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Hydrolysis of fructans from *Agave tequilana* Weber var. azul during the cooking step in a traditional tequila elaboration process

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Abstract

In traditional tequila production, the heads of *Agave tequilana* Weber var. azul are cooked in brick ovens to hydrolyze the fructan content and release fermentable sugars. The juice generated during cooking (known as "cooking honey") was collected periodically in a tequila distillery and characterized to study the efficiency of fructan hydrolysis. The complex structure of fructans from *A. tequilana* was confirmed. The generation of 5-(hydroxymethyl)-furfural, an increase in absorbance and °Brix, and a decrease in pH and apparent average degree of polymerization of fructans during cooking were observed. The conversion of fructans in the flowing honey increased gradually from 20% at the onset of cooking to 98% after 25.5 h, where fructose represented more than 80% of the total carbohydrates. The proportion of non-hydrolyzed fructans in the cooking honey collected before this time resulted in a total ethanol loss of 6% in the tequila distillery investigated.

Keywords: *Agave tequilana*; Tequila; Fructans; Inulin; Hydrolysis; Cooking process

1. Introduction

The famous Mexican alcoholic beverage known as tequila is produced from the fermentation and subsequent distillation of the juice obtained from *Agave tequilana* Weber var. azul, commonly called blue agave. The head (stem) of this plant is rich in fructans, that is, polymers or oligomers composed mainly of fructose units attached to a sucrose molecule, which are easily degradable by thermal treatments. Traditionally, during tequila elaboration, *A. tequilana* heads are steam cooked in brick ovens for approximately 36 h in order to hydrolyze the fructans and release principally fructose as a fermentable sugar. More recently, several tequila distilleries have replaced their brick ovens for autoclaves in order to decrease cooking time. The use of autoclaves, however, may result in a lack of some characteristic compounds which have an essential organoleptic influence on the final product. Currently, more than 65% of distilleries still use the traditional ovens for cooking, and approximately 50% of the total volume of tequila currently produced is elaborated with this system.

During the cooking step, a part of the vapor is condensed and accumulates in the oven (or autoclave). The condensed steam begins to extract sugars and other compounds from the agave heads by diffusion, generating a sweet juice known as "cooking honey" that is collected during this step. In addition, oxidation and dehydration products of sugars that play an important role on the flavour of the final product are also generated during this step (Lamas Robles, Sandoval Fabián, Osuna Tenes, & Gschaedler Mathis, 2004). The cooked agave is then milled in order to extract a second sweet juice known as "agave

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juice". Cooking honey and agave juice are then mixed, fermented, and double-distilled to obtain tequila *blanco*. An additional step of maturation in oak casks can then occur to obtain tequila *reposado* (at least 2 months of maturation), *añejo* (at least 1 year), and *extra-añejo* (at least 3 years) (Secretaría de Economía, 2005b).

The presence of fructans is not unique to the agave plant, as they are also found in about 15% of higher plants where they constitute mainly reserve carbohydrates. Five different groups of fructans are found in nature and can be distinguished according to the type of linkage between the fructose units and the position of the glucose moiety (stemming from the sucrose, initial acceptor) in the structure. These groups consist of inulins, neoseries inulins, levans, neoseries levans, and the mixed fructans group. The corresponding structures are shown in Fig. 1.

According to recent studies, the fructans of *A. tequilana* Weber var. azul consist of a complex mixture, principally containing highly branched fructans and neo-fructans (mixed fructans group), as those described in Fig. 1e

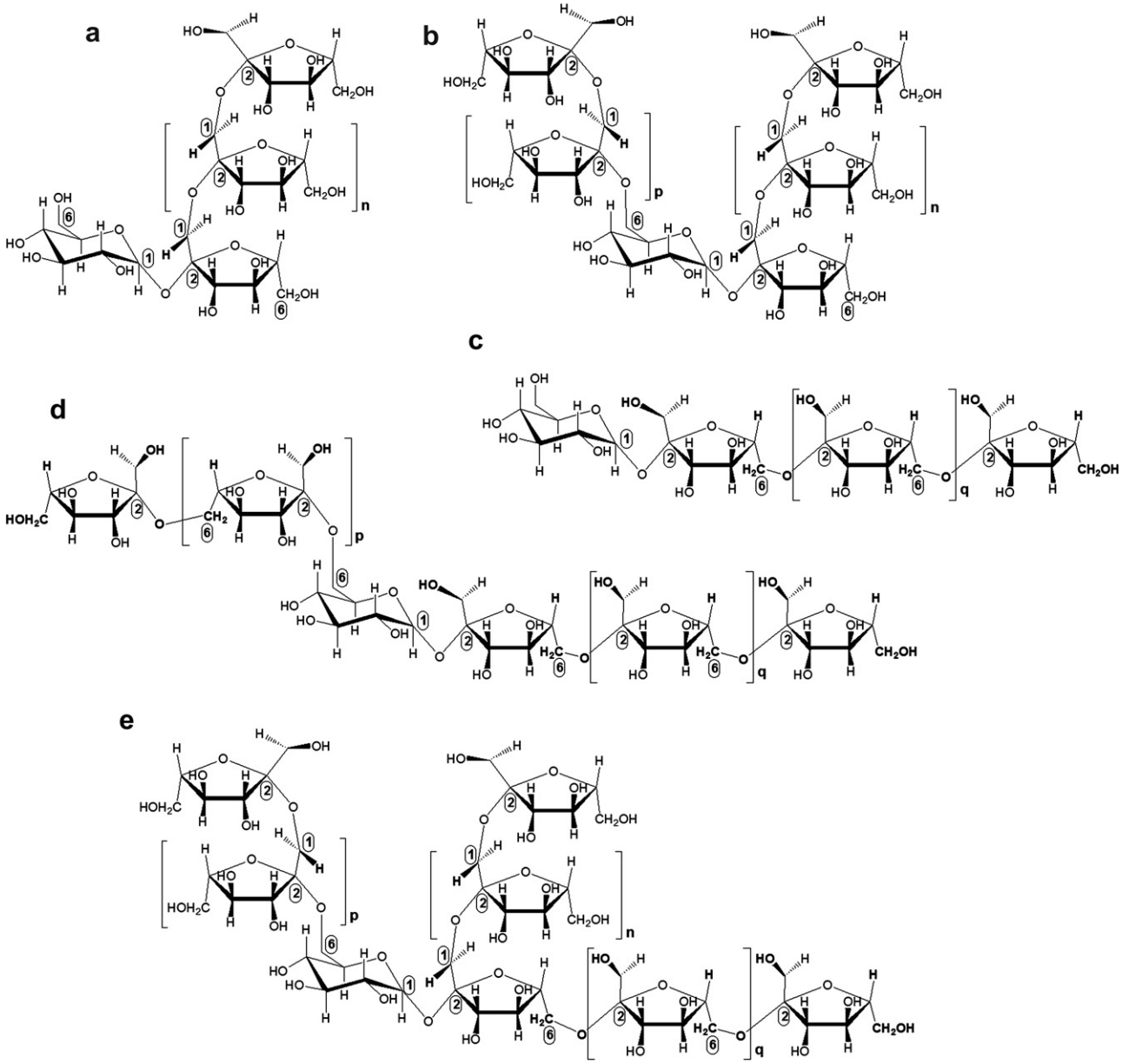

Fig. 1. Fructan groups: (a) Inulins are composed of one linear β-(2–1)-linked fructosyl chain attached to the fructosyl residue of the sucrose starter. (b) Neoseries inulins are composed of two linear β-(2–1)-linked fructosyl chains, one attached to the fructosyl residue of the sucrose, the other attached to the glucosyl residue. (c) Levans are composed of one linear β-(2–6)-linked fructosyl chain attached to the fructosyl residue of the sucrose. (d) Neoseries levans are composed of two linear β-(2–6)-linked fructosyl chains, one attached to the fructosyl residue of the sucrose, the other attached to the glucosyl residue. (e) Mixed fructans present the two types of linkages (β-(2–1) and β-(2–6)) between fructose moieties. The fructans of this group may be branched if the two types of linkages are present on the same fructose moiety, and they may contain the internal glucose typical of neoseries. This figure is adapted from Ritsema and Smeekens (2003).

(Lopez, Mancilla-Margalli, & Mendoza-Diaz, 2003; Mancilla-Margalli & Lopez, 2006). It is important to note that, although agave sugars are commonly referred to in the tequila industry as "inulin", this is inaccurate as fructans from the inulin series represent in fact a small fraction of the total fructan content.

The principal aim of this work was to study the hydrolysis of fructans from *A. tequilana* during the cooking step of a traditional tequila production process by analyzing the cooking honey released from the ovens. Indeed, there are currently very few reports regarding this subject (Mancilla-Margalli & Lopez, 2002). The efficiency of cooking to fully hydrolyze fructan oligosaccharides as well as the corresponding loss of fermentable sugars and the consequent loss of alcohol are discussed herein.

2. Materials and methods

2.1. Extraction of water-soluble carbohydrates (WSC) from A. tequilana Weber var. azul

Fifty grams of pulp produced from the transversal cutting of six mature *A. tequilana* heads were placed in a mixer with 1.5 l of distilled water at 80 °C and agitated for 5 min to extract the WSC content. The obtained suspension was then filtered in preparation for analysis.

2.2. High performance anion exchange chromatography (HPAEC) of A. tequilana WSC

Separation of the WSC in the extract obtained from the *A. tequilana* heads was performed by HPAEC with pulsed amperometric detection (HPAEC-PAD, Bio-LC50 system, detector ED40, Dionex, Sunnyvalle, CA, USA) using an analytical CarboPac PA-100 column (4 × 250 mm; Dionex, Sunnyvalle, CA, USA). The column temperature was 35 °C, and a sodium acetate gradient in 150 mM NaOH was used at a flow rate of 1 ml min^{-1}. The elution program consisted of 6 mM sodium acetate (0–10 min), 6–500 mM (10–190 min), and 6 mM (190–200 min). As fructan standards, 1-kestose, nystose (Fluka, Sigma Aldrich Chemie, Steinheim, Germany) and 1,1,1-kestopentaose (Megazyme International, Bray, Co., Wicklow, Ireland), DP3, DP4 and DP5 from the inulin series, respectively, were used.

2.3. Sampling of the cooking honey

Three independent sampling events were performed within a one-year period (June 2005, April 2006, and June 2006) at the tequila distillery of Casa Pedro-Domecq (Pernod Ricard), located in the region of *Los Altos* (Jalisco, México). In each sampling event, the flowing honey released from the cooking of *A. tequilana* heads was collected in 4 h intervals at the unique exit of a brick oven for the duration of the cooking process (about 36 h). The volume of the oven was 100 m^3 and contained approximately 50 metric tonnes of agave heads. The cooking honey sampled was assumed to be homogeneous and representative of the conditions in the oven. All laboratory analyses were carried out for each sample, and the data presented corresponds to the average of values obtained from the three sampling events. Five additional independent samples were collected from the reception tanks of cooking honey before it was used in fermentation. This last sampling was performed during the same week of production.

2.4. Determination of color, pH, °Brix and temperature

Each sample of cooking honey was diluted fivefold with water and its absorbance intensity was measured at 490 nm using a spectrophotometer (Cintra 6 system, GBC Scientific Equipment, Dandenong, VIC, Australia). During sampling, the temperature of each sample collected was also measured. °Brix values were determined using certified densimeters (sacarimeters, F. Mántey B., Mexico city, Mexico) at 20 °C. The pH of each sample was measured using a digital potentiometer (model Accumet, Fisher Scientific Company, Suwanee, GA, USA).

2.5. Determination of the percent hydrolysis of fructans in the cooking honey

The percent hydrolysis of fructans present in the cooking honey was calculated by the ratio between the direct reducing sugars (DRS) and the total reducing sugars (TRS). DRS and TRS correspond to the reducing sugars present before and after total acid hydrolysis, respectively, and were quantified by the Fehling method as is described in the Mexican Standard for the determination of direct and total reducing sugars for alcoholic beverage industry (Secretaría de Economía, 2005a). For acid hydrolysis, 5 ml of sample were diluted with 20 ml of distilled water and hydrolyzed with 5 ml of HCl at 50% (v/v) for 5 min at 68–70 °C, as is described in the Mexican Standard.

2.6. Sugars determination

Fructose, glucose, and disaccharides (DP2) were separated and subsequently quantified by high performance liquid chromatography (HPLC Varian Pro Star System, Palo Alto, CA, USA) using an Aminex® HPX-87C column (300 × 7.8 mm; Biorad, Hercules, CA, USA) and refractive index detection. Elution was performed with degassed ultrapure water at a flow rate of 0.5 ml min^{-1} and a column temperature of 80 °C. Fructan ($\geqslant$DP3) concentration was estimated from the area of the exclusion peak. Total sugar concentration was estimated from the total area of the chromatogram. Standards of D-glucose and D-fructose were obtained from Sigma (Sigma Aldrich Chemie, Steinheim, Germany). As DP2 and fructan standards, sucrose and inulin from chicory (Sigma) were used, respectively.

2.7. Quantification of 5-(hydroxymethyl)-furfural (HMF) in the cooking honey

HMF was quantified by HPLC using a C18 5 μm column (250 × 4 mm; Bischoff, Leonberg, Germany) and UV detection at 284 nm. Elution was performed with degassed ultrapure water at a flow of 0.9 ml min^{-1} and a column temperature of 60 °C. The HMF standard was obtained from Aldrich (Sigma Aldrich Chemie, Steinheim, Germany).

2.8. Determination of the apparent average degree of polymerization (DP) of fructans

Most fructans are composed of one molecule of glucose and two or more molecules of fructose. Thus, free glucose, free fructose, and free sucrose were first quantified by enzymatic methods in each sample with the Sucrose/D-Fructose/D-Glucose Assay Kit (Megazyme). Then, each sample was hydrolyzed with HCl as previously described, and the total glucose and total fructose contents were determined. The calculations for the different sugars were made according to the instructions of the manufacturer. The following formula was used to determine the average degree of polymerization (DP) of fructans:

$$DP_{average} = (F_{total} - F_{free} - 0.52S_{free}) / (G_{total} - G_{free} - 0.52S_{free}) + 1$$

where F, G, and S represent the fructose, glucose, and sucrose concentrations, respectively.

3. Results and discussion

3.1. WSC extracted from A. tequilana Weber var. azul

The WSC content of agave heads sampled was found to be equal to 28.3 g/100 g (fresh weight) ± 0.1% and 86.7 g/100 g (dry weight) ± 1.3%. These high values are in agreement with those previously reported (Cedeño, 2003; Mancilla-Margalli & Lopez, 2006) and show the very good potential of *A. tequilana* Weber var. azul as a sugar source.

Extracted carbohydrates were then analyzed by HPLC and showed to consist of fructans ⩾DP3 (93.4%), free disaccharides (2.0%), free glucose (0.8%), and free fructose (3.8%). The average DP of fructans in the extract was determined and found to be equal to 13.6 ± 1.3. Lopez et al. (2003) reported a range of fructan DP from 3 to 29 units in blue agave. The average DP found in the current study is compatible with this data.

Separation of fructans in the extract by HPAEC showed a complex pattern of oligosaccharides compared with the linear β-(2–1) linked series of chicory (Fig. 2). It confirms the results previously reported, that the nature of fructans of *A. tequilana* is more complex than the simple inulin series (Lopez et al., 2003; Mancilla-Margalli & Lopez, 2006). In these cited works, the authors report that the fructans from *A. tequilana* Weber var. azul and other *Agave* species contain a complex mixture of highly branched fructans and highly branched neo-fructans (referred to as agavins), with each type of linkage (β-(2–1) and β-(2–6)) between fructose moieties.

Kestose, nystose, and 1,1,1-kestopentaose (DP3, DP4 and DP5 from the inulin series, respectively) were all detected in our extract, however. Thus, the complex chromatogram obtained might be due to the presence of all existing isomers for each DP with each type of linkage (β-(2–1) or β-(2–6)), and glucose position (internal or terminal), belonging to the different groups presented in Fig. 1. The HPAEC-PAD chromatogram presented is the first reported concerning carbohydrates extracted from *A. tequilana* Weber var. azul. The closest HPAEC-PAD chromatogram previously reported is that obtained with carbohydrates extracted from *Phormium tenax* and *Phormium cookianum* (Sims, Cairns, & Furneaux, 2001), which were also described as a complex mixture with branched fructans and fructans neoseries.

3.2. Evolution of color and pH of the cooking honey

In the studied distillery, at the onset of cooking, the oven is first completely closed in order to allow for pressurization. This step lasts about 5–6 h. When the desired overpressure is reached (about 17,000 Pa, corresponding to an approximate steam temperature of 100 °C considering the distillery altitude), the oven is purged slowly in order to avoid a pressure decrease and the cooking honey begins to flow. After about 30 h of cooking, the steam injection is stopped and cooking of the agave heads follows for six additional hours with the residual steam.

As shown in Fig. 3a, the cooking honey became darker during the cooking step, as can be observed by an increment in absorbance intensity. It should be noted, however, that during the first 4 h of cooking, the absorbance decreased slightly before a sharp increase was observed. This behaviour may be due to the fact that in the first hours of flowing, the cooking honey contains soil and other residues from the surface of the agave heads that can make the honey darker.

As the absorbance of honey increased, its pH decreased regularly as a function of cooking time, from pH 4.8 at the beginning of honey flow (5.5 h) to pH 4.1 at the end of the cooking (36 h, Fig. 3a).

The increment in absorbance concomitant with the decrement in pH is in agreement with that previously reported on the cooking of agave heads (Mancilla-Margalli & Lopez, 2002), and is characteristic of Maillard reaction systems (Apriyantono & Ames, 1993). Indeed, the browning color is in general directly correlated with the generation of Maillard compounds, which have an important influence on the flavour of the final product. Mancilla-Margalli and Lopez (2002) report that in addition to the Maillard reaction, caramelization of sugars can take place under the cooking conditions of agave heads and might partici-

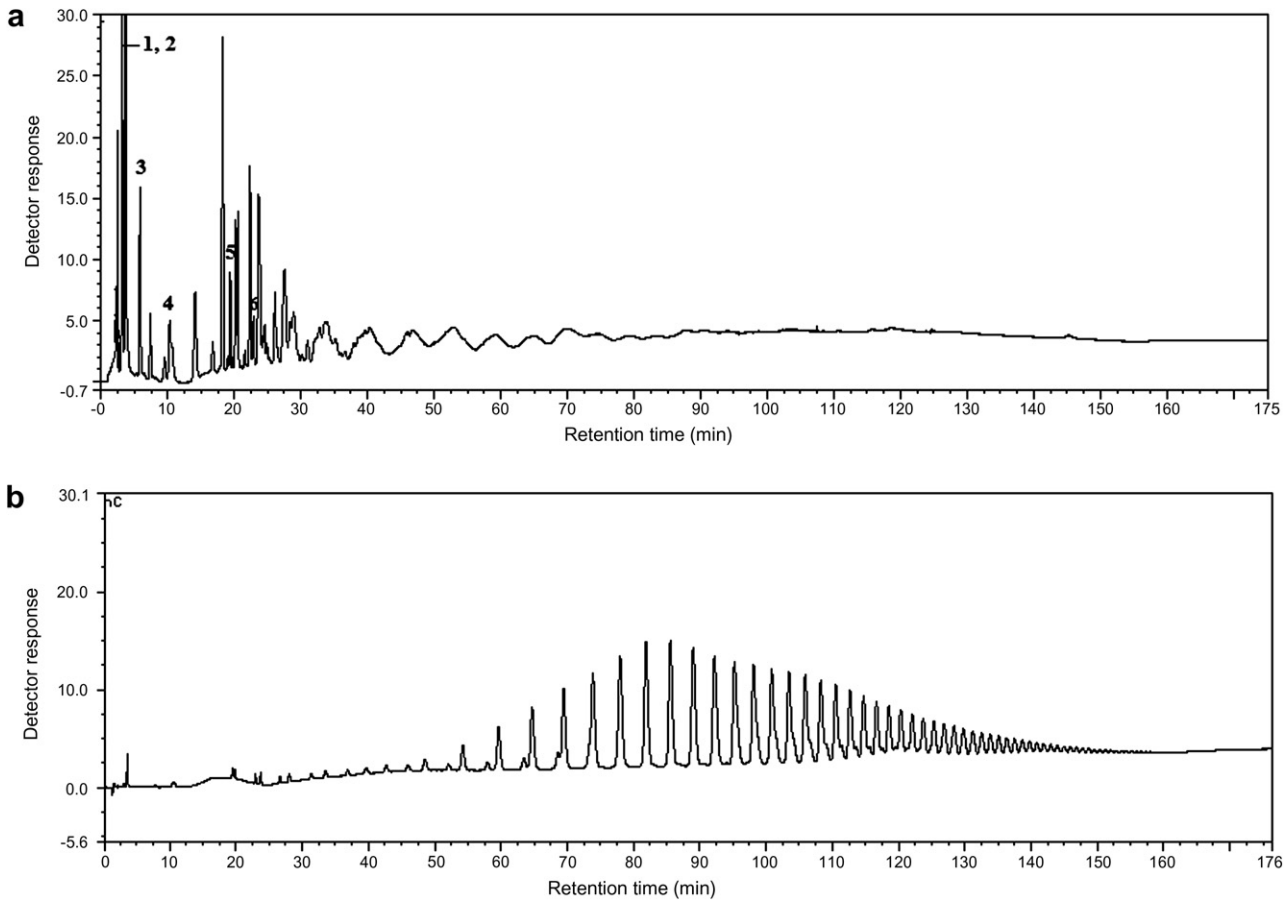

Fig. 2. HPAEC-PAD chromatograms of (a) water-soluble carbohydrates of agave head, and (b) inulin from chicory (SIGMA). Carbohydrates were identified by comparison with standards of known retention time. (1) Glucose, (2) fructose, (3) sucrose, (4) 1-kestose, (5) nystose, (6) 1,1,1-kestopentaose.

pate in browning of the cooking honey. It is essential to note that the caramel flavour is one of the most important descriptors of tequila flavour, and that the caramelization of sugars during cooking certainly plays a role in the caramel note of the final product.

Finally, the pH decrease may be due to the formation of organic acids or to the inability of amino moieties to act as bases when the amino compounds have reacted during Maillard reactions (Mancilla-Margalli & Lopez, 2002). In addition, it is also possible that organic acids initially present in the plant as well as other acidic compounds are liberated in the cooking honey.

3.3. Evolution of total carbohydrates content, °Brix and temperature of the cooking honey

Fig. 3b shows the evolution of carbohydrate content, °Brix and temperature of the honey released during the cooking step. A strong correlation between °Brix and total carbohydrate content is observed in this figure, and confirms that the use of densimeters in the tequila distilleries is a good tool to determine sugar concentrations. Carbohydrate content and °Brix increased regularly during the cooking process; carbohydrates increased from 175.7 g/kg when the flow of cooking honey begins to 289.5 g/kg at the end, whereas °Brix increased from 16.6 to 30.3 during the same period. This drastic increase in both carbohydrate content and °Brix cannot be explained only by the hydrolysis of fructan oligomers. We can assume that with an increased cooking time, more sugars are solubilized due to a decrease in agave consistency and subsequently, better diffusion conditions are encountered for these soluble compounds.

With regards to temperature, two different periods were distinguished: (i) during the first 30 h of cooking, where the temperature varied between 66.5 and 78.5 °C, and (ii) during the last 6 h, where the temperature decreased to less than 50 °C. This observed decrease is logical and corresponds to the termination of vapor injection.

3.4. Evolution of HMF concentration of the cooking honey

Concentration of HMF in the honey released during cooking increased from an initial value of about 50 mg/kg to more than 3000 mg/kg at the end of cooking (Fig. 4). This important increase in HMF concentration illustrates the production of Maillard compounds as well as the dehydration of carbohydrates during the cooking process

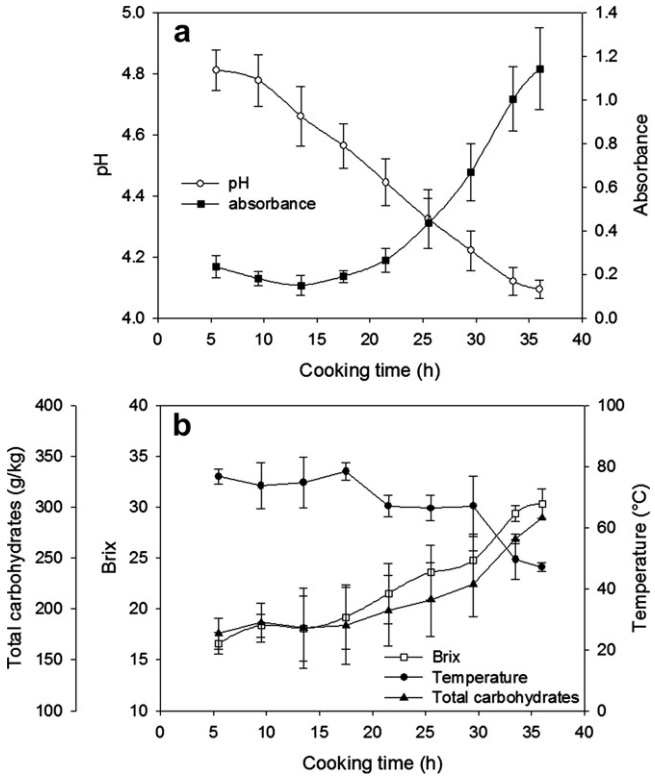

Fig. 3. (a) Evolution of the rate of browning (absorbance at 490 nm) and pH of the honey generated during the cooking process of agave heads. (b) Evolution of total carbohydrates content, °Brix and temperature.

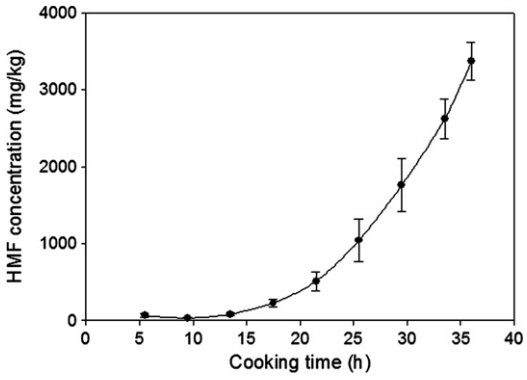

Fig. 4. Evolution of HMF concentration in the honey generated during the cooking process of agave heads.

(Mancilla-Margalli & Lopez, 2002; Shimamura, Ukeda, & Sawamura, 2000). The observed increase is consistent with previously obtained data. Indeed, Marcilla-Margalli and Lopez (2002) describe HMF as one of the more important Maillard compounds found in cooking honey obtained from *A. tequilana*, with values reaching more than 4000 ppm. It is essential to note the importance of HMF in tequila production. On the one hand, this compound plays, as mentioned previously, an important role on the flavour of the final product; its partial contribution on the characteristic aroma of cooked agave is recognized (Lamas Robles et al., 2004), and its presence is thus absolutely indispensable in the final product. On the other hand, however, HMF and other furfurals are known to have a significant inhibitory effect on yeasts, and particularly on *Saccharomyces cerevisiae*, which is the principal yeast present in the fermentation step of tequila production (Azhar, Bery, Colcord, Roberts, & Corbitt, 1981; Palmqvist & Hahn-Hagerdal, 2000; Taherzadeh, Eklund, Gustafsson, Niklasson, & Liden, 1997; Taherzadeh, Gustafsson, Niklasson, & Liden, 2000). Negative effects on growth rate, fermentation rate and cell composition have all been reported for HMF concentrations in the 1000–5000 mg/l range (Azhar et al., 1981). In tequila production, however, the cooking honey is mixed with agave juice in the formulation of the fermentation wort, and as a result, the HMF concentration is certainly lower at the beginning of the fermentation. Moreover, in the case of tequila, the pH during the fermentation step is generally 4.5 (Cedeño, 2003), and it has been previously reported that at this pH the inhibitory effects of the compounds resulting from a Maillard model system are almost eliminated (Tauer, Elss, Frischmann, Tellez, & Pischetsrieder, 2004).

3.5. Evolution of sugar composition in the cooking honey – evolution of hydrolysis and apparent average DP of fructans

Fig. 5 illustrates the efficiency of fructan hydrolysis and the corresponding release of fructose during cooking. Indeed, one can see the progressive disappearance of fructans concurrently with the appearance of fructose. The estimated proportion of fructans decreased from more than 80% to 5% of the total carbohydrates. On the contrary, fructose proportion increased from 12% to 80%. The composition of honey leveled after 25.5 h of thermal treatment, and only a slight variation in composition was observed thereafter. As expected, the glucose proportion increased during the cooking step (from 2% to 8% of the total carbohydrates), as a result of fructan hydrolysis. The more surprising behaviour was that of DP2, where a slow increase was observed during the first 20 h of cooking (from approximately 4.0% to 9.5% of total carbohydrates), before decreasing during the final hours (up to 6.5% of total carbohydrates). This behaviour may result from a

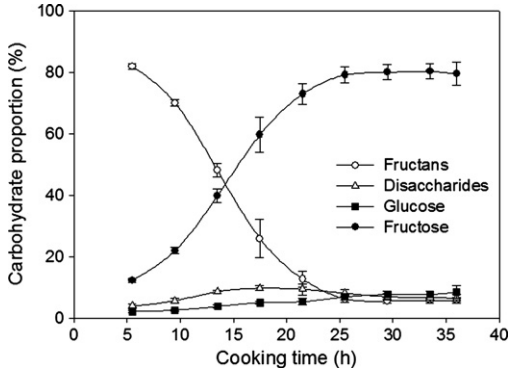

Fig. 5. Evolution of the carbohydrate composition in the honey generated during the cooking process of agave heads.

combined effect of the apparition of DP2 resulting from fructan hydrolysis and/or from other reactions concomitantly with the thermal hydrolysis of DP2. The presence of residual DP2 was unexpected, but it represents a low proportion of the total carbohydrates, and might result in part from the co-elution of other compounds with DP2, thus causing an increased response in the chromatogram. Concerning residual fructans, this explanation is even more probable, as their concentration was estimated based on the area of the exclusion peak, which is likely to contain interferences such as from salts. The presence of interfering compounds is reinforced by the analysis of Fig. 6 that shows that at the end of the cooking process, fructan oligomers reached about 98% hydrolysis and thus only 2% of carbohydrates are not totally hydrolyzed (far less than the sum of residual fructans and DP2 detected by chromatography). This level of hydrolysis demonstrates the good efficiency of heat application in degrading fructans.

The evolution of the apparent average DP showed, as expected, a decrease from approximately 14 to 3 over the course of the complete cooking process (Fig. 6). The initial value was close to the average DP determined for fructans extracted from uncooked agave showing a limited hydrolysis during the pressurization of the oven. A lower value was expected, however, due to the appearance of free fructose during this period (Fig. 5). This fact could be explained by a difference in raw material, as average DP of uncooked agave was measured on a different lot of agaves than what corresponded to the cooking honey samples. DP can differ from plant to plant and it has been reported previously that fructan structure, DP and fructan concentration are highly dependent on environmental conditions (temperature, luminosity, soil nutrients, etc.) and the development stage of the plant (age, time of the year, etc.) (Chatterton & Harrison, 1997; Mancilla-Margalli & Lopez, 2006; Sims, 2003; Sims et al., 2001). The deviation of the final value from one might be explained by the method of calculation of apparent average DP. Indeed, in order to calculate DP, the presence of one glucose moiety per molecule is assumed. Thus, for example, if at the end of cooking residual dimers of fructose remain, the absence of the glucose moiety tends to cause an increase in the apparent DP. For example, one molecule of sucrose and one fructose dimer will be seen as a fructan of apparent average DP of 4 (1 glucose moiety and 3 fructose moieties).

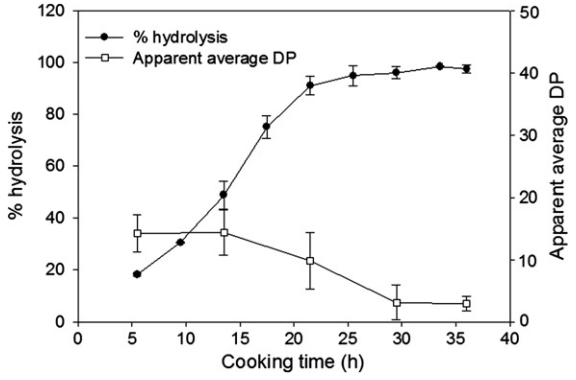

Fig. 6. Evolution of the percent hydrolysis and the apparent average DP (degree of polymerization) of the fructans contained in the honey generated during the cooking process of agave heads.

Finally, we can note that various works have reported the formation of di-D-fructose dianhydrides resulting from the thermal degradation of inulin (Blize, Manleyharris, & Richards, 1994; Bohm, Kaiser, Trebstein, & Henle, 2005; Christian, Manley-Harris, Field, & Parker, 2000; Manley-Harris & Richards, 1996). These compounds are cleavable by acid into two fructose monomers (Bohm et al., 2005). In this way, we could assume that these compounds appear during cooking of agave heads as well. Thus, they might appear during agave cooking and be the interfering compounds assumed before, that is, they might be responsible of the presence of a residual peak corresponding to disaccharides at the end of the cooking (Fig. 6). Moreover, these compounds might explain the deviation of final apparent average DP from 1, and why the percent hydrolysis measured does not exceed 98%, since the determination of the average DP and of the percent hydrolysis include a step of acid hydrolysis.

3.6. Analysis of cooking honey samples collected from the reception tank: determination of theoretical loss of alcohol

Figs. 5 and 6 show that with the onset of cooking honey flow, very little hydrolysis was observed (percent hydrolysis: <20%, proportion of fructans: >80%: proportion of fructose: 12%) and that hydrolysis evolved regularly until reaching a maximum of 98% after 25.5 h of cooking. This means that during the first 25 h of treatment, the collected honey contained a proportion of carbohydrates that cannot be used by yeasts during the fermentation step due to incomplete hydrolysis. In order to estimate the corresponding theoretical loss of alcohol, we sampled the cooking honey from the reception tank immediately prior to the fermentation step, within the same week of production. It is important to note that the cooking honey sampled at this level corresponds to the mix of cooking honey released during the whole cooking process, that is to say a mix between honey slightly hydrolyzed (onset of flow) and honey increasingly hydrolyzed (cooking evolution). In addition, the cooking honey sampled from the reception tank can potentially be a mix of cooking honeys from different ovens.

The estimation of alcohol loss was done as follows: the theoretical fermentable sugar concentration (assuming total hydrolysis; TRS value in Table 1) was compared to the fermentable sugar concentration measured in the cooking honey (glucose + fructose + 1.0526 DP2, taking into account that 1 g of sucrose corresponds to 1.0526 g of glucose + fructose) to determine the loss of fermentable sugars. The Gay–Lussac equation was then used to determine the corresponding theoretical loss of ethanol (1 mol

Table 1
Carbohydrate composition of different cooking honeys sampled in the reception tank

Sample	Estimated fructans (g/l)	DP2 (g/l)	Glucose (g/l)	Fructose (g/l)	DRS (g/l)	TRS (g/l)	% Hydrolysis	Fermentable sugar loss (g/l of cooking honey)	Theoretical ethanol loss (ml pure ethanol/l of cooking honey)	Fermentable sugar loss and theoretical ethanol loss (%)
1	94.2	11.6	6.3	45.0	62.4	144.9	43.1	81.4	52.7	56.2
2	76.3	13.8	7.7	65.4	87.2	155.7	56.0	68.1	44.1	43.7
3	74.4	16.1	8.6	67.0	90.7	158.8	57.1	66.4	43.0	41.8
4	27.6	21.0	13.7	116.5	145.6	174.7	83.3	22.5	14.5	12.9
5	133.3	10.8	5.1	40.8	57.3	174.1	32.9	116.8	75.7	67.1
Average	81.2	14.6	8.3	66.9	88.6	161.6	54.5	71.0	46.0	44.0

Theoretical losses in fermentable sugars and ethanol.

of hexose permits the formation of 2 mol or 116.6 ml of pure ethanol, considering 100% fermentation efficiency).

Table 1 shows the important variability in the level of hydrolysis of cooking honey prior to the fermentation step. Indeed, the conversion of fructans was found to vary between 32.9% and 83.3%, due potentially to the manner in which the tanks are actually filled in industry. Table 1 shows that in each case, however, there was an important loss in fermentable sugars due to incomplete hydrolysis during thermal treatment. The average loss of fermentable carbohydrates for the five samples was 71.0 g/l of cooking honey, though this value reached 116.8 g/l of cooking honey in extreme cases (sample 5). The average loss of fermentable carbohydrates corresponded to a loss of pure ethanol of 44.0% considering only the cooking honey. Considering the whole process of tequila production and the proportion of cooking honey employed in the fermentation step, the loss of ethanol represents about 6% of the total ethanol produced for the specific distillery where this work was carried out.

4. Conclusions

In the present study, fructan hydrolysis has been evaluated during a traditional brick oven cooking process of *A. tequilana* Weber var. azul. The results of this evaluation show that an efficient fructan hydrolysis of 98% is indeed observed, however, only after 25.5 h of cooking. Consequently, the major part of the cooking honey, which is released and collected prior to this time, contains a large proportion of non-hydrolyzed, and therefore, non-fermentable carbohydrates. This non-hydrolyzed proportion accounted for a 6% loss in the total ethanol produced by the specific distillery investigated in this study. As previously cited, several tequila distilleries have replaced the brick ovens by autoclaves in order to decrease cooking time. It has not been well studied, however, the effect of autoclave use on the efficiency of fructan hydrolysis or on the organoleptic qualities essential to tequila. In this way, the traditional brick oven cooking process may be preferable, and precise studies are now needed which investigate improvements in cooking conditions or the use of additional cooking honey treatments (thermal, acid and/or enzymatic), that may improve the efficiency of production without changing the characteristic flavour of the final product.

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