cells, cellular compounds, and the products of their metabolism. Yeast can carried out two chemoorganotrophic metabo-lisms: respiration and fermentation. Under aerobic conditions yeast can growth using sugar producing biomass. However, under anaerobic conditions, fermentation process occurs, the yeasts produce less biomass and high quantities of alcohol and CO2 [8].

Two different yeasts were used for fermentation standardization, lyophilized brewer yeast and fresh bakery yeast. For both yeasts, the same fermentation process conditions were used. The temperature was controlled at 25 °C during all the fermentation time. The fermentation process was standardized with a sucrose solution and after a solution of piloncillo dissolved in water. Temperature during alcoholic fermentation needs to be the one that allows and adequate fermentation process, right development of the aro-matic compounds, obtaining a product with the desire characteristics. Yeast growth optimal temperature is around 25 °C. Lower temperatures cause a slower fermentative process, due to a reduction of the viable yeast population [31]. The use of specific in-oculum of Saccharomyces makes it possible to reduce the variations due to the devel-opment of uncontrolled indigenous populations and to ensure a good development of alcoholic fermentation [27].

In addition to temperature, pH is an important factor that need to be controlled in alco-holic fermentation, because it allows the control of bacterial contamination as well as the effect on yeast growth, fermentation rate and alcohol formation. In different industries, specific flavor compounds are produced through the fermentation process. Optimal pH values for bioprocess are between the range of 4 to 5. At this pH value is possible to obtain optimal yeast development. The pH value for the growth medium of lyophilized and fresh yeasts was 4.75 and 4.87 respectively [5].

The fermentation time for both yeasts was 160 h, and the growth medium was prepared with the same nutrients at the same concentrations (ammonium sulfate (0.3 g L-1), di-basic ammonium phosphate (0.24 g L-1), magnesium sulfate heptahydrate (0.024 g L-1). In Figure 1, the Brix degrees obtained are shown as a function of time for both yeasts.

In Figure 1 is possible to observe that during the fermentation time, the fresh baking yeast initially consumes the substrate almost immediately, while the lyophilized brew-er yeast does not significantly consume the substrate, even when the process parame-ters were adjusted and controlled all the time, this may be because the freeze-dried yeast was inactive and therefore the fermentation process was not carried out correctly. Therefore, the selected yeast to carry out the fermentation process at the established conditions was the bakery yeast.

3.3 Fermentation process

The material of the containers in which the fermentation is carry out plays an im-portant role in the flavor of the mezcal. In this work were used 5 L plastic containers for the wort fermentation.

During the fermentation process, the sugars are transformed into CO2 and alcohol. This operation lasts from 2 to 7 days at a temperature of 25 to 30 °C and is concluded when the sugars have been spent.

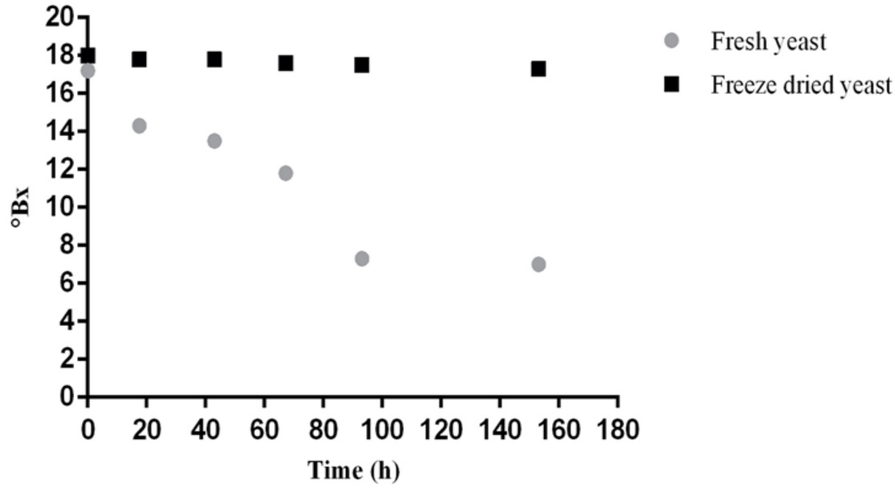

Figure 1. Behavior of soluble solids as a function of time of lyophilized brewer yeast and fresh bakery yeast.

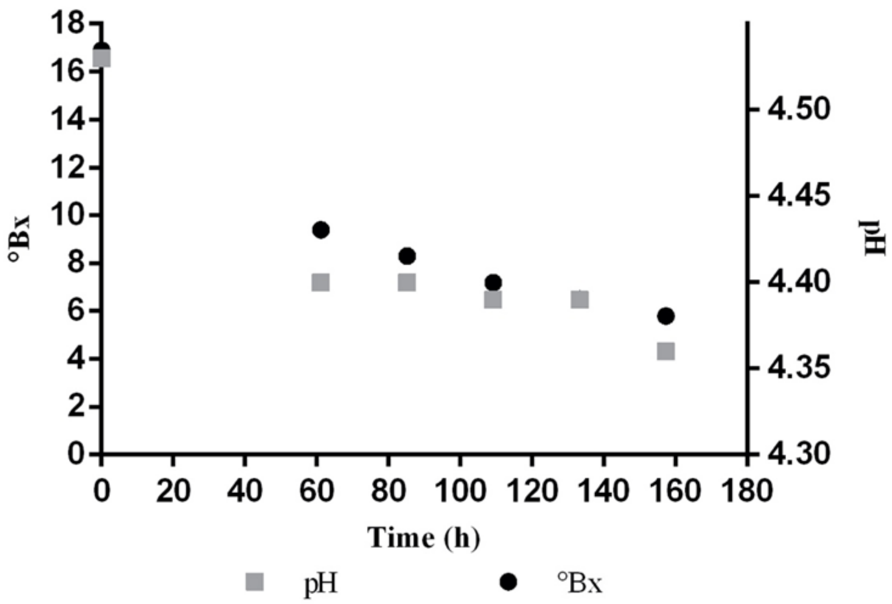

Figure 2. Soluble solids and pH behavior as a function of time

The Embden-Meyerhof pathway is the metabolic route used by the yeasts to the fer-mentation of sugars of agave syrup [5].

Figures 2 and 3 show that the sugar content in the medium (Brix degrees) and the pH decrease. The slight variation of pH prevents contamination of the medium as well as the development of undesirable metabolites in the process that affect the final characteristics of the product. The yeast consumes the substrate and ethanol is produced; after 60 h the consumption of substrate begins to be slower. As ethanol concentration increase, the cellular concentration in the medium decreases. This behavior can be explained with the Crabtree effect.

According with Peña and Arango in 2009 [26]. when Saccharomyces cerevisiae is under

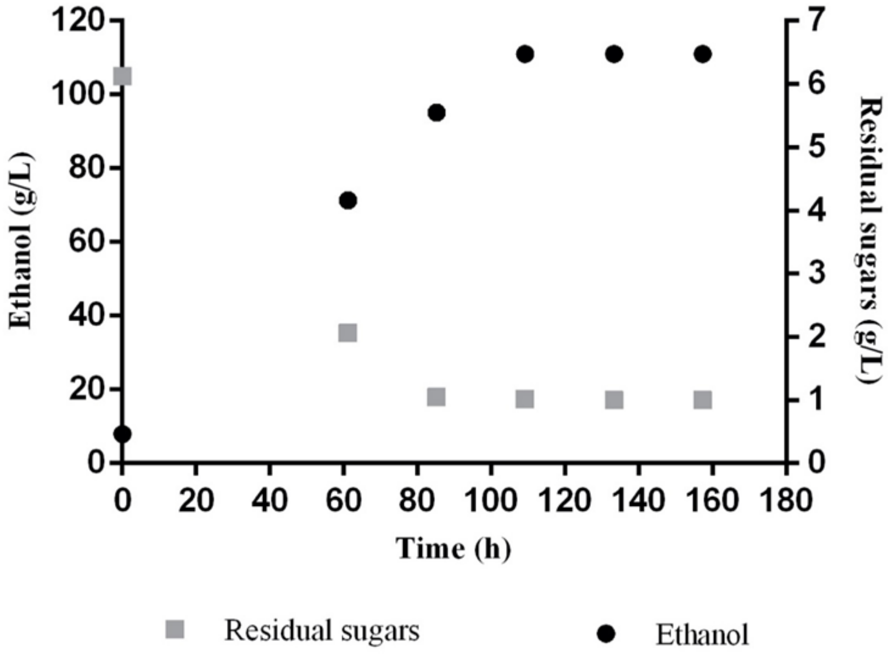

Figure 3. Residual sugar content and ethanol production as a function of time.

high dissolved oxygen concentration conditions and sugar concentration exceeds 0.16 g L-1 or 9 g L-1, the yeast converts its oxidative metabo-lism to oxide reductive or fer-mentative one, increasing the production of ethanol. At the beginning of fermenta-tion, strong alcoholic and sweet odors are perceived, which diminishes over time until they have a vinegary odor and a liquid with dark brown color. The end of fermentation is established at a point of equilibrium between the amount of produced yeast biomass, the sugar content, and the production of alcohol [19, 28]. After the fermenta-tion pro-cess, the wort was left to rest to promote the generation of important aromatic com-pounds in the product.

3.4 Distillation

Tequila composition is very com-plex, several compounds have been reported as re-sponsible for taste and aroma; these compounds in-clude alcohols, fatty acids, esters, aldehydes, terpenes, phenols, sulfur compounds, and others. Some of these compounds come directly from the aga-ve and suffer chemical transformations during the different stages of the pro-cess. However, others are developed because of cooking, fermentation disti-llation, and maturation processes [20].

Distillation is the process by which ferments are separated in two groups by heat and steam pressure. The first groups are alcoholic richness products (tequila), and the sec-ond group is called vinasses or fermentation slu-dges which includes waste products as water and yeast. Distillation is ca-rried out in copper or stainless-steel pod stills, or in continuous distilla-tion towers [20]. In the present work, distillation process was carried out in distillation apparatus. After fermen-tation, the wort is distilled twice. The first distillation is known as smashing; during this distillation, 3480 g of wort were intro-duced into the rotary eva-porator, of which a 10% cut of the so-called heads (higher alcohols) was made, followed by obtaining the ordinary distil-late (65%) and finally a cut of the 25% of vinasses (medium depleted in alcohol, dead yeasts, non-fermentable sugars, and minerals). In this distillation stage, an ordinary 14°GL distillate was obtained. The second distillation, known as rectification, was car-ried out by differential distillation to obtain a higher alcohol concentration. During this second distillation, 25% heads cut was made. Distillation was continued until reach 40-60% of alcohol volume. Then a 20% of tails cut was carried out. The final distillate contains 55°GL. After the addition of the fruit juices, the alcohol content was diluted to 35°GL, as is established by NOM-006-SCFI-2012 [10].

During the distillate agave process obtaining, the yields in each of its stages were calculated. From 3480 g of wort during the smashing distillation, 348 g of heads (10%), 2262 g of ordinary distillate (65%), and 870 g of vinasse (25%) were obtained. During the rectification stage, from 2262 g of ordinary distillate, 565.5 g of heads (25%), 1244 g of white distillate (55%), and 452.4 g of tails (20%) were obtained. According to the producers who provided the agave heart, 1 L of tequila is obtained by processing approximately 7 kg of agave. The theoretical yield from the agave heart used in this work showed that is possible to obtained 1.668 L of agave distillate. However, only 1,569 L of distillate was obtained, the difference was due to the losses during agave heart cooking and grinding. The agave distillate yield was 94.23%.

After the agave distillate was obtained, were developed formulations of agave distillate added with blueberry and apple juice. The fruit juices were added, as replacers from mixers, due to the high sucrose content of mixers. Fruit juices are perceived by the population and health professionals as healthier beverages because they come from natural sources. Also, the adding of these juices gave the agave distillate special organoleptic characteristics derived from the added fruits [17].

3.5 Formulations of agave distillate added with fruit juices

A percentage of the weight of blueberry and apple was lost during the scalding process. This loss was less in apple than in the blueberry, probably due to the higher water con-tent present in the apple. Different formulations of agave distillate added with fruit juices were developed, also was developed a formulation contained agave distillate and macerated fruits. The following formulations were proposed: Formulation 1: 20 mL of blueberry juice and 20 mL of apple juice; Formulation 2: 30 mL of blueberry juice and 20 mL of apple juice; Formulation 3: 20 g of macerated blueberry and 20 g of macer-ated apple. Each formulation was added with 100 mL of agave distillate.

3.6 Sensory evaluation

Sensory evaluation measures and quantified products or ingredients characteristics, which can be perceived by the human senses, using their organs and their integrative capacity [25]. The persons who carried out the evaluation are called judges [13]. In this study, consumer judges were used; the person who participates as a consumer must be, precisely, a consumer of the product under study. Judges must communicate to the researcher his point of view about his acceptance or rejection, the order of his preference when confronting several samples, and the level of satisfaction of the sam-ples presented to them [25].

The judges evaluated the following characteristics: color, aroma, flavor, and consisten-cy. The results obtained for each evaluated and weighted attribute for each of the three formulations are shown in Table 1

As it can see in Table 1, there is no significant difference between the attributes of Formulations 2 and 3 as aroma, flavor, and consistence. To the color attribute, the judges indicate that reddish is an unusual color of distillate products. However, 50% of the total judges liked the color of formulation 3, obtaining a significant

Attribute	Formulation 1	Formulation 2	Formulation 3
Color	28^{b}	28^{b}	36^{a}
Aroma	44^{b}	34^{a}	34^{a}
Flavor	18^{b}	22^{a}	22^{a}
Consistence	-12^{b}	24^{a}	24^{a}
Weighted	78^{c}	108^{b}	**116^{a}**
n=32, different letters in the same row indicates significant difference ($p \leq 0.05$) by Tukey's test			

Table 1. Sensory evaluation results to the different formulations.

difference with formulations 1 and 2. For formulation 3, the judges indicates that the presence of fruit solids is not an attractive characteristic. Formulation 3 of agave distil-late with macerated blueberry and apple, obtained the highest value of the weighted, being perceived as the most accepted among the judges.

3.6 Costs projection

In this analysis only variable costs were considered, which refer to: raw material and packaging material, these costs are a function of the production volume. The costs of each of the raw materials were according to the supplier. The cost to produce 1 L of agave distillate with blueberry and apple juice is $326.00 MX, it is observed that this cost is like agave distillated commercial drinks. The elimination of commonly used mixers presents a remarkable advantage from the point of view of human health.

4. CONCLUSION

A distillate of agave added with macerated blueberry and apple was obtained, as well as the biotechnological development at the Pilot Plant level. The developed product in this work is considered agave distillate, although it was developed with the raw materi-al and with parameters established by NOM-006-SCFI-2012, it is not considered Te-quila because it was not made within the territory established by the statement.

The Agave distillate developed comes from musts of vegetable origin, the water used to make this product was for human consumption to comply with regulations. The product contains 12 Bx, this value is like commercial products (mixers), however; the additional benefits is the qualities offered by cranberry and apple macerates due to their antioxidant capacity as well as the contribu-tion of vitamins, minerals, and enzymes. For this reason, the Agave distillate with notes of blueberry and apple developed presents a consumption alternative for the elimination of mixers and provides additional benefits.

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