Content of Free Amino Acids in Huitlacoche (*Ustilago maydis*)

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The free amino acid content of frozen and lyophilized huitlacoche (*Ustilago maydis*) was investigated. Huitlacoche or cuitlacoche is an edible corn smut fungus consumed in Mexico, and it is becoming internationally known as a delicacy. Free amino acids are crucial nonvolatile compounds involved in the overall taste of many food products. The components were isolated from the mushroom by extraction with methanol/chloroform/water at room temperature. A prepurification on a cation resin was performed before derivatization to *N*-trifluoroacetic-*N*-methyl ester form. The identification and quantitation were performed on a capillary gas chromatography and gas chromatography−mass spectrometry. Lysine (3.21 mg/g dry weight) was the most abundant amino acid followed by glycine, valine, leucine, and glutamic acid. Besides 14 common amino acids, γ-aminobutyric acid, ornithine, and tricholomic acid were also found. The concentrations of all amino acids were in the range of 0.08−3.21 mg/g.

Keywords: *Free amino acids; huitlacoche; Ustilago maydis; GC−MS; identification; quantitation*

INTRODUCTION

Peptides and free amino acids contribute highly to the overall food taste, although not to food odor. Several studies have been conducted to determine free amino acids in edible mushrooms such as *Agaricus bisporus* (Dijkstra and Wikén, 1976) and *Pleurotus ostreatus* (Bano and Rajarathnam, 1988). Among their major components are glutamic acid, alanine, and proline for *A. bisporus* and glutamic acid, ornithine, and aspartic acid for *P. ostreatus*.

Free amino acids play an important role in the taste of food, either as flavor precursors (Strecker degradation) or as key compounds in the umami taste, mainly glutamic and aspartic acids. Amino acids have also been related to aroma biogenesis; this subject has been investigated in vegetables such as tomato by Yu *et al.* (1967) and in fruit like banana by Tressl and Drawert (1973). Therefore, the free amino acid content in a food might explain, in a way, the wide difference of volatile constituents in fruits and vegetables.

The determination of free amino acids profile of mushrooms might be of great value for their nutritional, chemical, and biochemical composition. The aim of this work is to determine the type and amount of free amino acids in frozen and lyophilized huitlacoche by gas chromatography (GC) and gas chromatography−mass spectrometry (GC−MS).

MATERIALS AND METHODS

Sample. Huitlacoche (*Ustilago maydis*) was bought in the Ecatepec open markets in the State of Mexico. The galls were separated from the corn using a knife and mixed all together. The homogenized samples were frozen at −80 °C, half was kept at −80 °C, and the other half was lyophilized and stored at −4 °C under nitrogen. All reagents and solvents were analytical reagent grade. Instrument helium was high-purity grade and was passed through a molecular sieve and an oxygen trap.

Extraction Technique. Sample analysis was conducted as follows: frozen (5 g) and its equivalent of lyophilized (0.7 g) huitlacoche plus norvaline (internal standard 1.4 mg) were extracted with methanol/chloroform/water (48:20:12) at room temperature for 12 h and reextracted with 20 mL of water for 3.5 h. The extracts were combined and diluted to 100 mL. A 25 mL sample aliquot was extracted with 20 mL of $CHCl_3$ and concentrated to 5 mL and passed through a Bio-Rad resin H^+ 200−400 mesh.

Derivatization. The amino acids in the eluent were converted first to their methyl ester form with 600 μL of acetyl chloride in 2 mL of dry methanol, and then the esters were acylated with 1 mL of trifluoroacetic anhydride and 1 mL of CH_2Cl_2 at 75 °C for 1 h. Samples were evaporated under a stream of nitrogen and redissolved in 200 μL of CH_2Cl_2.

Gas Chromatography (GC). The derivatives of the free amino acids were separated in a HP5890 Series II gas chromatograph equipped with a flame ionization detector and a 25 m × 0.33 μm × 0.20 mm i.d. methyl silicone capillary column (HP-1). Operating conditions were as follows: N_2 carrier gas, 0.44 mL/min; detector, 250 °C; injector, 180 °C; injection volume, 1 μL. The column was held for 3 min at 60 °C and programmed at 6 °C/min to a final temperature of 230 °C for 40 min.

Gas Chromatography−Mass Spectrometry (GC−MS). Identification of the compounds was made by means of GC−MS using a HP-MS detector 5972. The MS spectra of the extract components were compared with the authentic standards. Quantitative determinations were made using norvaline as an internal standard, and correlation factors were calculated for all standards. The same operating conditions were used except for the column, which was a HP-5 MS.

RESULTS AND DISCUSSION

Figures 1 and 2 show the GC profiles for the standards and sample analyses as their *N*-trifluoroacetyl-*N*-methyl ester form. The derivatization procedure, especially the preparation of methyl esters instead of butyl esters, presented no problems. GC profiles on a HP-1 of standards and samples were also obtained (not shown). Table 1 presents the retention times observed for standards on GC and GC−MS analysis. Some amino

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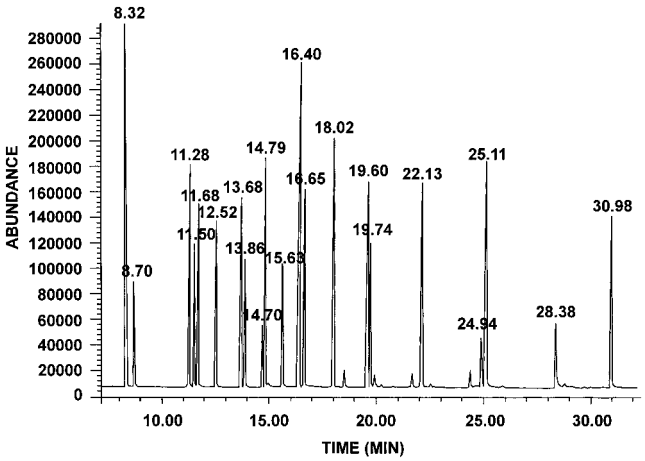

Figure 1. Gas chromatography−mass spectrometry profile of *N*-trifluoroacetyl-*N*-methyl esters of amino acid standards analysis.

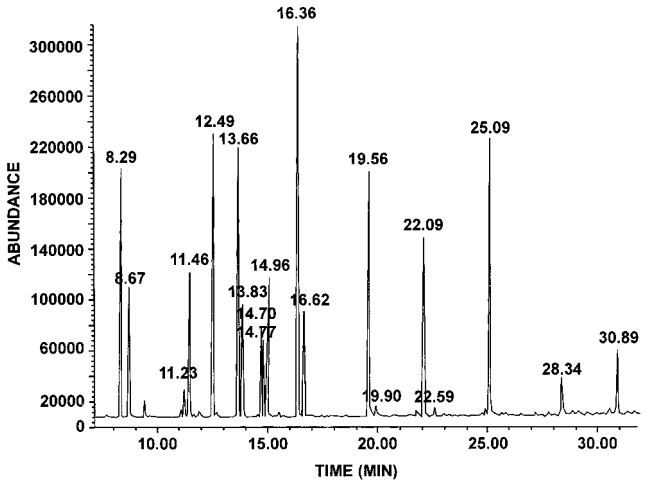

Figure 2. Gas chromatography−mass spectrometry profile of *N*-trifluoroacetyl-*N*-methyl esters of amino acids in huitlacoche (*Ustilago maydis*).

acids such as tyrosine and serine gave two peaks on a GC−MS analysis; however, tyrosine also gave two peaks on the GC run. This was due to the generation of mono- and ditrifluoroacetylated forms due to the presence of an hydroxide group in these two compounds. On the other hand, asparagine and glutamine coeluted with aspartic and glutamic acid on both GC runs, presenting retention times of 16.525 and 19.776, respectively. A disadvantage of the GC studies was that proline also coeluted with aspartic acid; however, this was not an inconvenience for the GC−MS studies that permitted the quantitation of both amino acids separately, as the two amino acids did not coeluted during these studies. Arginine (28.052) and cystine (33.420) were only observed in the GC profile but not in the GC−MS runs. An advantage of the GC−MS runs was that this tandem technique allowed the identification of three nonproteic amino acids such as γ-aminobutyric acid (14.96), ornithine (22.59), and tricholomic acid (30.89). Most of these compounds were identified by comparing their mass spectra with the authentic compounds, the library in the GC−MS, or the mass spectra reported by Leimer *et al.* (1977). Table 2 shows the response factors based on GC studies; these factors agree with data published by Islam and Darbre (1972). The determination of the response factors was necessary for accuracy and precision in the amino acids quantitation since their large structural differences could lead to quantitation errors.

Table 1. Retention Times for *N*-Trifluoroacetyl-*N*-methyl Esters of Amino Acid Standards in Gas Chromatography[a] and Gas Chromatography−Mass Spectrometry[b] Analysis

amino acid	GC	GC−MS
alanine	8.53	8.32
glycine	8.61	8.70
threonine	11.59	11.28
serine	11.79	11.68
		14.70
valine	11.93	11.50
norvaline	12.90	12.52
leucine	14.13	13.68
isoleucine	14.36	13.86
norleucine	14.76	14.79
cysteine	15.76	15.63
aspartic acid	16.52	16.40
proline	16.52	16.65
hydroxiproline	18.02	18.02
glutamic acid	19.77	19.60
methionine	19.96	19.74
phenylalanine	22.48	22.13
tyrosine	25.19	24.94
	28.74	28.38
lysine	25.40	25.11
arginine	28.05	
tryptophan	31.56	30.98
cystine	33.42	

[a] GC. [b] GC−MS.

Table 2. Response Factors for *N*-Trifluoroacetyl-*N*-methyl Esters of Amino Acid Standards by Gas Chromatography Analysis[a]

amino acid	R_t	I_a	R_f
alanine	8.533	0.85	1.13
glycine	8.619	0.90	0.18
threonine	11.59	0.65	0.68
serine	11.79	0.75	0.48
valine	11.93	0.75	0.69
norvaline	12.90	0.50	1.00
leucine	14.13	0.65	1.10
isoleucine	14.36	0.65	0.68
norleucine	14.76	0.80	0.93
cysteine	15.76	0.80	0.33
aspartic acid	16.52	0.75	1.09
proline	16.57	0.75	0.81
hydroxyproline	18.02	0.85	0.73
glutamic acid	19.77	0.65	0.80
methionine	19.96	0.60	0.76
phenylalanine	22.48	0.60	1.39
tyrosine	25.19	0.70	0.94
lysine	25.40	0.70	0.65
arginine	28.05		
tryptophan	31.56	0.70	0.89
cystine	33.42		

[a] R_t, retention time in min. I_a, injected amount in μg. R_f, response factor relative to the standard.

The total free amino acid values were 21.62 and 20.91 mg/g on a dry matter basis (Table 3) for frozen and lyophilized huitlacoche, respectively. This analysis revealed that huitlacoche contains almost all of the essential amino acids and many nonessential amino acids. Essential amino acids comprise 10.24 mg/g of the total amino acids determined.

Lysine (3.21 mg/g) was the most abundant amino acid, amounting to 14.84% of the total amino acids. Other abundant amino acids included glycine, leucine, and aspartic acid, which collectively accounted for 29.97% of the total amino acids in the samples. The low methionine levels reported for frozen and lyophilized huitlacoche tally with those reported by Bano and Rajarathnam (1988) of 0.23 mg/g and Dijkstra and Wikén (1976) of zero. On the other hand, huitlacoche

Table 3. Composition of Free Amino Acids[a] in Frozen and Lyophilized Huitlacoche (mg/g Dry Basis)

amino acid	frozen	lyophilized
alanine	1.05 ± 0.20	1.30 ± 0.06
glycine	2.44 ± 0.18	2.63 ± 0.05
threonine	0.62 ± 0.24	0.65 ± 0.10
serine	1.02 ± 0.30	0.98 ± 0.27
valine	1.46 ± 0.10	1.59 ± 0.05
leucine	2.24 ± 0.60	2.00 ± 0.06
isoleucine	1.32 ± 0.09	1.40 ± 0.10
γ-aminobutyric acid	1.19 ± 0.30	0.75 ± 0.38
aspartic acid	1.80 ± 0.20	1.83 ± 0.12
proline	0.75 ± 0.20	0.66 ± 0.04
glutamic acid	1.90 ± 0.50	1.79 ± 0.23
methionine	0.15 ± 0.06[b]	0.06 ± 0.01[b]
phenylalanine	1.16 ± 0.22	1.06 ± 0.05
ornithine	0.08 ± 0.01	0.08 ± 0.05
lysine	3.21 ± 0.60	2.97 ± 0.20
tyrosine	1.00 ± 0.10	0.80 ± 0.26
tricholomic acid	0.23 ± 0.10	0.36 ± 0.32

[a] Mean values and standard error of six independent determinations. [b] Statistically significant (0.025 ± 0.001).

Table 4. Comparison of Free Amino Acids in Huitlacoche (*U. maydis*), *Agaricus bisporus*, and *Pleurotus ostreatus* (mg/g Dry Basis)

amino acid	*U. maydis*[a]	*A. bisporus*[b]	*P. ostreatus*[c]
alanine	1.31	4.20	2.21
glycine	2.63	0.35	0.77
threonine	0.65	0.96	1.28
serine	0.98	1.18	1.19
valine	1.59	1.09	1.26
leucine	2.00	1.78	1.30
isoleucine	1.40	1.20	0.54
γ-aminobutyric acid	0.75	3.33	0.53
aspartic acid	1.83	1.33	2.44
proline	0.66	3.45	0.52
glutamic acid	1.79	4.71	5.84
methionine	0.06		0.23
phenylalanine	1.06	0.76	1.08
ornithine	0.08		3.68
lysine	2.97		1.32
tyrosine	0.80		1.87
tricholomic acid	0.36		
total	21.62	24.34	26.06

[a] This work. [b] Dijkstra and Wikén (1976). [c] Bano and Rajarathnam (1988).

extracts were deficient in sulfur-containing amino acids. Deficiencies in sulfur-containing amino acids have been observed in many other mushrooms. The amino acid γ-aminobutyric has been found in a wide variety of plants, and it is relevant to record its presence in huitlacoche even if its value is much lower than that reported for *A. bisporus* and some what larger than that for *P. ostreatus*. This amino acid is not a constituent of protein hydrolysates, nor are ornithine (0.08 mg/g) and tricholomic acid (0.36 mg/g), which were also found in huitlacoche.

Among the 17 amino acids found in huitlacoche, only the methionine content presented differences between extracts, 0.15 mg/g for frozen and only 0.06 mg/g for lyophilized huitlacoche. These differences could be due to losses during the derivatization or the lyophilization process damaged the sample to some extent.

Table 4 shows that the total content of free amino acids in huitlacoche is quite similar to previous work on *A. bisporus* by Dijkstra and Wikén (1976) and *P. ostreatus* by Bano and Rajarathnam (1988), which reported 24.34 and 26.06 mg/g, respectively. However, the number and relative amounts of free amino acids present in huitlacoche are different from other mushrooms. Qualitatively, there are no big differences; nevertheless, quantitatively the differences are quite large.

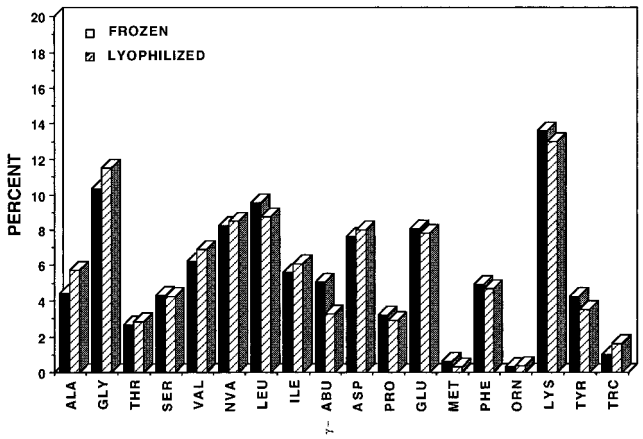

Figure 3. Profile of the percent of amino acids in frozen and lyophilized huitlacoche (*Ustilago maydis*).

Finally, Figure 3 shows the amino acid composition of frozen and lyophilized samples on a percent basis. In this bar graph, the main amino acids in the samples were lysine, glycine, leucine, aspartic acid, glutamic acid, and valine; all of these represent 56% of the total free amino acids. Under these experimental conditions, there do not appear to be any substantial differences between frozen and lyophilized huitlacoche. However, a statistical difference was found for methionine (Table 3).

No references were found regarding determination of the free amino acids in huitlacoche. However, Valverde and Paredes-López (1993) reported that the most abundant amino acids from huitlacoche proteins are aspartic acid, glutamic acid, serine, and lysine. Richards and Haskings (1957), Kurtz and Ericson (1962a,b), and Sánchez-Marroquín *et al.* (1969) reported that fermentation of *Ustilago maydis* strains produced mainly lysine and threonine. It is important to point out that huitlacoche extracts contain three of the four amino acids related with the umami taste; these four amino acids are glutamic, aspartic, tricholomic, and ibotenic acids (Nishimura and Kato, 1988).

Conclusions. Huitlacoche contains important amounts of flavoring amino acids that might play a significant role in its flavor. From the data obtained, it appears that huitlacoche may be a valuable supplement for the human diet, although it is mostly eaten for its flavor. Frozen and lyophilized samples presented significant differences only for methionine.

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