fermentation

MDPI

Article

Development of an *Araucaria araucana* Beer-like Beverage: Process and Product

Angélica Durán [1], José Reyes-De-Corcuera [2], Gustavo Garay [1], Pedro Valencia [1] and Alejandra Urtubia [1,*]

[1] Departamento de Ingeniería Química y Ambiental, Universidad Técnica Federico Santa María, Av. España 1680, Valparaíso 2390123, Chile; angelica.duran@usm.cl (A.D.); g.garay.palma@gmail.com (G.G.); pedro.valencia@usm.cl (P.V.)

[2] Department of Food Science and Technology, University of Georgia, Athens, GA 30602, USA; jireyes@uga.edu

* Correspondence: alejandra.urtubia@usm.cl

Abstract: The seed from the *Araucaria araucana* (in Spanish, piñon) tree, native to Chile and Argentina, is sold mainly as raw seed. Engineering a process to add value to piñon has the potential to positively impact local indigenous communities with very little ecological impact because it is routinely harvested in the wild. This study evaluated the feasibility of using 100% piñon, or as a blend with barley malt, to produce a beer-like beverage, while also evaluating consumer acceptance of the beverage's piñon characteristics. Prototypes generated based on 93% piñon and 7% oat (enzymatic treatment of α-amylase, glucoamylase, protease and β-glucanase), as well as 50% piñon and 50% barley (no external enzymatic treatment), were evaluated. Overall acceptability by a consumer acceptance panel (21 consumers) rated the 100% piñon and the piñon–barley malt blend 5/9 and 7/9, respectively. The piñon–barley malt blend prototype stood out for its low level of carbohydrates, high potassium content and banana and clove aromas.

Keywords: piñon; fermentation; α-amylase; glucoamylase; beer-like beverage; valorization

check for updates

Citation: Durán, A.; Reyes-De-Corcuera, J.; Garay, G.; Valencia, P.; Urtubia, A. Development of an *Araucaria araucana* Beer-like Beverage: Process and Product. *Fermentation* **2021**, *7*, 170. https://doi.org/10.3390/fermentation7030170

Academic Editors: Ogueri Nwaiwu and Mohamed Gedi

Received: 26 July 2021
Accepted: 25 August 2021
Published: 28 August 2021

1. Introduction

Between 2015 and 2018, the global consumption of beer increased by 2.7% [1]. In Chile, the per capita consumption of beer increased from 25 L to 50 L between 2001 and 2018, and 7.2 million hectoliters were produced in 2016 [2]. That year, the craft beer industry accounted for 1% of the beer market, with an annual growth of 15–20% and around 331 microbreweries in Chile [3]. This growth suggests that there is market for new craft beer and new beer-like products. In addition, very few gluten-free beers are available for individuals with celiac disease, who represent 0.5–1.0% of the population [4]. There is also a growing number of consumers who, despite the fact that they do not suffer from celiac disease, prefer a gluten-free diet [5]. Therefore, there is an opportunity for the development of new alternatives to conventional beers. From a microeconomic perspective, the development and sustainable industrialization of products with protected denomination of origin are particularly important to small agricultural communities with unique crops and processes. The pine nut of *Araucaria araucana* K. Koch (in Spanish, piñon), also known as the monkey-puzzle tree, is a native and ancestral seed that only grows in Chile and Argentina. It is a species of high conservation value. It was declared a national monument by CITES (Convention on International Trade in Endangered Species World Flora and Fauna). The international trade of its wood and subproducts is forbidden [6]. However, the international trade of products derived from piñon is not subject to prohibition. A. Araucana occupies an area of 239,378 ha in Chile and 180,000 ha in Argentina [7]. In its best conditions, this ancient tree generates its first seeds at 15 to 25 years old [8]. The production yield of piñon is 197 kg/ha and 455 kg/ha in Chile and Argentina, respectively [9,10]. In Chile, there is no detailed information on the proportion of piñon collected with respect to total production [11]. However, some authors highlight the importance of this seed in the Mapuche Pehuenche native community, based on its economic, sociocultural and religious value [12]. The communities mainly collect piñon by hand in a sustainable way and sell it primarily as a raw seed. The locals then cook, roast or grind the seeds into flour at home. Although overharvest would threaten the sustainability of the *A. araucana* forest, much of the seed is wasted because it is not harvested. Therefore, the rational harvest and processing of piñon is amenable to the creation of a circular economy around it. Historically, piñon has been used to produce a fermented beverage known as mudai [6]. It was formerly obtained by chewing the piñon seed, which hydrolyzed part of the starch and facilitated the subsequent alcoholic fermentation of the paste mixed with water [13]. The starch, protein and lipid composition of peeled piñon seeds is very similar to that of barley, as shown in Table 1, making it suitable for alcoholic fermentation. As with beer fermentation, a wort rich in hydrolytic enzymes is needed to make the nutrients available for fermentation [14]. However, the germination of piñon to produce a malt equivalent to barley malt in beer fermentation is not practical because piñon has a long germination time and a slow germination rate, as shown in Table 2. Therefore, the degradation of piñon starch must be aided by the addition of exogenous enzymes to produce fermentable sugars.

Table 1. Comparison between barley and piñon seed [15,16].

Component	Composition of Barley Seed (Dry Base)	Composition of Peeled Piñon Seed (Dry Base)
Starch	54–65%	64%
Protein	9–14%	7.8%
Lipids	2–3%	1.1%
Moisture (Total seed)	12–16%	43%

Table 2. Germination properties and maximum moisture content of barley for malting and piñon seed [17,18].

Property	High Quality Barley for Malting	Piñon Seed
Germination rate [%]	>95	28 ± 3
Germination time [days]	3	22 ± 3
Maximum moisture content [%]	13.5	43

Several sources of sugars and/or starch have complemented or completely replaced barley in beer production. The total replacement of barley with other grains is used, for example, in the production of gluten-free beer [19]. Others have reported the use of raw sorghum and exogenous enzymes such as α-amylase, glucoamylase and protease to produce a beer-like beverage [20,21]. The piñon seed is a gluten-free source of nutrients, and thus a potential ingredient for gluten-free beer [22].

Our overall goal of this research was to develop a product for a beer-like craft beverage that benefits local communities with responsible stewardship for the sustainable use of natural resources. Several beer-like prototypes from piñon were developed, and their sensory acceptability was evaluated to determine the one with greatest consumer acceptability.

2. Materials and Methods

2.1. Storage and Crushing of Piñon

Piñon was acquired from the towns of Lonquimay and Melipeuco, Region of Araucanía, Chile. The material was selected upon visual inspection, and the seeds were cleaned with absorbent paper (to eliminate dust) and stored in low-density polyethylene bags at 4 °C (for up to 4 months) to prevent dehydration of the product, as recommended by Galleti, Lizana and Gálmez [23]. Prior to each trial, the seeds were cut using a PSV C15 bowl cutter from Talsa (Valencia, Spain) for 3 min at a blade speed of 2840 rpm.

2.2. Enzymes

Glucoamylase from *A. niger*, formulation AMG® 1100, α-amylase from *B. amyloliquefaciens*, formulation BAN® 800 MG, protease from *B. licheniformis* and *B.amyloliquefaciens*, formulation Protamex® and β-glucanase from *H. insolens*, formulation Ultraflo® L from Novozymes (Bagsvaerd, Denmark) was used. The BAN® 800 MG enzyme was used at a concentration of 0.054 g/kg piñon, the AMG® 1100 enzyme at a concentration of 1.17 g enzyme/kg piñon, the Protamex® enzyme at a concentration of 0.022 g/kg piñon and the Ultraflo® L at a concentration of 3.93 mL/kg piñon. The initial mash pH was 5.2. Enzyme concentrations were selected based on the manufacturer's recommended dosage, as well as the starch content (Glucoamylase and α-amylase), protein content (Protease) and β-glucane (β-glucanase) content of piñon.

2.3. Yeast

For the determination of mash conditions, *Saccharomyces cerevisiae* Saflager S-23, typically used for lager-type beers (Fermentis, Belgium), was used in the first study to determine the effect of applying exogenous enzymes. For the production of the prototypes for sensory analysis, *Saccharomyces cerevisiae*, Safale S-04 that is typically used for ale beers (Fermentis, Belgium), was used. The initial dosage was 6×10^6 cells/mL. The inoculum was prepared with sterile demineralized water at a ratio of 1:10 for dry yeast mass to water for 30 min at 25 °C.

2.4. Preliminary Fermentation Trials

Preliminary fermentations were carried out in 4 L reactors to verify whether the yeast was able to metabolize the mash based on piñon (with enzymatic treatment). For this, the crushed piñon was mixed with water at a ratio of 1 kg piñon/3 kg water (initial adjustment of pH to 5.2 with phosphoric acid) in a 4 L fermenter model LiFlus GS from Biotron (Gyeonggi-do, Korea) with an OS-20-S stirrer model from Dragon Lab (China), which was used with a constant agitation of 120 rpm. The crushed piñon and water were brought to 80 °C, the enzyme α-amylase BAN® 800 MG was added and the mixture was held at 80 °C for 60 min. Then, the mixture was brought to 70 °C, the enzyme glucoamylase AMG® 1100 was added and the mixture was held at 70 °C for 60 min. As a preliminary test, these two enzymes were considered because they transform starch and oligosaccharides into sugars with a lower molecular weight. The wort of the piñon was filtered from a conical stainless-steel holder with 2 mm diameter grooves covered with 2 layers of sterile gauze measuring 20 × 24 cm. The volume of the wort obtained was 2.56 L, and it was cooked by boiling for 60 min on a hot plate with a magnetic stirrer model SP131320-33 (Thermo Scientific). During the cooking process, different hops were added: 1.54 g of Saaz hops pellets, 4.0%AA (minute 2), 2.18 g of Hallertau hops pellets, 5.5%AA (minute 25) and 1.15 g of Cascade hops pellets 6.0%AA (minutes 60) in order to create an equivalent of 15 IBU (International Bitterness Units).

Then, the container with wort was cooled to 20 °C by immersion in an ice water bath. The density was adjusted to 1.045 g/mL by adding sterile water. Immediately after cooling, the wort was aerated for 30 min with an air pump connected to a 0.22 μm MCE filter. Finally, the pH was adjusted to 5.2, and rehydrated Saflager S-23 yeast was added at an approximate concentration of 10^6 cells/mL. Lager fermentation was performed at a constant temperature of 14 °C and was carried out in a 4 L multifermenter model YT from Ingenieria Ltd. (Limache, Chile) with constant stirring at 50 rpm (simple paddle stirrer from Ingenieria Ltd.). Samples were taken every 12 h during the first 3 days and every 24 h during the following 3 days. Reducing sugars, density, nitrogen assimilated by yeast and pH were all quantified. The experiments were performed in triplicate.

2.5. Development of Piñon Prototype Beverages

2.5.1. Mashing

Piñon beer-like prototypes were developed in 22 ± 2-L batches in a 65 L stainless steel tank. A 1:3 piñon-to-water ratio was used for mashing (13 kg piñon). Water was standardized at 67 ppm of Ca^{+2}, 29 ppm of Mg^{+2}, 62 ppm of SO_4^-, 94 ppm of Na^+, 35 ppm of Cl^- and 79 ppm of HCO_3^- using sodium bicarbonate from Loba Chemie (India), calcium chloride from Merk (Germany), magnesium sulphate from Merk (Germany), sodium chloride from Loba Chemie (India) and magnesium chloride from CDH (India). Initially, the piñon–water mixture was kept at 80 °C for 30 min. Then, the temperature was lowered to 60 °C using a copper coil immersed in the tank, with a cooling rate of 10,376 J/s. 0.054 g/kg piñon of α-amylase and 1.17 g/kg piñon of glucoamylase, as well as 0.022 g/kg piñon of protease and 3.93 mL/kg piñon of β-glucanase, were added.

The mash was recirculated using a centrifugal pump model MP-20R (Xinxishan, China) with a flow rate of 32 lpm for 5 min, and then filtered using a perforated plate with 2 mm round holes placed at the bottom of the tank (false bottom), and then transferred to another 65 L tank. Simultaneously, water at 70 °C was added at a 1:1 piñon-to-water ratio (13 kg piñon). The resulting wort was boiled for 60 min using a Vulcan 21A commercial range (China), with a heating rate of 2008 J/s, and 38 g of hops (equivalent to 15 IBU) was added. Finally, the wort (22 ± 2 L) was cooled to 20 °C using the copper coil, with a cooling rate of 10,376 J/s. The fermentation was carried out in 30 L polypropylene fermenters in a room kept at 20–22 °C for 7 days using Safale S-04 yeast. The fermented product was divided to produce prototypes 1.1 and 1.2, which were brought to maturation stage and were stored at 5 °C for 7 days in a Nordik 480 plus refrigerator from Mademsa (Chile). In order to verify whether there was an additional contribution of aromas or flavor, shelled piñon (previously boiled for 30 min in water) was added to prototype 1.2 at a concentration of 2% *w/v* during the last 3 days of maturation. Finally, both prototypes were carbonated by adding dextrose at 8 g/L and keeping the batches at 20–22 °C for 2 weeks in 330-mL glass bottles, and then they were stored at 5 °C.

2.5.2. Expert Sensory Analysis

Prototypes 1.1 and 1.2, were tasted by a beer tasting judge certified by the Beer Judge Certification Program (BJCP), who characterized the aroma, taste, mouthfeel, appearance and overall quality of the beverages.

2.5.3. Prototype Development for Overall Acceptance Tests

Based on the expert feedback on prototypes 1.1 and 1.2, six new prototypes were developed. Starch source, type of mashing (exogenous enzymes vs. malted barley), dry-hopping (last 3 days of maturation, East Kent Golding in pellets (EKG)) and carbonation method (sugar or honey) were adjusted, as shown in Table 3. In general, the mashing, sparging and fermentation processes were performed as described in Section 2.5.1, with the following changes: no dry-hopping was conducted for prototypes 2.1, 2.2, 3.1 and 3.2, and mashing temperature was reduced to 67 °C (prototypes 3.1, 3.2 and 3.3), according to the temperature used for the mashing of barley malt. Swaen©Ale malt was used in prototypes 3.1, 3.2 and 3.3 as an alternative to exogenous enzymes, and Na^+ concentration was reduced to 74 ppm by reducing the amount of NaCl added to the mixture.

Table 3. Prototypes for general acceptability test.

Prototype	Starch Source	External Enzymatic Treatment	Dry-Hopping	Carbonation Technique
2.1	93% piñon, 7% oat	Yes	No	8 g/L dextrose
2.2	93% piñon, 7% oat	Yes	No	8.8 g/L honey
2.3	93% piñon, 7% oat	Yes	1 gEKG/L beer	8 g/L dextrose
3.1	50% piñon, 50% malt	No	No	8 g/L dextrose
3.2	50% piñon, 50% malt	No	No	8.8 g/L honey
3.3	50% piñon, 50% malt	No	1 gEKG/L beer	8 g/L dextrose

2.5.4. Overall Acceptance Test and Sensory Characterization of the Best Evaluated Beverages

An overall acceptance test was performed by a panel of 21 beer consumers in an age range from 20 to 45 years old. Aroma, appearance, flavor, mouthfeel and overall impression were the attributes chosen to describe the 6 prototypes. Each attribute was evaluated using a hedonic scale from 1 to 9 with "Dislike extremely" and "Like extremely" corresponding to the lowest and highest scores, respectively. Each consumer was given a rating chart along with six samples, a glass of water and bread. The samples, at a volume of approximately 20 mL and a temperature of 10 °C, were served at random in disposable 30 mL cups. Each sample served was identified with random three-figure numbers. To avoid influence among the samples, the consumers were asked to drink water and eat flour bread between each tasting. The test was performed in groups of 4 to 6 consumers, who were each separated both to the front and to the sides with white wall panels, and the base of the table on which the samples were served was also white. Finally, the best prototype evaluated was tested by the beer tasting judge.

2.6. Analytical Methods

The concentration of reducing sugars was determined by the "method of 3.5-dinitrosalicylic acid" [24]. Starch concentration was determined with the iodine–iodide method, where 0.25 mL of the sample was mixed with 2 mL of demineralized water and 0.25 mL of an iodine solution (0.01 N iodine and 0.02 N iodide) and homogenized, and absorbance was measured at a wavelength of 640 nm and compared to that of a water blank. Starch concentration was determined by interpolation with a standard curve of soluble starch as a function of absorbance. Yeast assimilable nitrogen was determined by the formaldehyde index method [25].

Probable alcoholic strength was calculated as:

$$\text{Probable alcoholic strength} = \frac{(G_{fi} - G_{in}) \times 2 \times PM_{eth}}{\rho_{eth} \times 10} \quad (1)$$

where: G_{in}: Initial molar glucose concentration [mol/L]; G_{fi}: Final molar glucose concentration [mol/L]; PM_{eth}: Ethanol molar mass [g/mol]; ρ_{eth}: Ethanol density [g/mL].

2.7. Statistical Analysis

For the overall acceptability tests, Tukey's honestly significant difference test was used.

3. Results and Discussion

3.1. Preliminary Fermentation Studies

Preliminary fermentation studies were performed in 4 L reactors for the purpose of verifying the feasibility of the yeast consuming the sugars generated with enzymatic treatment in the mash. They also studied the evolution of fermentable sugars and starch during the mash time. Figure 1 presents the mashing and fermentation results.

Between 40 min and 90 min, the solubilized starch stabilized at a value of approximately 80 g/L; therefore, we decided to start mashing at a temperature of 80 °C for 30 min in subsequent experiments. As the concentration of starch remained constant and not all the present starch was transformed before the glucoamylase enzyme was added, it was inferred that this could have been product inhibition produced by the α-amylase enzyme. This phenomenon is consistent with the literature [26], where it has been reported that the enzyme Novamyl® (α-amylase from *B. Subtilis*), which transforms starch into oligosaccharides and maltose, presents competitive product inhibition by maltose, maltotriose, oligosaccharides of up to 7 glucose units, α- and γ-cyclodextrins.

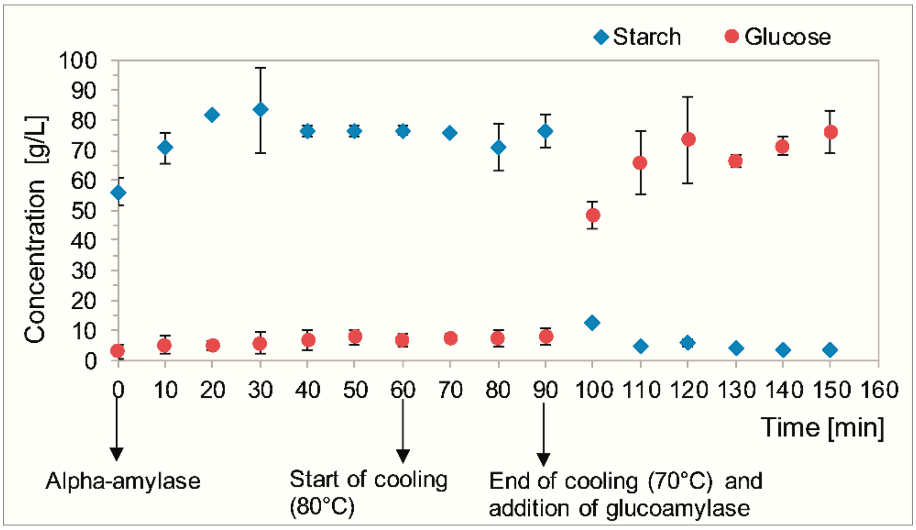

Figure 1. Evolution of starch and reducing sugar concentrations in mashing time *n* = 2.

It was noted that, during the cooling period (60 to 90 min) from 80 °C to 70 °C, there was no crystallization of starch or sugars (constant concentrations). After glucoamylase was added and until 110 min, the concentration of starch decreased, which reinforced the suggestion that the glucoamylase enzyme decreased the effect of the product inhibition (oligosaccharides) of α-amylase by transforming it into glucose, which was reflected in the increase in reducing sugars.

Based on these assays, we decided to start mashing at a temperature of 80 °C (30 min) in future experiments, and then add the enzymes together (because when added separately, product inhibition occurs). Moreover, to better mimic conventional mashing, β-glucanase and protease were added at an intermediate working temperature of the four enzymes of 60 °C.

Figure 2 shows fermentation evolution as a function of the changes in the concentration of reducing sugars and yeast assimilable nitrogen, and as a function of the changes in pH and density. All these variables decreased initially and remained constant after 96 h, indicating the successful fermentation of sugars produced by the addition of exogenous α-amylase and glucoamylase, and the fermentation was complete within 96 h.

The kinetics of the fermentation of piñon are consistent with the fermentation of lager barley beer [27]. Glucose is the first sugar consumed by yeast, and when this resource runs out, maltose and maltotriose are used as energy sources [28]. During the exponential phase (24–84 h), the rate of glucose consumption was 1.2 g/L h, and it was 1.7 g/L h for assimilable nitrogen. The fermentation had a lag phase of 24 h, and this duration is within the range reported in the literature for barley beer (12–24 h) [27].

The wort's initial concentration of reducing sugars was 100.2 ± 5.1 g/L (Table 4), which corresponds to the upper range of concentrations (75.0–95.6 g/L) typically found in barley wort [29], as well as within the upper range of concentrations (61.1–101.7 g/L) found in sorghum wort [20]. By the end of the fermentation, 96% of the reducing sugars were consumed, indicating the successful completion of the fermentation. This high reducing sugar concentration results in a probable alcohol content of 6.1% *v/v* in pinon beer, which is greater than in American Lager beers (4.2–5.3%) [30].

The initial wort density was slightly higher than that of sorghum wort produced by exogenous enzymatic treatments [20], but lower than barley wort used for American Lager beers [30].

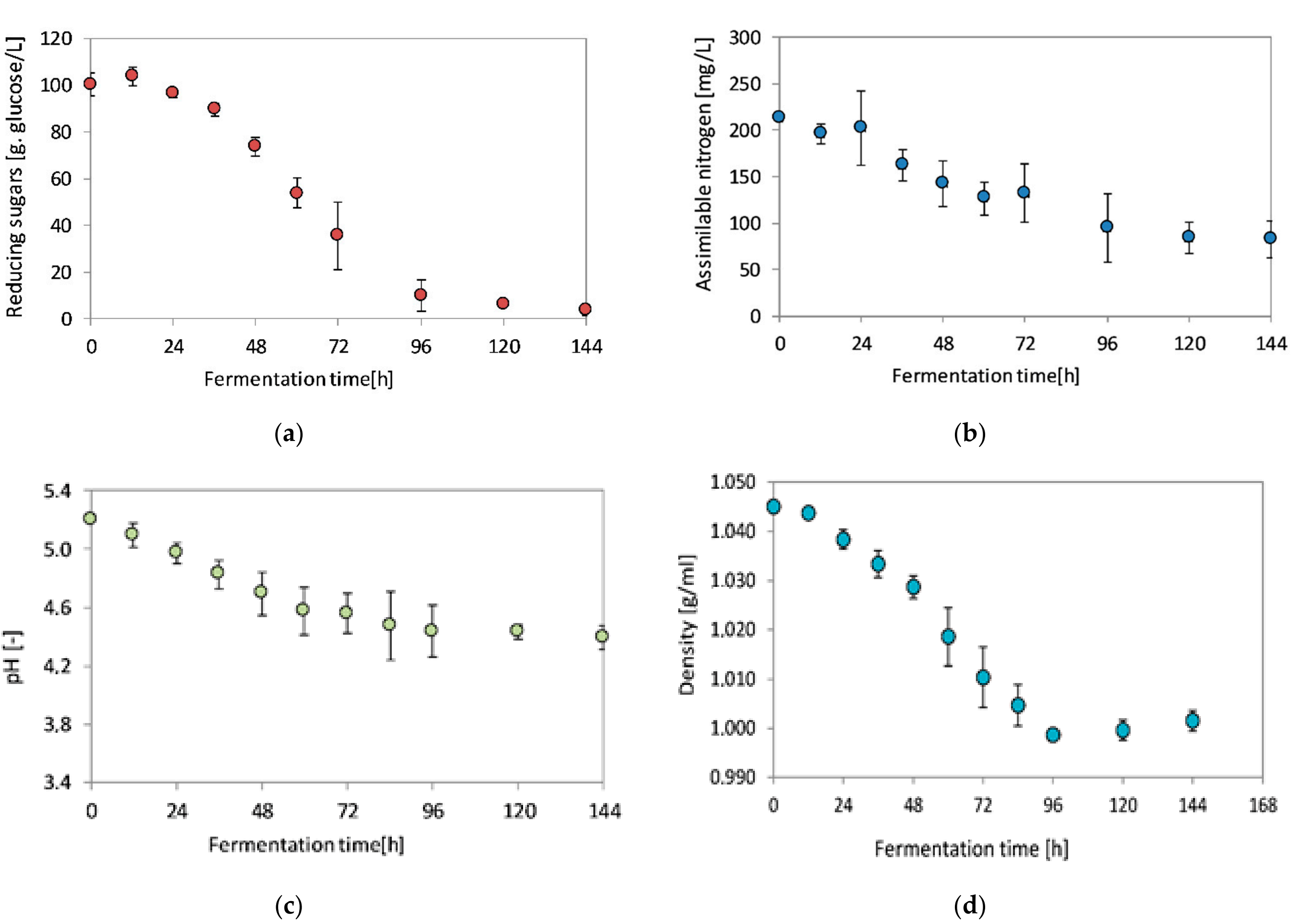

Figure 2. Evolution of: (**a**) reducing sugars, (**b**) yeast assimilable nitrogen, (**c**) pH and (**d**) density during the fermentation of piñon wort $n = 3$.

Table 4. Average results (with standard deviation) of different parameters before and after fermentation.

Parameter	Before Fermentation	After Fermentation
Reducing sugar [g/L]	100.2 ± 5.1	3.7 ± 2.2
Density [g/mL]	1.045 ± 0.000	1.002 ± 0.002
Assimilable nitrogen [mg/L]	213.2 ± 3.4	83.0 ± 20.2
pH [-]	5.2 ± 0.0	4.4 ± 0.1
Probable alcoholic strenght	0	6.1 ± 0.3

During the fermentation process, the pH decreased from 5.2 to 4.4. As in the other parameters, pH remained constant after 96 h. The decrease in pH (in conjunction with the increase in yeast cells) reflects the production of buffer substances, such as free amino nitrogen (FAN), and some organic acids, such as acetic acid, lactic acid, pyruvic acid, succinic acid, caproic acid and caprylic acid [31].

It was concluded that the yeast did not experience any catastrophic nutritional deficiencies during the fermentation process.

3.2. Development of Piñon Prototypes

3.2.1. Sensory Analysis of Prototypes 1.1 and 1.2

The sensory analysis of preliminary prototypes can be seen in Table 5.

The main difference between the two prototypes (1.1 and 1.2) is due to the addition of piñon during maturation, which contributes aromas of coconut and aniseed. The piñon added during maturation contributed a bit of acidity and a slight diacetyl taste, so it was decided not to make this addition in future experiments, as it does not significantly change the general impression and adds acidity, which is not desirable at this time.

Table 5. Expert sensory analysis of piñon prototype 1.1 and 1.2.

Attribute	Prototype 1.1	Prototype 1.2
Aroma	Prominent clove and banana, there is a slight caramel, dried peach and a subtle aroma of honey and nuts.	Prominent banana and clove, there is a character of vanilla, coconut, slight caramel, dried peach and a subtle aroma of honey, nuts and aniseed.
Appearance	Orange amber, it has opacity and a low retention white foam.	Orange amber, it has opacity and a low retention white foam.
Taste	In the mouth, it is saline, with subtle notes of walnut and nut shell. It has a fruity flavor, like dried peach and cooked apricots.	It is saline and has a slight final sourness, with subtle notes of walnut and nut shell. It has a fruity flavor like dried peach and cooked apricots. It also has a slight diacetyl character.
Mouthfeel	Light body, low carbonation, dry and fresh finish.	Light body, low carbonation, dry and fresh finish.
Overall impression	It needs small adjustments, such as regulating fermentation temperature, if one wants to lower the aroma of banana and dried peach. It is also important to regulate mineral salts in order to lower the saline character presented. Increase foam retention, although this is more complicated, as it does not have grains.	It needs small adjustments, such as regulating fermentation temperature, if you want to lower the aroma of banana and dried peach. It is also important to regulate mineral salts in order to lower the saline character presented. Increase foam retention, although this is more complicated as it does not have grains.

At the aromatic level, the descriptors were found have an attractive potential. The predominant aroma descriptors of the preliminary prototypes (clove and banana) are desirable in Weissbier, Dunkles Weissbier, Weizenbock, Dubbel Belga and Triple Belga type beers [30] that are currently on the market. The notes of honey and caramel tend to be found in beers using caramel or crystal malts and are desirable in pale ale or wheat wine [30]. This suggests the potential value of an existing niche that prefers these aromas.

In contrast to other gluten-free beers, such as those produced using raw teff, malted amaranth, oats, malted corn or malted buckwheat [32], prototypes 1.1 and 1.2 had a low foam retention.

The foam retention of the preliminary prototypes was low and, therefore, in terms of appearance, the prototypes would potentially not be acceptable. Foam is an important quality parameter that affects consumers' decisions when buying craft beer. Consumer preferences regarding foam level vary depending on country and region, from a moderately low foam (Scottish) to a high foam level beer (Belgian) [33].

Among the Beer Judge Certification Program styles [30], there are no blends that combine fruity flavors with salty notes, which could be a disadvantage for consumers not used to this trend. However, in last year, two contemporary styles have stood out: the Leipzig-style gose and contemporary-style gose [34], which indicates that salt may be added to beer in small quantities. Based on this, an early trend towards the use of new and different flavors in beer is observed. Nevertheless, based on the description of traditional styles, it was decided to reduce the concentration of salt in the future prototypes in order to lower saline intensity. Moreover, [35] describe that consumers opt for craft beer because of the variety of flavors, such as malted barley, chestnut and honey; therefore, the notes of walnut and nut shell could potentially be attractive for consumers.

For consumers, color is an indicator of flavor, and [36] indicate that an orange color is positively associated with citrusy, fruity and sweet flavors. The fruity notes of the prototype could meet consumers' prior expectations based on its orange color.

3.2.2. Overall Acceptance of Six Prototypes

Based on the expert evaluation of prototypes 1.1 and 1.2, six new prototypes were developed.

Panel members were asked to indicate their personal level of satisfaction (like/dislike), with respect to the attributes of aroma, appearance, flavor, mouthfeel and overall impression, on a scale from 1 to 9. A summary of all acceptability parameters assessed by the consumer panel is presented in Table 6.

Table 6. General acceptability of prototypes 2.1, 2.2, 2.3, 3.1, 3.2 and 3.3. Note: values with the same letter are statistically equal ($t < 0.05$).

Attribute	Prototype 2.1	Prototype 2.2	Prototype 2.3	Prototype 3.1	Prototype 3.2	Prototype 3.3
Aroma	5.0 ± 1.7 b	5.3 ± 1.4 bc	5.0 ± 1.5 b	6.4 ± 1.2 ac	6.4 ± 1.0 ac	6.6 ± 1.4 a
Appearance	4.6 ± 1.4 b	4.6 ± 1.3 b	4.9 ± 1.5 b	7.4 ± 1.0 a	6.3 ± 1.5 a	6.9 ± 1.2 a
Taste	4.1 ± 1.9 b	4.2 ± 1.7 b	4.3 ± 1.9 b	7.1 ± 1.1 a	6.1 ± 2.0 a	7.0 ± 1.2 a
Mouthfeel	4.8 ± 1.8 b	4.5 ± 1.6 b	4.7 ± 1.7 b	7.6 ± 1.4 a	6.2 ± 2.0 a	7.0 ± 1.3 a
Overall impression	5.0 ± 1.5 b	5.2 ± 1.4 b	4.9 ± 1.0 b	7.0 ± 1.4 a	6.3 ± 1.5 a	6.6 ± 1.4 a

In an effort to develop an acceptable gluten-free beer-like beverage, oat was added to the piñon base at a final concentration of 7% because [37] reported that oat improves foam formation and stability. However, the oat–piñon prototypes scored lower in visual appearance than the 1:1 barley–piñon prototypes, which can be attributed to the lower foam retention of the oat–piñon prototypes. This is consistent with other studies that report that a moderate level of foam stability is preferred to low stability [38].

Prototypes 3.1, 3.2 and 3.3 (prepared with 50% malt and 50% piñon, with no enzymatic treatment) were the best evaluated in terms of appearance, flavor, mouthfeel and general impression, with no significant differences when using dextrose carbonation, honey or adding dry-hopping to the maturation process. The average scores for the piñon-malt beer range from "6: Like slightly" to "7: Like moderately", which is a good starting point to scale the prototype to the commercial level; however, it is suggested that work could be conducted to improve the average to "8: Like very much" or "9: Like extremely."

Prototypes 2.1, 2.2 and 2.3 (made with 93% piñon and external enzymatic treatment) showed no differences in terms of the evaluation of their appearance, flavor, mouthfeel or general impression. The average ratings are between "4: Dislike slightly" and "5: Neither like nor dislike"; therefore, these would not be potential candidates to consider as "beer," as they would not be attractive to consumers (in comparison to the prototype that uses piñon as an additive to barley).

There is a clear difference in consumer trends showing a preference towards the piñon–malt prototypes (3.1, 3.2 and 3.3). Flavor is perhaps the most important quality characteristic of beer [39]. Some of the ingredients that influence it come from the raw materials used (barley, hop, yeast metabolism) [28]. It is inferred that the different origins of the raw materials influence the flavor and, therefore, consumer acceptance.

As prototypes 2.1, 2.2 and 2.3 had a score in flavor between 4 (slightly dislike) and 5 (neither like nor dislike), it was suggested that piñon could be used as an ingredient with malt and not as the primary grain. Thus, a fermented beverage based on 93% piñon and 7% oat would not be a potential product of preference for the craft beer consumer niche, but it could be redesigned as a "fermented beverage" and marketed to another consumer segment.

For the purpose of the following analyses, the prototype used was the one with the highest average score in terms of general impression, which was prototype 3.1.

3.2.3. Sensory Analysis of the Best Evaluated Prototype

The expert sensory analysis of the best prototype from the consumer panel is summarized in Table 7.

The best evaluated prototype shows primary notes of banana, pepper and dried peach, which are attractive for consumers, since these are notes found in different commercial styles. According to [40], giving more importance to the aroma increases the probability of craft beer consumption by 3.2%. The banana and clove notes are of special interest, as these are only found naturally in wheat styles and not those prepared with US-04 yeast.

Table 7. Sensory analysis of the best evaluated prototype.

Attribute	Prototype 3.1
Aroma	Banana, pepper, dried peach, a slight note of cloves and green apple is perceived.
Appearance	Light amber color, cloudy, white foam with good retention.
Flavor	Slightly saline flavor, balanced with its slight acidity. Hops are perceived medium. It also has notes such as dried peach and green apple. Slightly sweet finish.
Mouthfeel	Medium body, medium-high carbonation, slightly astringent.
Overall impression	Slightly saline with a sweet finish. Check the present quantity of piñon content. Check the vitality of the fermentation, to reduce the taste of green apple.

As it has good foam retention, the prototype would be potentially attractive since, as mentioned above, consumers prefer beers with moderately low to high foam levels [33], which was confirmed by the acceptance ratings given by consumers in Section 3.2.2.

The attributes prioritized by beer consumers depend on geographic location. For example, it has been found that consumers in Korea find that a beer's CO2 level is more important than its bitterness, duration of aftertaste, foam volume or density. Moreover, [41,42] mention that beers with a low level of carbonation are related to flat and dull beers, while highly carbonated beers generate a refreshing and lively effect; thus, the medium to high carbonation of the prototype could be attractive for consumers, which confirms the results of Section 3.2.2.

There is a balance between slightly salted notes, acidity, hops and a sweet finish. This is confirmed by consumer impressions with a rating of "7: Like moderately". The perceived saltiness can be attributed to the added NaCl and perhaps to the Na+ present in piñon. Thus, to further improve the quality of this beer-like beverage, a lower concentration of Na+ in water is required.

3.2.4. Analysis of Best Prototype

A characterization was performed of the prototype best evaluated by consumers (prototype 3.1). Table 8 presents the results:

Table 8. Proximal analysis, ethanol content, calcium, potassium concentration and parameters before and after fermentation of prototype 3.1.

Attribute	Result
Moisture	94.1 g/100 mL
Protein	0.3 g/100 mL
Total fat	0.2 g/100 mL
Total dietary fiber	0.9 g/100 mL
Available carbohydrates	0.7 g/100 mL
Energy	39 kcal/100 mL
Alcohol content	5.9% vol/vol
Calcium	4.5 mg/100 mL
Potassium	131 mg/100 mL
Before fermentation	**Result**
Density	1.054 g/mL
pH	5.5 [-]
After fermentation	**Result**
Density	1.005 g/mL
pH	4.3 [-]

Prototype 3.1 had a similar fermentation behavior to the preliminary fermentation trials, where the final density and pH were 1.005 and 4.3, respectively. These findings allow us to conclude that the yeast did not experience a catastrophic nutritional deficit during this stage.

The caloric content of the barley–piñon beer prototype is within the calorie range of traditional beers, which varies between 30 and 40 kcal/100 mL [43].

The piñon–malt beer has a low carbohydrate content, since a 330 mL portion contains four times fewer carbohydrates than traditional beer and two times fewer carbohydrates than light beer [44].

The potassium content of a 330 mL piñon-malt beer would cover 12% of the recommended daily dose to reduce blood pressure and the risk of cardiovascular disease [45]. Moreover, it has five times more potassium than traditional beer [44].

The dietary fiber in the beer would cover 7–10% of the recommended value for adults [46] and it also has a smaller fiber content than traditional beer [44].

Prototype 3.1 (piñon–malt) has the same protein content as traditional beer and 0.2 g/100 mL of extra total fat. These quantities are not significant with respect to daily recommended values. It is observed that the calcium content is 16% lower than traditional beer [44]; although, in both cases, calcium content is immaterial at only 1% of the recommended daily value [47].

Prototype 3.1 is differentiated positively due to its low carbohydrate level and high potassium content, while its banana and clove aromas are attractive and found only in wheat beers and not those produced with S-04 yeast. This prototype is a possible candidate to be sold on the market thanks to its attractive properties.

4. Conclusions

Piñon from the *Araucaria araucana* tree can be fermented to produce an alcoholic beverage. The alcoholic beverage produced from piñon alone has modest potential for consumer acceptability. However, it is recommended that such a drink be named a "fermented beverage" rather than a "beer-like fermented beverage" because it has a neutral rather than outstanding impression on beer consumers.

When used as an additive to barley malt, piñon offers attractive features that differentiate it from a barley beer (clove and banana aromas, low carbohydrate content and high potassium). The greater acceptance observed in the piñon–malt blend indicates that the utilization of piñon as an ingredient added to barley malt has greater potential for success in the market.

The acceptability of prototypes 3.1, 3.2 and 3.3 could continue to be improved by, for example, reducing their salinity or adding malts to complement their flavor and aroma.

Author Contributions: Conceptualization, A.D., J.R.-D.-C., A.U.; methodology, A.D., P.V., G.G., A.U.; validation, A.D.; formal analysis, A.D., P.V., A.U., J.R.-D.-C.; investigation, A.D., G.G.; resources, A.U.; data curation, A.D., A.U.; writing—original draft preparation, A.D., G.G., A.U.; writing—review and editing, A.D., J.R.-D.-C., A.U.; visualization, A.D., A.U.; supervision, A.U.; project administration, A.D. and A.U.; funding acquisition, A.D. and A.U. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by CONICYT, grant number VIU16E0124 and the Incentive Program for Scientific Initiation (PIIC Scholarship) of Universidad Técnica Federico Santa María.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data and intellectual property belong to Universidad Técnica Federico Santa María.

Acknowledgments: Authors give thanks to Blumos S.A, Chile and ANID PIA/APOYO AFB180002.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Beer, K. Global Beer Consumption by Country in 2018. 2019. Available online: https://www.kirinholdings.co.jp/english/news/2019/1224_01.html (accessed on 21 February 2021).
2. ACECHI Minuta Estudio Sobre la Contribución de la Industria de la Cerveza a la Economía en Chile, Realizado por Acechi (2018) 2019. Available online: https://acechi.cl/nuestra-industria/ (accessed on 21 December 2020).
3. Olivos, P. Cuantificación de los Principales Actores del Sector Privado del Turismo en Cerveza Artesanal en Chile y Estudio de sus Canales de Difusión, Memoria para optar al título de Ingeniero Comercial, Universidad Técnica Federico Santa María, Chile, 2018. Available online: https://repositorio.usm.cl/handle/11673/49097 (accessed on 21 December 2020).
4. Espino, A.; Castillo, C.; Guiraldes, E.; Santibáñez, H.; Miquel, J.F. Encuesta nacional online aplicada en pacientes con enfermedad celíaca en Chile. *Rev. Med. Chil.* **2011**, *139*, 841–847. [CrossRef]
5. Estévez, V.; Araya, M. La dieta sin gluten y los alimentos libres de gluten. *Rev. Chil. Nutr.* **2016**, *43*, 428–433. [CrossRef]
6. Herrmann, T.M. Indigenous knowledge and management of *Araucaria araucana forest* in the Chilean Andes: Implications for native forest conservation. *Biodivers. Conserv.* **2006**, *15*, 647–662. [CrossRef]
7. Gutierrez, R. Bosques de araucaria, peligros para una especie milenaria. *Ambiente* **2016**, *4*, 31–41.
8. Taha, E.; Casanova, G.; Navarro, R. *Resultados y Lecciones en Producción, Técnicas de Postcosecha y Desarrollo de Productos a Partir del Piñón*; FIA: Santiago, Chile, 2010; ISBN 978-956-328-093-7.
9. Lizana, L. *Araucaria araucana (mol.) k.koch: Un Recurso Promisorio*; Facultad de ciencias agronómicas Universidad de Chile: Santiago, Chile, 2009; ISBN 978-956-19-0667-9.
10. Sanguinetti, J. Producción de semillas de *Araucaria araucana* (Molina) K. Koch durante 15 años en diferentes poblaciones del Parque Nacional Lanín (Neuquén-Argentina). *Austral Ecol.* **2014**, *24*, 265–275. [CrossRef]
11. Donoso, S.R.; Zhou, J.; Galdames, E.; Pacheco, C. Producción, permanencia y germinación de semillas de *Araucaria araucana* (Mol.) K. Koch en bosques naturales, aprovechados por comunidades indígenas del sur de Chile. *Interciencia* **2014**, *39*, 338–343.
12. Herrmann, T.M. Knowledge, values, uses and management of the *Araucaria araucana* forest by the indigenous Mapuche Pewenche people: A basis for collaborative natural resource management in southern Chile. *Nat. Resour. Forum* **2005**, *29*, 120–134. [CrossRef]
13. García, J. Tradición tecnológica y variaciones técnicas en la producción cerámica mapuche. *Complutum* **2009**, *20*, 153–171.
14. Kunze, W. *Germinación de la Cebada*, 1st ed.; VLB Berlin: Berlin, Alemania, 2006; ISBN 3-921 690-54-4.
15. Bamforth, C.W. *Brewing New Technologies*; e-book; CRC Press LLC: Boca Raton, FL, USA, 2006; ISBN 978-1-84569-173-8.
16. Leite, K.; Henrique, T.; De Oliveira, G.; Moraes, I.; Sinigaglia, R.; Rita, M.; Rodrigues, K.L.; Araújo, T.H.; Schneedorf, J.M.; de Ferreira, C.S.; et al. A novel beer fermented by kefir enhances anti-inflammatory and anti-ulcerogenic activities found isolated in its constituents. *J. Funct. Foods* **2016**, *21*, 58–69. [CrossRef]
17. Brewing and Malting Barley Research Institute Quality factors in malting barley. *Brew. Malt. Barley Res. Inst. Publ.* **2016**, *1*, 1–2.
18. Duplancic, M.A.; Martínez, E.; Cavagnaro, B.; Herrera, M.; Navas, A.L. Factores que inciden en la germinación de *Araucaria araucana* (Araucariaceae) del bosque xérico. *Rev. la Fac. Ciencias Agrar.* **2015**, *47*, 71–82.
19. Hager, A.-S.; Taylor, J.P.; Waters, D.M.; Arendt, E.K. Gluten free beer—A review. *Trends Food Sci. Technol.* **2014**, *36*, 44–54. [CrossRef]
20. Bajomo, M.; Young, T. The properties, composition and fermentabilities of worts made from 100% raw sorghum and commercial enzymes. *J. Inst. Brew.* **1993**, *99*, 153–158. [CrossRef]
21. Bajomo, M.; Young, T. Fermentation of worts made from 100% raw sorghum and enzymes. *J. Inst. Brew.* **1994**, *100*, 79–84. [CrossRef]
22. Araya, M.; Parada, A. Poniendo al día la enfermedad celíaca. *Rev. Médica Clínica Las Condes* **2011**, *22*, 204–210. [CrossRef]
23. Galletti, L.; Lizana, A.; Gálmez, C. Conservación de piñones. *Simiente* **2004**, *74*, 66–67.
24. Miller, G.L. Determination of reducing sugar by DNS method. *Anal. Chem.* **1959**, *31*, 426–428. [CrossRef]
25. Pszczółkowski, P.; Ceppi de Lecco, C. *Manual de Vinificación, Guía Práctica para la Elaboración de Vinos*; Ediciones Universidad Católica de Chile: Santiago, Chile, 2011; ISBN 978-956-14-1214-9.
26. Christophersen, C.; Otzen, D.E.; Norman, B.E.; Christensen, S.; Schäfer, T.; Denmark, B. Enzymatic Characterisation of Novamyl, a Thermostable a-Amylase. *Starch/Stärke* **1998**, *1*, 39–45. [CrossRef]
27. Priest, F.G.; Stewart, G.G. *Handbook of Brewing*, 2nd ed.; Taylor & Francis Group: Boca Raton, FL, USA, 2006.
28. Olaniran, A.O.; Hiralal, L.; Mokoena, M.P.; Pillay, B. Flavour-active volatile compounds in beer: Production, regulation and control. *J. Inst. Brew.* **2017**, *123*, 13–23. [CrossRef]
29. Boulton, C.; Quain, D. *Brewing Yeast and Fermentation*; Blackwell Science Ltd.: Oxford, UK, 2001; ISBN 0632054751.
30. BJCP 2015 Beer Style Guidelines. Available online: https://www.bjcp.org/docs/2015_Guidelines_Beer.pdf (accessed on 21 December 2020).
31. Rodriguez, H. Determinación de Parámetros Físico-Químicos para la Caracterización de Cerveza Tipo Lager, Elaborada por la Compañía Cervecera Kunstmann, S.A, Tesis Presentada como Parte de los Requisitos para Optar al Grado de Licenciado en Ingeniería en Alimentos, Universidad Austral de Chile, Chile, 2003. Available online: http://cybertesis.uach.cl/tesis/uach/2003/far696d/sources/far696d.pdf (accessed on 21 December 2020).
32. Cela, N.; Condelli, N.; Caruso, M.C.; Perretti, G.; Di Cairano, M.; Tolve, R.; Galgano, F. Gluten-Free Brewing: Issues and Perspectives. *Fermentation* **2020**, *6*, 1–26. [CrossRef]

33. Donadini, G.; Fumi, M.D.; de Faveri, M.D. How foam appearance influences the Italian Consumer's beer perception and preference. *J. Inst. Brew.* **2011**, *117*, 523–533. [CrossRef]
34. Yeo, H.Q.; Liu, S.Q. An overview of selected specialty beers: Developments, challenges and prospects. *Int. J. Food Sci. Technol.* **2014**, *49*, 1607–1618. [CrossRef]
35. Da Costa Jardim, C.; de Souza, D.; Cristina Kasper Machado, I.; Massochin Nunes Pinto, L.; de Souza Ramos, R.; Garavaglia, J. Sensory Profile, Consumer Preference and Chemical Composition of Craft Beers from Brazil. *Beverages* **2018**, *4*, 3–12. [CrossRef]
36. Koch, C.; Koch, E.C. Preconceptions of taste based on color. *J. Psychol. Interdiscip. Appl.* **2003**, *137*, 233–242. [CrossRef]
37. Suárez, M. Cerveza: Componentes y Propiedades, Trabajo fin de Magíster en Biotecnología Alimentaria, Universidad de Oviedo, España. 2013. Available online: https://digibuo.uniovi.es/dspace/bitstream/handle/10651/19093/TFM_%20Maria%20Suarez%20Diaz.pdf?sequence=8 (accessed on 21 December 2020).
38. Van Doorn, G.; Timora, J.; Watson, S.; Moore, C.; Spence, C. The visual appearance of beer: A review concerning visually-determined expectations and their consequences for perception. *Food Res. Int.* **2019**, *126*, 1–11. [CrossRef]
39. Verhagen, L.C. Beer Flavor. In *Comprehensive Natural Products II Chemistry and Biology*; Elsevier: Oxford, UK, 2010; p. 967997. [CrossRef]
40. Aquilani, B.; Laureti, T.; Poponi, S.; Secondi, L. Beer choice and consumption determinants when craft beers are tasted: An exploratory study of consumer preferences. *Food Qual. Prefer.* **2015**, *41*, 214–224. [CrossRef]
41. Hong, J.H.; Choi, J.H.; Lee, S.J. Investigation of sensory attributes contributing to beer preference among Koreans by using fuzzy reasoning. *J. Inst. Brew.* **2017**, *123*, 49–57. [CrossRef]
42. Langstaff, B.S.A.; Lewis, M.J. The mouthfeel of beer—A review. *J. Inst. Brew.* **1993**, *99*, 31–37. [CrossRef]
43. Sánchez, C.L.; Franco, L.; Bravo, R.; Rubio, C.; Rodríguez, A.B.; Barriga, C.; Cubero, J. Cerveza y salud, beneficios en el sueño. *Rev. Esp. Nutr. Comunitaria* **2010**, *16*, 160–163. [CrossRef]
44. Bamforth, C.W. *Beer: Health and Nutrition*; Blackwell Science Ltd.: Oxford, UK, 2009; ISBN 9780126692013.
45. Organización Mundial de la Salud OMS Directrices: Ingesta de Potasio en Adultos y Niños—Resumen. Available online: http://apps.who.int/iris/bitstream/handle/10665/85226/WHO_NMH_NHD_13.1_spa.pdf (accessed on 21 March 2021).
46. Vilaplana, M. Aspectos nutricionales y terapéuticos de la fibra dietética. *Offarm* **2015**, *13*, 96–100.
47. Cerezo de Ríos, S.; Ríos-Castillo, I.; Brito, O.A.; López de Romaña, D.; Olivares, G.M.; Pizarro, A.F. Consejería nutricional incrementa el consumo de alimentos ricos en calcio, pero la ingesta se mantiene por debajo del requerimiento diario TT—Nutritional counseling increases consumption of calcium-rich foods, but mean intake remains below the daily r. *Rev. Chil. Nutr.* **2014**, *41*, 131–138. [CrossRef]