

Generation of Maillard Compounds from Inulin during the Thermal Processing of *Agave tequilana* Weber Var. azul

NORMA A. MANCILLA-MARGALLI AND MERCEDES G. LÓPEZ*

Unidad de Biotecnología e Ingeniería Genética de Plantas, Centro de Investigación y Estudios Avanzados del IPN, Apartado Postal 629, 36500 Irapuato, Gto., Mexico

During the cooking process of *Agave tequilana* Weber var. azul to produce tequila, besides the hydrolysis of inulin to generate fermentable sugars, many volatiles, mainly Maillard compounds, are produced, most of which may have a significant impact on the overall flavor of tequila. Exudates (agave juice) from a tequila company were collected periodically, and color, °Brix, fructose concentration, and reducing sugars were determined as inulin breakdown took place. Maillard compounds were obtained by extraction with CH₂Cl₂, and the extracts were analyzed by GC-MS. Increments in color, °Brix, and reducing sugars were observed as a function of time, but a decrease in fructose concentration was found. Many Maillard compounds were identified in the exudates, including furans, pyrans, aldehydes, and nitrogen and sulfur compounds. The most abundant Maillard compounds were methyl-2-furoate, 2,3-dihydroxy-3,5-dihydro-6-methyl-4(*H*)-pyran-4-one, and 5-(hydroxymethyl)furfural. In addition, a series of short- and long-chain fatty acids was also found. A large number of the volatiles in *A. tequilana* Weber var. azul were also detected in tequila extracts, and most of these have been reported as a powerful odorants, responsible for the unique tequila flavor.

KEYWORDS: *Agave tequilana*; inulin; Maillard compounds; tequila; GC-MS

INTRODUCTION

The well-known Maillard reaction results from an interaction between amino compounds, usually amino acids or proteins, and reducing carbohydrates (1). This reaction leads to the formation of compounds that, because their volatility, influence the overall flavor of a product (2, 3). Since Ruckdeschel in 1914 reported aroma generation by Maillard pathways, the food industry has patented flavor formation processes from the heated aqueous mixtures of amino acids and reducing sugars. In the same way, thermal treatments of foods as well as their basicity are favorable conditions for the generation of these compounds (4).

Tequila is one of the most consumed Mexican liquors worldwide and is made from *Agave tequilana* Weber var. azul, a native plant of Mexico (5), the only raw material appropriate to produce this beverage (6). During tequila production, stems of *A. tequilana* are submitted to a cooking process for at least 32 h at ~100 °C. The exudates (agave juice or cooking honey) obtained are then fermented and double-distilled to generate tequila blanco (white). This product can be matured from 3 to 12 months in oak casks to produce tequila reposado (rested) or from 1 to 5 years to produce tequila añejo (aged) (7).

The main reason for the cooking process during tequila production is to hydrolyze the inulin, the principal polysaccharide in the core of the agave pine plants. Inulin is thus converted

into free sugars, mainly fructose (6), for their subsequent fermentation. Shu (8) in 1998 showed that inulin heated with asparagine, even under mild conditions, produced Maillard molecules in an analogous way to fructose.

The cooking conditions of the agave pines such as high temperature, low pH (4.5), time, and humidity are highly favorable to the Maillard reaction (9). Therefore, the main objective of this study was to determine the Maillard compounds generated in the thermal processing of *A. tequilana* Weber var. azul during tequila production.

MATERIALS AND METHODS

Materials. The sweet liquid generated during the cooking of *A. tequilana* Weber var. azul pines, known as exudates or cooking honey, was collected from an oven of a tequila company every 4 h during the whole cooking process (32 h). The following determinations were performed with each exudate.

Color and pH Determination. One hundred microliters of each exudate was diluted to a final volume of 1 mL with water. The absorbance of each sample was read in a 1 cm quartz cell at 490 nm with a visible spectrophotometer (Spectronic 20, Bausch and Lomb). The pH of all samples was also determined.

Sugar Determination. Total and reducing sugars were determined by spectrophotometric methods described previously (10, 11). Fructose concentration was also measured according to the procedure of Somani et al. (12). Ten microliters of each sample was used to measure Brix grades using a refractometer (Sper Scientific, 0–80%).

Maillard Compounds. Ten milliliters of each exudate was diluted with the same volume of water and extracted with 6 mL of dichlo-

* Corresponding author (telephone 52-462-623-9632, fax 52-462-624-5996; e-mail mlopez@ira.cinvestav.mx).

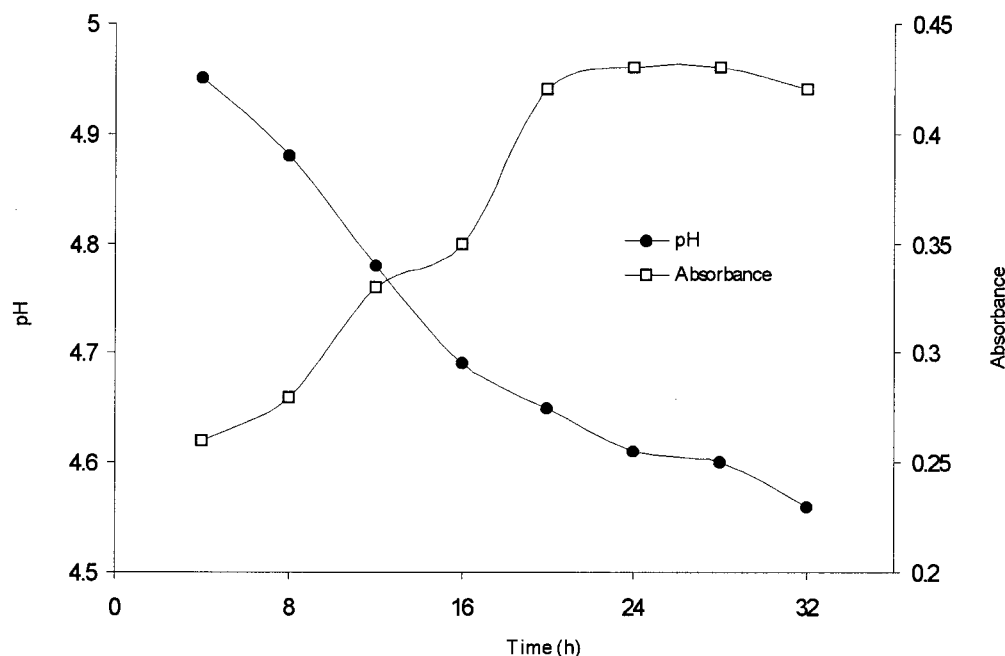


Figure 1. Rate of browning and pH value changes in exudates generated from the cooking process of *A. tequilana* Weber var. azul during tequila elaboration. Exudates were collected every 4 h for 32 h. Absorbance was read at λ 490 nm.

romethane in a separatory funnel. The phases were separated by centrifugation for 10 min at 10000 rpm at 4 °C. This step was repeated three times. The organic phases were combined and passed through an anhydrous Na_2SO_4 column and completely evaporated in a Kuderna-Danish apparatus.

Gas Chromatography-Mass Spectrometry (GC-MS). The evaporated samples were diluted with 25 μL of CH_2Cl_2 , and 1 μL was injected in a splitless mode on a gas chromatograph (Hewlett-Packard 5890 series II) coupled to mass spectrometer (Hewlett-Packard 5972 series) as a selective detector for the characterization of all compounds present in the extracts. Compound separations were carried out using an HP-FFAP column (25 m \times 0.32 mm i.d. \times 0.52 μm film). Helium was used as carrier gas (2 mL/min) with a starting temperature of 40 °C for 5 min followed by a temperature program of 20 °C/min to 100 °C for 1 min, followed by a second rate of 3 °C/min to a final temperature of 230 °C for 40 min. Injector and detector temperatures were 220 and 240 °C, respectively. Because chromatograms were so complex, quantitative data were obtained by injection of methyl undecanoate as an external standard in an independent run under the same conditions as the samples. Peaks were identified by comparison with the mass spectra library and commercial standards when possible.

RESULTS AND DISCUSSION

Color and pH Determination. Agave exudate color became darker as a function of time. **Figure 1** shows the absorbance increments observed, the lowest set at 0.26 at 4 h and the highest 0.44 at 28 h. Contrary to the absorbance increments, pH values decreased from 4.95 to 4.56. Although the pH changes were not very drastic, the tendency was always to be more acidic. The absorbance increments concomitant with the pH decrements are characteristics observed in many Maillard systems (13). On the basis of these parameters, it can be concluded that the Maillard reaction was occurring during the cooking process of agave pines. The browning color is usually directly correlated with the Maillard reaction based on the generation of compounds such as furfural, 5-(hydroxymethyl)furfural, and pre-melanoidins, although carotenoids or other pigments might be contributing to the brown color. Besides the Maillard reaction, caramelization of the sugars could take place under these conditions and might also lead to browning. Fructose is known to contribute

more noticeably to browning via caramelization than glucose (14). The pH decrease might also be due to the formation of organic acids in the sample or to the inability of amino moieties to act as bases when the amino compounds have reacted.

Sugar Determination. Inulin is the principal carbohydrate present in *A. tequilana* Weber var. azul. Measurement of °Brix shows the efficiency of the hydrolysis of this polysaccharide made of fructose, indicated as reducing sugars content. **Figure 2** shows the correlation between °Brix and sugar determinations throughout the cooking process. A drastic increment in °Brix values was observed during the first 8 h followed by a constant value to 32 h. A similar pattern was shown during reducing sugar determination. Fructose concentration and total sugar content displayed a similar behavior, with maximum concentrations being reached at 16 h and then almost constant concentrations after this time. In addition, the high fructose concentration observed confirms the presence of inulin as the major carbohydrate on the agave pines.

Identification of Maillard Compounds. Chromatograms obtained from the exudates of agave pines at different times (4, 8, 12, 16, 20, 24, and 28 h) showed very complex profiles (data not shown). Generally, >240 components were identified in each exudate. Acids, alcohols, and furans were the most abundant compounds, followed by aldehydes, ketones, aromatic compounds, terpenes, pyrans, and nitrogen and sulfur compounds. Thirty-six percent of the volatile compounds found in agave exudates have been reported previously as Maillard reaction products that might be generated by different pathways. Hollnagel and Kroh (15) reported that large differences in the volatile profiles from mono- and oligosaccharides exist. Whereas monosaccharides undergo reactions to yield simple heterocyclic compounds, disaccharides and oligo- and polysaccharides generate heterocyclic compounds that still possess either glycosidic substituents or anhydrous sugars, respectively. However, we did not find any of these compounds, because cooking conditions favored the total hydrolysis of inulin toward fructose generation. **Table 1** lists the appearance and disappearance of some compounds, which must be related to a dynamic transformation process. For instance, the concentration of some volatiles, such

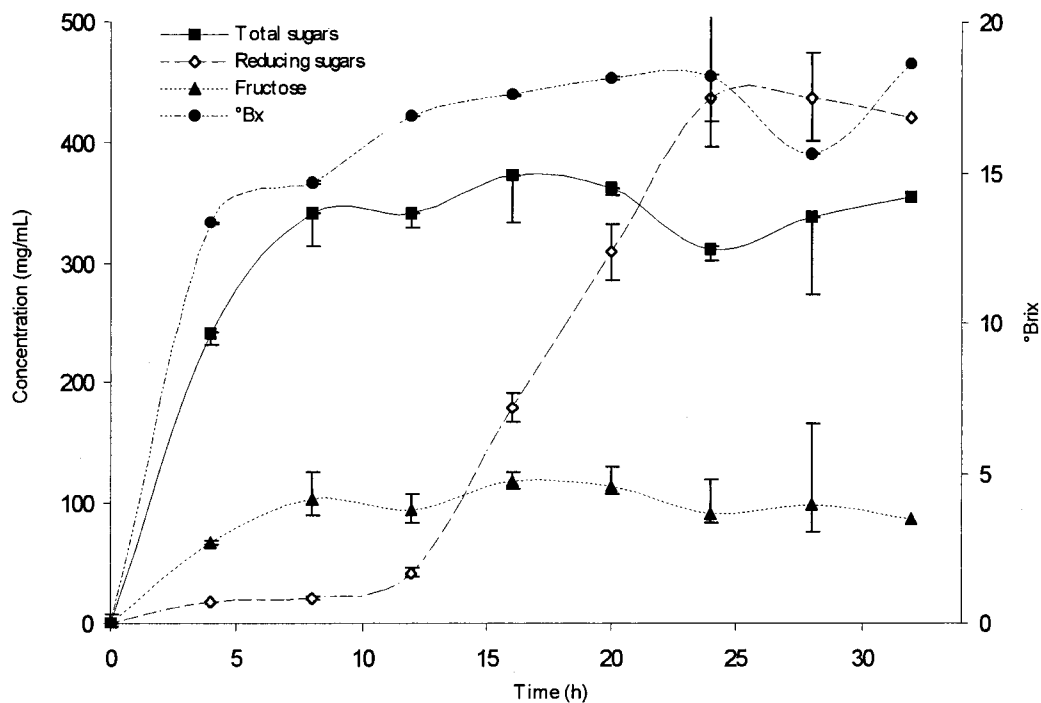


Figure 2. Correlation between °Brix and sugar content as indicator of inulin breakdown during cooking of agave pines. Determinations were done in exudates obtained every 4 h during the whole process. Bars represent SE and, where not shown, were smaller than the symbol.

as 3-hydroxy-2-butanone and 1,2-butanediol, decreased with time. Therefore, polyols and hydroxylated ketones might be considered as precursors of compounds such as pyridine, furfural, 2-furanmethanol, 5-methylfurfural, methyl-2-furoate, and 5-(hydroxymethyl)furfural, concentrations of which increased throughout the cooking process. **Table 1** also lists the principal compounds found in the agave pine exudates and their abundance with cooking time. The presence of short and long fatty acids in the extracts, from acetic acid (C_2) to octadecanoic acid (C_{18}), was used to calculate the Kovats indices (KI). The presence of heterocyclic compounds is further evidence that a Maillard reaction took place during the cooking of agave pines. Furans represented the majority followed by pyrans, sulfur compounds, and ketones. Other compounds such as benzyl alcohol, benzaldehyde, 3-hydroxy-2-butanone, and acetic acid have also been reported as Maillard compounds (2, 13, 16, 17). The exudates also contained compounds considered to be relevant to the final tequila flavor profile. Among them, the lactone β -damascenone has been reported to result from the thermal degradation of carotenoids (18). Terpenes such as linalool, phytol, and (*Z*)- and (*E*)-geraniol probably come from the agave plant per se. On the other hand, the series of organic acids found in the exudates might come from cell membranes that could be degraded during the cooking treatment, although acetic acid is also generated by the Strecker degradation of alanine (19). Phenolic compounds such as vanillin, syringaldehyde, phenol, and phenol derivatives may be formed by the thermal degradation of lignin. It is important to emphasize that is believed that both vanillin and syringaldehyde are formed during the resting or aging of alcoholic beverages through contact with the wood casks; however, these compounds were found in tequila exudates, and it is known that their concentrations increase during aging. Alcohols such as 1-hexanol, 3-methyl-1-butanol, and phenylethyl alcohol have been considered to be fermentation products, responsible for the sweet notes of most alcoholic beverages (20), but their presence in agave exudates suggests that fermentation is not the only

pathway for their formation. Another alcohol present in the exudates was 1-octen-3-ol. Its presence is characteristic of shellfish and tomato, and it has been considered the most important odorant in mushrooms such as huitlacoche (*Ustilago maydis*) and austern pilzen (*Pleurotus* sp.) (21).

Only one amino acid derivative was detected and quantified in the exudates: *N*-acetylalanine was observed only in the first 8 h. The presence of amino heterocyclic compounds such as pyrazines, pyranones, and pyrroles is evidence of the presence of other amino acids in the agave pines. Most of these compounds were detected between 20 and 32 h. Phenylacetaldehyde and phenylacetic acid are known to come from the interaction of phenylalanine and sugars by Strecker degradation (19). On the other hand, the presence of sulfur compounds indicated that amino acids such as cysteine and methionine were also present in *A. tequilana* pines; for example, 2,4,5-trimethylthiazole has been shown to come from ribose–cysteine interaction (22).

The kinetic patterns of the cooking process showed a great variation, but three principal groups can be differentiated. In the first group, most compounds showed a maximum concentration during the first 4 h. Hydroxylated ketones, polyols, organic acids, unsaturated compounds, and terpenes such as hydroxylinalool belong to this group. A second group includes substances such as 3-methyl-2-(5*H*)-furanone, 2-furancarboxylic acid, and 2(5*H*)-furanone among others, which reached their maximum at 20 h; after this time, their concentrations decreased. This behavior is related to the disappearance of amino-containing compounds.

Figures 3 and 4 show profile changes of some minor and major compounds over time, respectively. 3-Hydroxy-2-butanone, like 1,2-butanediol, showed a drastic decrease in the first 8 h, subsequently declining slowly. They can therefore be considered as precursors of other compounds such as furfural, which increased after this time. It is important to mention that cyclotene (**Figure 3**) has been reported previously from the interaction between fructose or glucose with glutamine, threo-

Table 1. Maillard and Other Important Compounds Found in Exudates of *A. tequilana* Weber Var. azul at Four Different Times during the Cooking Process^a

compound	KI ^b (k _r)	4 h	12 h	20 h	28 h	compound	KI ^b (k _r)	4 h	12 h	20 h	28 h
alcohols						organic acids					
1-butanol	(6.11)		0.37	0.43	0.23	acetic	200	14.03	6.13	22.82	2.60
3-methyl-1-butanol	(6.87)	0.42	1.35			propanoic	300	3.14	1.24	1.65	0.81
1-hexanol	(9.16)	0.41	0.88	0.82	0.95	butanoic	400	15.25	6.96	7.27	5.42
1-octen-3-ol	(10.85)		0.75	0.86	0.49	pentanoic	500	10.04	2.12	5.63	2.33
1,2-butanediol	345	6.14	1.18	1.97	1.04	hexanoic	600	6.30	4.66	13.03	5.38
1,3-butanediol	365	1.41	1.18	0.93	0.77	heptanoic	700	9.09	4.96	1.86	3.76
1,2-ethanediol	405	5.83	2.86	4.76	4.36	octanoic	800	9.65	3.10	8.17	7.02
benzyl alcohol	635	6.64	2.60	4.28	3.78	nonanoic	900	9.79	3.13	2.59	2.42
phenylethyl alcohol	666	14.70	3.73	6.26	4.58	decanoic	1000			12.03	
3-methylphenol	826		3.33	2.59	6.48	benzoic	1165	8.31	2.26	8.28	5.62
aldehydes						dodecanoic	1200	11.25	6.09	5.30	17.42
benzaldehyde	294		0.36	2.81		benzeneacetic	1304	13.11	5.49	41.61	12.81
phenylacetaldehyde	420		1.94		3.85	tetradecanoic	1400	36.78	11.73	13.92	20.06
vanillin	1186	19.08	17.73	23.82	16.15	pentadecanoic	1500	1.36	4.85	12.55	12.89
syringaldehyde	1656	5.98	11.72	17.81	17.05	3-phenyl-2-propanoic	1560	12.75	19.59	5.36	14.48
4-hydroxybenzaldehyde	1657	18.38	14.04	19.98	14.49	hexadecanoic	1600	5.01	32.23	73.72	86.28
amino acids and derivatives						heptadecanoic	1700	10.65		8.56	16.98
<i>N</i> -acetylalanine	(5.00)	1.00				octadecanoic	1800	5.98	17.41	43.46	50.18
furans						oleic	(59.56)	35.23	15.93	18.39	33.93
tetrahydro-2-methylfuran	(6.61)	0.18	0.24	0.18	0.44	pyrans					
5-ethenyltetrahydro- α,α -5-trimethyl-2-furanmethanol	(10.74)	0.93	1.79	1.65	3.53	2,3-dihydro-4(<i>H</i>)-pyran-4-one	287			2.19	1.91
furfural	230	6.90	6.42	11.02	14.87	maltol	718	9.38	7.08	26.01	7.59
1-(2-furanyl)ethanone	275	1.72	1.94	5.49	2.58	2,3-dihydroxy-3,5-dihydro-6-methyl-4(<i>H</i>)-pyran-4-one	999	25.18	22.23	17.29	72.67
5-methylfurfural	351				3.87	2,3-dihydro-2-methyl-4(<i>H</i>)-pyran-4-one	1022	17.40	5.55	58.87	13.09
2-furanmethanol	437		7.89	15.34	16.27	sulfur compounds					
2-furancarboxylic acid	710		1.23	7.38	1.88	4-methylthiazole	359	0.61		0.71	
methyl-2-furoate	756	16.67	10.88	12.20	54.09	2,3-dimethylthiophene	809	9.77			
5-(hydroxymethyl)furfural	1139	106.7	54.72	816.01	522.80	2,3-dihydrothiophene	845			7.84	
furanones						2,4,5-trimethylthiazole	1436		1.61		
dihydro-2-methyl-3(2 <i>H</i>)-furanone	(7.81)		0.59	0.63	2.11	4,5-dimethyl-2-propylthiazole	1438				7.07
dihydro-5-methyl-2(3 <i>H</i>)-furanone	393		1.53	2.04	0.77	2-cyclopropylthiophene	(59.61)		4.06		
3-methyl-2(5 <i>H</i>)-furanone	491			2.13	1.56	terpenes					
2(5 <i>H</i>)-furanone	528	5.01	1.39	6.26	2.68	linalool	305	19.15	31.18	8.24	24.19
ketones						linalyl propanoate	462	16.11	12.89	9.01	16.92
3-hydroxy-2-butanone	(8.25)	29.95	9.21	3.01	2.83	(<i>Z</i>)-geraniol	561	5.52	5.99	3.28	3.78
1-hydroxy-2-propanone	(8.55)	4.23	3.09	3.52	4.05	(<i>E</i>)-geraniol	606	9.71	14.46	3.81	3.54
butyrolactone	414	3.72	3.49	6.44	4.68	phytol	734	8.32	6.24	15.01	8.25
cyclotene	589	8.28	4.62	12.32	3.77	1-hydroxylinalool	1070	22.77	14.34	15.91	10.81
β -damascenone	1273		1.97			squalene	1738	25.06	4.93	8.27	21.07
nitrogen compounds											
pyridine	(6.92)	5.95	16.11	4.84	6.44						
2-hydroxypyridine	695			3.46	0.71						
1 <i>H</i> -pyrrole-2-carboxaldehyde	756			12.19	54.08						
2-pyrrolidinone	791				6.72						
4-ethyl-2-methylpyrrole	1171		4.04		6.97						
3,4-diethyl-2-methylpyrrole	1683	13.72									
2-methoxy-5-(2-methylpropyl)-pyrazine	1762		4.32								

^a Concentration of compounds in ppm. ^b Kovats index based on short and long fatty acids; k_r , retention time.

nine, or serine (4, 22–24). Meanwhile, butyrolactone is an important fructose degradation product (2), and its constant concentration could indicate continued fructose degradation to butyrolactone and thereafter to other compounds.

Maltol, 2,3-dihydroxy-3,5-dihydro-6-methyl-4(*H*)-pyran-4-one, and 2,3-dihydro-2-methyl-4(*H*)-pyran-4-one were among the most abundant compounds, reaching concentrations >60 ppm. According to some reports (4, 16, 17, 24, 25), 2,3-dihydroxy-3,5-dihydro-6-methyl-4(*H*)-pyran-4-one could be formed by the interaction between fructose or glucose with amino acids such as alanine, phenylalanine, serine, leucine, threonine, glycine, glutamine, or lysine and 2,3-dihydro-2-

methyl-4(*H*)-pyran-4-one from fructose–alanine interaction alone (4, 25).

Finally, the third group comprised compounds that increased throughout whole process. Furfural, maltol, and 5-(hydroxymethyl)furfural increased throughout the whole cooking time, and the latter compound was the most abundant (not shown in **Figure 4**), with a concentration of >4000 ppm. Its high concentration is reasonable and expected because it forms from dehydration and degradation of carbohydrates as well as from the Maillard reaction (26).

The Maillard reaction has been extensively investigated fundamentally in model systems. However, its complexity in

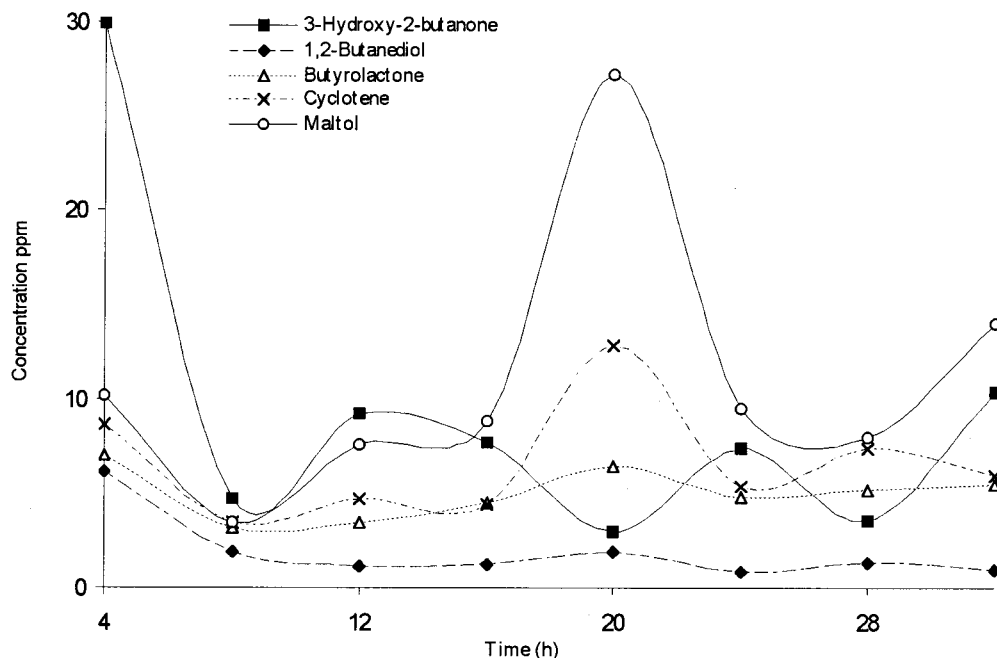


Figure 3. Time course of the formation of some representative minor Maillard compounds found in exudates obtained during cooking of agave pines. 3-Hydroxy-2-butanone and 1,2-butanediol might be precursors of other compounds.

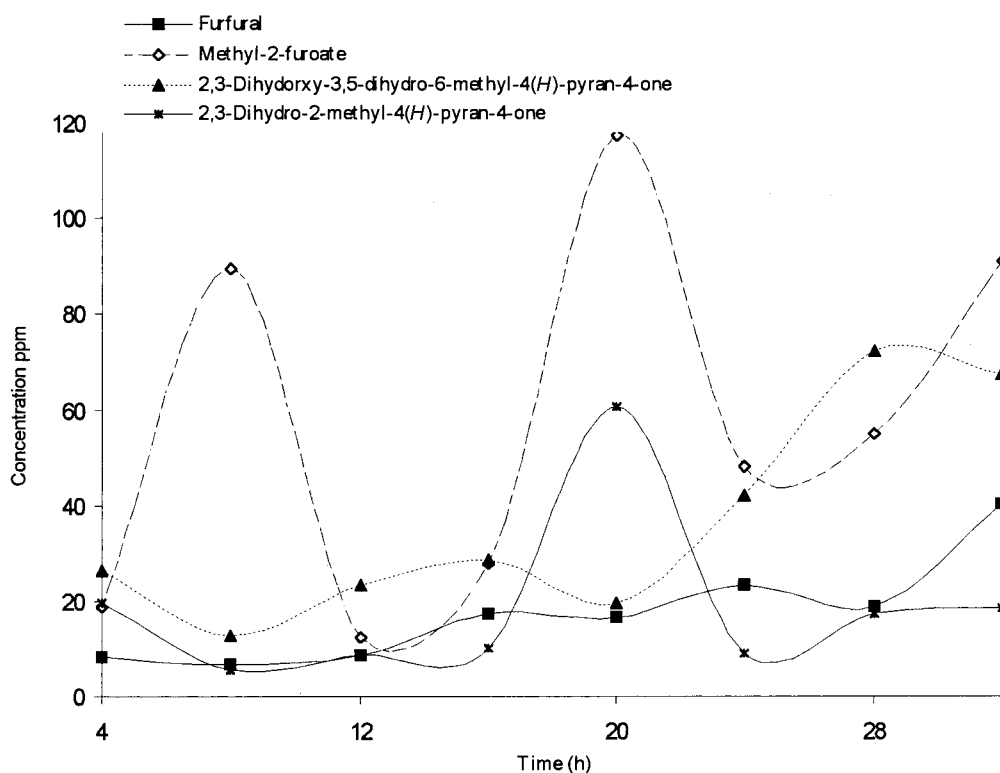


Figure 4. Profile changes of some major Maillard compounds generated during cooking of agave pines.

food systems due to the presence of many different reducing sugars and amino groups and multiple conditions can produce a wide variation of Maillard compounds. Also, a single compound might be generated by more than one pathway. The great abundance of fructose with respect to glucose in agave pines favors the Heyns rearrangement, and specific compounds might be used as markers to identify this type of rearrangement. Although fructose and glucose are able to generate the same type of compounds through isomerization, it is possible to distinguish between Heyns and Amadori rearrangements by measuring isotopic fractionation. Furfural has been shown to

be formed from the thermal processing of wheat, rye, barley, and chicory (27). All of these crops have in common fructans as reserve carbohydrates, similar to agave. Furfural might therefore be formed by the Heyns pathway.

Due to the larger amount of carbohydrates with respect to amino acids or proteins in agave, caramelization processes might also take place, generating α -dicarbonyl intermediates via retro-aldolization, which are precursors of oxygen heterocyclic compounds (15). However, the contribution of caramelization might be insignificant, and the presence of sulfur, amino heterocyclic molecules, and products of Strecker degradation

is indicative of the Maillard reaction being the main process during the cooking of agave pines. It is well-known that Maillard reaction products develop flavor and influence sensory characteristics of food systems. During tequila elaboration Maillard products are generated mainly during agave cooking, having an impact on the flavor characteristic of this beverage. Furans and pyrans impart sweet notes; benzaldehyde in the exudates adds green, floral, and flower notes with a threshold of 4 ppb; and phenylacetaldehyde has a flowery odor (19). Vanillin has a sweet, creamy, and vanilla odor, and β -damascenone is known for its woody, sweet, fruity, and floral descriptors. Many of these compounds have received much attention as biologically active constituents in foods for their antioxidative properties (28).

Many volatiles generated during the cooking stage are transformed to other compounds in the subsequent processes (fermentation, resting, and/or aging). However, some of them persist in the final product, influencing the overall tequila flavor. 3-Methyl-1-butanol and phenylethyl alcohol come from the Maillard reaction; however, their concentrations are higher in the product due to the fermentation process. Fatty acids do not show a constant level because acetic, propanoic, octanoic, and dodecanoic acids are present in higher concentrations in the product, whereas others such as hexanoic and tetradecanoic acids are most abundant in the exudates. On the other hand, furanoic compounds were lost during tequila production because their concentrations are notably lower than in exudates, except for 2-acetylfuran. The same pattern was observed for linalyl propanoate.

Benn and Peppard (18) reported that 3-methyl-1-butanol, linalool, phenylethyl alcohol, decanoic acid, vanillin, and β -damascenone are the most powerful odorants in tequila. These compounds are mainly generated during the cooking process, but some of them increased in subsequent steps. On the other hand, López (5) reported other Maillard compounds not present in the exudates studied here. Among these, 2-acetyl-5-methylfuran, 3-furfuryl alcohol, and 3,4,5-trimethylpyrazole are significant. Maillard compounds usually vary according to the type and concentration of reactant and also the reaction conditions (17, 29). Therefore, the differences in Maillard compounds can be due to cooking parameters used in each distillery, in addition to the quality and age of raw material, in this case *A. tequilana* Weber var. azul.

Inulin hydrolysis throughout the whole cooking process of *A. tequilana* Weber var. azul can be monitored by measuring °Brix, which reflects reducing sugars production. On the other hand, the role of fructose during the Maillard reaction can be followed by measuring the presence of many heterocyclic compounds containing oxygen, sulfur, and nitrogen. Additionally, the abundance of 5-(hydroxymethyl)furfural, furfural, and methyl-2-furoate provides information on the original concentration of carbohydrates compared to amino-bearing substances at the beginning of the process. Besides Maillard molecules, the cooking treatment also generated thermal breakdown products. Maillard compounds formed during cooking of agave pines are largely dependent on cooking parameters; therefore, the differences in the flavor characteristics between tequilas can be changed, directed, and/or controlled not only throughout the resting (reposado) and/or aging (añejo) process but also during the cooking step.

LITERATURE CITED

- Bedinghaus, A. J.; Ockerman, H. W. Antioxidative Maillard reaction products from reducing sugars and free amino acids in cooked ground pork patties. *J. Food Sci.* **1995**, *60*, 992–995.
- Shaw, P. E.; Tatum, J. H.; Berry, R. E. Base-catalyzed fructose degradation and its relation to non-enzymic browning. *J. Agric. Food Chem.* **1968**, *16*, 979–982.
- Ames, J. M.; Bailey, R. G.; Mann, J. Analysis of furanone, pyranone, and new heterocyclic colored compounds from sugar-glycine model Maillard systems. *J. Agric. Food Chem.* **1999**, *47*, 438–443.
- Chen, J.; Ho, C.-T. Comparison of volatile generation in serine/threonine/glutamine-ribose/glucose/fructose model systems. *J. Agric. Food Chem.* **1999**, *47*, 643–647.
- López, M. G. Tequila aroma. In *Flavor Chemistry of Ethnic Foods*; Shaidi, F., Ho, C.-T., Eds.; Plenum: New York, 1999; pp 211–217.
- Cedeño, M. Tequila production. *Crit. Rev. Biotechnol.* **1995**, *15*, 1–11.
- López, M. G.; Dufour, J. P. Tequila. Charm analysis of blanco, reposado, and añejo tequilas. In *Gas Chromatography-Olfactometry. The State of the Art*; Leland, J. V., Schieberle, P., Buettner, A., Acree, T. E., Eds.; American Chemical Society: Washington, DC, 2001; pp 62–72.
- Shu, C. K. Flavor components generated from inulin. *J. Agric. Food Chem.* **1998**, *46*, 1964–1965.
- López, M. G.; Mancilla-Margalli, N. A. Maillard compounds from the thermal processing of *Agave tequilana* Weber var. azul. In *Frontiers of Flavor Science*; Schieberle, P., Engel, K.-H., Eds.; Deutsche Forschungsanstalt für Lebensmittelchemie: Germany, 2000; pp 523–526.
- Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350–356.
- Somogyi, M. Notes on sugar determination. *J. Biol. Chem.* **1952**, *195*, 19–23.
- Somani, B. L.; Khanade, J.; Sinha, R. A modified anthrone-sulfuric acid method for the determination of fructose in the presence of certain proteins. *Anal. Biochem.* **1987**, *167*, 327–330.
- Apriyantono, A.; Ames, J. M. Xylose-lysine model systems: the effect of pH on the volatile reaction products. *J. Sci. Food Agric.* **1993**, *61*, 477–484.
- Brands, C. M. J.; Alink, G. M.; van Boekel, M. A. J. S.; Jongen, W. M. F. Mutagenicity of heated sugar-casein systems: effect of the Maillard reaction. *J. Agric. Food Chem.* **2000**, *48*, 2271–2275.
- Hollnagel, A.; Kroh, L. W. Degradation of oligosaccharides in nonenzymatic browning by formation of α -dicarbonyl compounds via a “peeling off” mechanism. *J. Agric. Food Chem.* **2000**, *48*, 6219–6226.
- Oberparleiter, S.; Ziegler, G. Amadori-verbindingen als aromastufen in kakao. *Nahrung* **1997**, *41*, 142–145.
- Negróni, M.; D’Agostina, A.; Arnoldi, A. Autoxidation in xylose/lysine model systems. *J. Agric. Food Chem.* **2000**, *48*, 479–483.
- Benn, S. M.; Peppard, T. L. Characterization of tequila flavor by instrumental and sensory analysis. *J. Agric. Food Chem.* **1996**, *44*, 557–566.
- Hofmann, T.; Munch, P.; Schieberle, P. Quantitative model studies on the formation of aroma-active aldehydes and acids by Strecker-type reactions. *J. Agric. Food Chem.* **2000**, *48*, 434–440.
- Garnero, J. Heterocyclic aroma compounds precursors. In *The Chemistry of Heterocyclic Flavouring and Aroma Compounds*; Vernin, G., Ed.; Ellis Horwood: Horwood, U.K., 1982; pp 17–71.
- Lizárraga-Guerra, R.; Helmut, G.; López, M. G. Identification of the most potent odorants in huitlacoche (*Ustilago maydis*) and austern pilzen (*Pleurotus* sp.) by aroma extract dilution analysis and static head-space samples. *J. Agric. Food Chem.* **1997**, *45*, 1329–1332.
- Chen, Y.; Xing, J.; Chin, C. K.; Ho, C. T. Effect of urea on volatile generation from Maillard reaction of cysteine and ribose. *J. Agric. Food Chem.* **2000**, *48*, 3512–3516.

- (23) Tressl, R.; Kersten, E. Formation of 4-aminobutyric acid specific Maillard products from [1-¹³C]-D-glucose, [1-¹³C]-D-arabinose, and [1-¹³C]-D-fructose. *J. Agric. Food Chem.* **1993**, *41*, 2278–2285.
- (24) Nishibori, S.; Bernhard, R. A. Formation of 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one from fructose and β -alanine under conditions used for baking. *J. Agric. Food Chem.* **1994**, *42*, 1080–1084.
- (25) Chen, J.; Ho, C.-T. Volatile compounds generated in serine-monosaccharide model systems. *J. Agric. Food Chem.* **1998**, *46*, 1518–1522.
- (26) Shimamura, T.; Ukeda, H.; Sawamura, M. Reduction of tetrazolium salt XTT by aminoreductone formed during the Maillard reaction of lactose. *J. Agric. Food Chem.* **2000**, *48*, 6227–6229.
- (27) Frank, O.; Hofmann, T. Characterization of key chromophores formed by nonenzymatic browning of hexoses and L-alanine by using the color activity concept. *J. Agric. Food Chem.* **2000**, *48*, 6303–6311.
- (28) Fuster, M. D.; Mitchell, A. E.; Ochi, H.; Shibamoto, T. Antioxidative activities of heterocyclic compounds in brewed coffee. *J. Agric. Food Chem.* **2000**, *48*, 5600–5603.
- (29) Wijewickreme, A. N.; Zrepcio, Z.; Kitts, D. D. Hydroxyl scavenging activity of glucose, fructose, and ribose-lysine model Maillard products. *J. Agric. Food Chem.* **1999**, *64*, 457–461.

Received for review August 2, 2001. Revised manuscript received October 26, 2001. Accepted October 29, 2001. We thank Conacyt for financial support. Part of this work has been presented during the 9th Weurman Flavour Research Symposium in Freising, Germany (1999).

JF0110295