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Furosine as Indicator of Maillard Reaction in Jams and Fruit-Based Infant Foods

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To investigate the presence of furosine in commercial samples of jams and fruit-based infant foods a simple method by ion-pair reversed-phase liquid chromatography is described. The yield of furosine during the hydrolysis with hydrochloric acid was optimized. The reproducibility in the repeatability and recovery of the method, expressed in relative standard deviation percentages, proved to be in the ranges of 4.1−8.3% and 1.0−4.4%, respectively. The recovery percentages of furosine varied between 86.7 and 95.3%. The obtained results support the suitability of the method. Furosine was detected in all studied samples. Although a high variability in the content of furosine was noticed, in general terms, the lowest levels of furosine were observed in samples of fruit-based infant foods and the highest were observed in jams of more than 60% sugar. These results could be due to different heat treatment, storage conditions, and/or differences in the values of water activity (a_w) and amounts of sugar. The results obtained in the present paper point out the usefulness of furosine as an indicator of Maillard reaction for jams and fruit-based infant foods.

KEYWORDS: Furosine; jams; fruit-based infant foods; quality

INTRODUCTION

Jams can be made from fresh fruits or intermediary products, such as pulps or slurries, by adding sugar and other ingredients (gelling agents, starch syrup, and acids) (*1*). Regarding the content of fruit and sugar, jams can be classified as follows: (a) standard jam (minimum 35% fruit as pulp and/or puree by mass and minimum 60% sugar by mass); (b) extra jam (minimum 45% fruit as pulp by mass and minimum 60% sugar); or (c) reduced sugar jam (minimum 35% fruit by mass and 30−55% sugar). In the case of standard jam the addition of fruit juice is also allowed (*2*, *3*).

Usual methods for jam manufacture include boiling at atmospheric pressure or under vacuum (*4*). In the first case, the ingredients are boiled in open kettles until the thickening of the products is achieved, in general, after 15−30 min (*1*). When an intense heat treatment is applied to remove the excess of water, the organoleptic properties of jam can be adversely affected (*5*). However, the process of boiling ingredients under controlled vacuum conditions reduces the time and the temperature of treatment, ensuring a better product quality. In some cases, a thermal treatment is carried out to stabilize the final product.

Fruit-based infant foods are manufactured similarly, with fruits that are triturated, mixed with sugar (in lower proportion than in jams), and other minor ingredients. To stabilize the product, the mixture is submitted to a thermal treatment (*6*).

Heat processing during manufacture and storage under inappropriate conditions induce a number of chemical changes, such as sugar caramelization and Maillard reaction that contribute to their characteristic flavor and color (*1*). The formation of Amadori compounds that takes place during the early stages is considered as the key step of the Maillard reaction. Evaluation of the early stages of this reaction can be achieved by the determination of furosine, formed during the acid hydrolysis of the Amadori compound, ϵ-deoxy-L-fructosyl-lysine (*7*). Although furosine has been proved to be a good quality indicator in dairy products (*8*, *9*), eggs (*10*), baby cereals (*11*), pasta (*8*), tomato products (*12*, *13*), soybeans, barley, and malt (*14*), honey (*15*), and treated meat products (*16*), no data have been previously reported either on the presence of Amadori compounds, or on the usefulness of furosine as quality indicators in jams and fruit-based infant foods.

The aim of the present study was to investigate the presence of furosine in commercial samples of jams from different fruits and with different levels of fruit and sugar, as well as in fruit-based infant foods, to assess the usefulness of furosine as an indicator of the Maillard reaction for this kind of products.

MATERIALS AND METHODS

Samples. Twenty samples of jams from various types of fruits (orange, lemon, apple, apricot, mulberry, bilberry, fig, pineapple, plum, strawberry, banana, mixture of fruits, and tropical fruits), with a shelf life of 1−3 years, were collected at the Spanish market: 10 samples with a content of sugar ≥ 60% (3 standard and 7 extra jams) and 10 samples with reduced sugar content (40−55%). Special attention was paid to peach jam, and thus, 18 peach samples were also analyzed: 6 extra jams (≥ 60% of sugar) and 12 reduced-sugar jams (31−55% of

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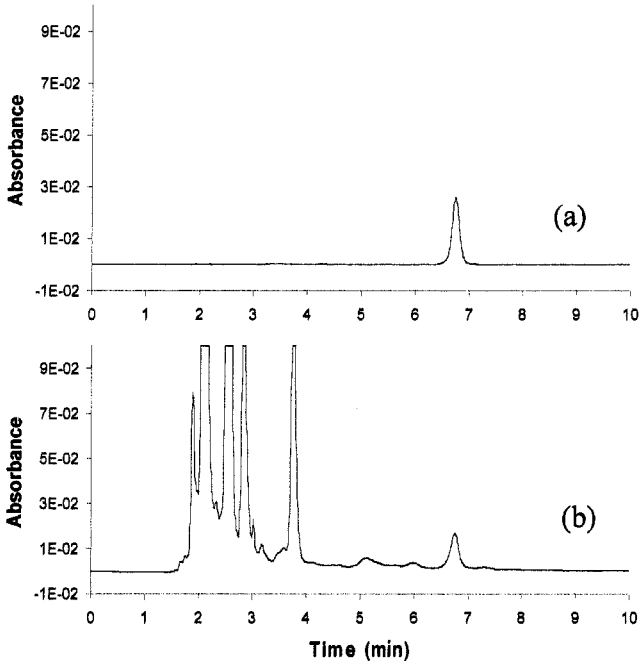

Figure 1. HPLC chromatograms of a standard of furosine (a) and an acid hydrolyzate of a commercial jam of fig (b).

sugar). In addition, 18 commercial samples of fruit-based infant foods (different fruits and mixtures of them) were also studied. Prior to analytical determinations, all samples were homogenized using an Ultra-turrax homogenizer (Janke & Kunkel Ika-Werk).

Analytical Determinations for the Characterization of Samples. Determinations of pH, a_w, moisture, and protein content were performed. The pH of samples was measured in a pH meter MP 225 with glass electrode (Mettler-Toledo GmbH, Schwerzenbach, Switzerland). Water activity was determined at 25 °C using a Novasina a_w Sprint TH-500 (Pfäffikon, Switzerland) previously calibrated with saturated solutions of different salts. Moisture was analyzed using the AOAC method 920.151 (*17*). Total nitrogen (TN) content was determined by means of AOAC (Kjeldahl) method 920.152 (*18*), and the protein level was calculated using 6.25 as conversion factor (TN × 6.25). All determinations were carried out in duplicate and the results were expressed as mean values.

Quantitation of Furosine. The quantitation of furosine was performed by HPLC analysis. The preparation of samples was carried out using a slight modification of the method for dairy products of Resmini et al. (*19*) as follows: samples (1 g) were hydrolyzed under inert conditions (helium) with 6 mL of 8 N HCl at 110 °C for 23 h in a screw-capped Pyrex vial with PTFE-faced septa. The hydrolyzate was filtered with a medium-grade paper filter. A 0.5-mL aliquot of the filtrate was applied to a Sep-Pack C_{18} cartridge (Millipore) pre-wetted with 5 mL of methanol and 10 mL of water, then eluted with 3 mL of 3 N HCl and evaporated at 40 °C under vacuum. The dried sample was dissolved in 1 mL of the mobile phase before HPLC analysis.

Chromatographic determination of furosine was carried out following the method of Delgado et al. (*20*), using a Spherisorb ODS(2) 5-μm column (250 mm × 4.6 mm; Phenomenex, Torrance, CA) at 25 °C. The mobile phase consisted of a solution of 5 mM sodium heptane sulfonate with 20% acetonitrile and 0.2% formic acid. The elution was isocratic and the flow rate was 1.2 mL/min. The UV detector was set at 280 nm and the injection volume was 50 μL. Quantitation was performed by the external standard method, using a commercial standard of pure furosine (Neosystem Laboratoire, Strasbourg, France). All analyses were done in duplicate and the data were the mean values expressed as mg/100 g protein.

Identification of Furosine. The identity of furosine was confirmed by HPLC−MS. Samples of jams and fruit-based infant foods (20 g) were dialyzed against water. The retentates were lyophilized, hydrolyzed with 6 mL of 8 N HCl, and treated as described above. HPLC−MS analyses were performed at ambient temperature on a Hewlett-Packard 1100 liquid chromatograph working in electrospray ionization mode, under atmospheric pressure and positive polarity (API-ES positive). Chromatographic conditions were as follows: column C_{18} Spherisorb ODS(2), 5 μm (250 mm × 4.6 mm; Phenomenex); mobile phase, water/acetonitrile/formic acid (79.8:20:0.2), at a flow rate of 0.7 mL min^{-1}.

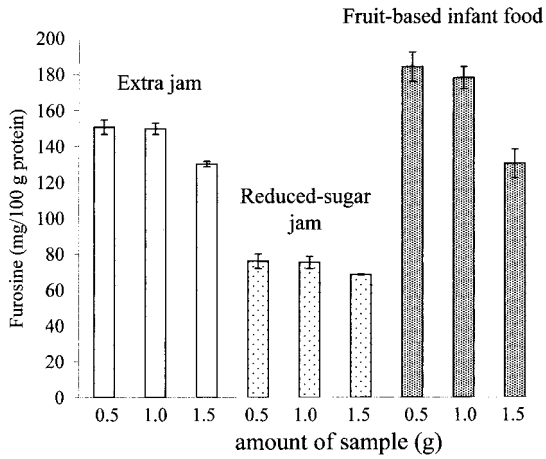

Figure 2. Effect of different amounts of sample on the formation of furosine in an extra and reduced-sugar jam and a fruit-based infant food.

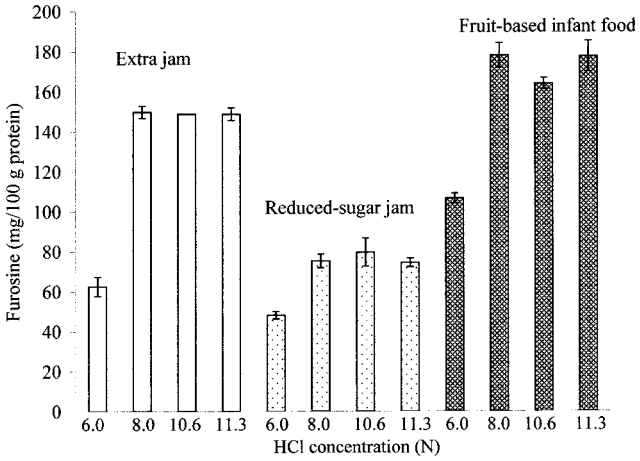

Figure 3. Effect of different concentrations of HCl on the formation of furosine in an extra and reduced-sugar jam and a fruit-based infant food.

RESULTS AND DISCUSSION

Figure 1 illustrates the HPLC chromatograms corresponding to a standard of furosine (**a**) and an acid hydrolyzate of a commercial jam (**b**). As observed, furosine was eluted in less than 7 min, and no interfering peaks were present at the furosine retention time. Peak identity was assigned by retention time, spike of standard to samples, and confirmed by HPLC−MS using the ion *m*/*z* 255 corresponding to furosine $[M + H]^+$ for selective monitoring.

With the aim of optimizing the yield of furosine during the hydrolysis with HCl, different amounts of sample (**Figure 2**) and concentrations of HCl were assayed (**Figure 3**). No differences were observed in the quantity of furosine formed when 0.5 and 1 g of sample were used, whereas a lower yield of furosine was detected in the case of the highest amount of sample assayed (1.5 g). With respect to the influence of the HCl concentration, as shown in **Figure 3**, the yield of furosine increased as the concentration of HCl increased from 6 to 8 N (*21*), but not with further increase to 10.6 or 11.3 N. According to these results, acid hydrolysis of samples was performed using 1 g of sample in 8 N HCl.

Table 1. Repeatability ($n = 5$) for Furosine Determination in Samples of Jams and Fruit-Based Infant Foods

	chromatographic repeatability		method repeatability	
sample	content (mg/100 g protein)	RSD (%)[a]	content (mg/100 g protein)	RSD (%)[a]
extra jam (peach)	154.6	2.4	142.8	8.3
reduced sugar jam (peach)	72.8	1.8	73.7	7.7
fruit-based infant food (several fruits)	175.9	4.5	172.4	4.1

[a] RSD, relative standard deviation.

Table 2. Recovery Percentage of Furosine (mg/100 g protein) in the Analysis of Jams and Fruit-Based Infant Foods

sample	amount in the sample	added amount	detected amount	recovery (%)
extra jam (apricot)	96.4	142.6	222.9	93.3
	96.4	285.2	348.6	91.3
	96.4	427.7	481.7	91.9
				92.2 ± 1.0
reduced sugar jam (peach)	51.5	126.4	164.3	92.3
	51.5	252.8	287.2	94.4
	51.5	379.3	403.0	93.5
				93.4 ± 1.0
fruit-based infant food (apple)	43.0	66.6	95.0	86.7
	43.0	133.2	168.0	95.3
	43.0	166.5	194.2	92.7
				91.6 ± 4.4

Table 3. Content of Furosine (mg/100 g protein) and Other Parameters in Commercial Jams

jams	fruit (%)[a]	sugar (%)[a]	pH	moisture (%)	a_w	protein (%)	furosine (mg/100 g protein)
				standard			
orange (sour)	35	63	3.31	33.4	0.854	0.227	119.4
orange (sweet)	35	63	3.22	32.7	0.847	0.237	137.3
lemon	35	63	3.30	32.3	0.819	0.107	186.7
				extra			
apple	50	63	3.54	34.3	0.854	0.294	72.6
apricot	50	60	3.34	32.4	0.823	0.323	104.5
mulberry	50	60	3.18	36.2	0.850	0.479	153.2
mixture of fruits	50	63	3.11	33.8	0.825	0.348	212.1
bilberry A	50	60	3.22	33.8	0.836	0.331	292.7
bilberry B	50	60	3.24	36.8	0.845	0.256	424.2
fig	50	60	4.39	36.6	0.874	0.556	448.3
				reduced sugar			
pineapple A	60	40	3.60	59.1	0.926	0.324	51.2
plum	45	54	3.40	43.2	0.890	0.393	66.3
pineapple B	48	52	3.65	47.1	0.918	0.313	71.2
orange (sweet)	45	53	3.31	43.8	0.900	0.331	75.9
apricot	55	48	3.41	50.2	0.925	0.465	79.3
strawberry	57	47	3.50	49.6	0.919	0.381	81.7
mixture of fruits	57	47	3.54	50.9	0.922	0.470	92.0
banana	60	-	4.45	63.0	0.939	0.682	168.5
tropical	45	55	3.54	41.1	0.870	0.283	186.7
lemon	40	50	3.24	48.6	0.903	0.201	224.3

[a] Manufacturer-reported values.

The chromatographic repeatability was evaluated by repeated injections ($n = 5$) of the same sample in different days; the repeatability of the entire method (including acid hydrolysis, sample preparation, and HPLC analysis) was determined by analyzing five aliquots of the same sample (**Table 1**). In all cases, the relative standard deviations (RSDs) were lower than 8.5%.

To test the recovery of furosine, known amounts of standard furosine were added to acid hydrolysates of commercial samples of jam (extra and reduced sugar) and fruit-based infant food (**Table 2**). The recovery range was 91.6−93.4% (RSD lower than 5%).

Table 4. Content of Furosine (mg/100 g protein) and Other Parameters in the Peach Jams

jams	fruit (%)[a]	sugar (%)[a]	pH	moisture (%)	a_w	protein (%)	furosine (mg/100 g protein)
				extra			
A	50	60	3.13	36.9	0.862	0.343	149.8
B	50	63	3.41	34.2	0.833	0.307	186.4
C	50	63	3.50	35.5	0.835	0.371	263.2
D	50	60	3.22	36.5	0.856	0.366	297.1
E	50	63	3.47	35.1	0.841	0.337	363.8
F	50	63	3.42	32.6	0.805	0.256	629.3
				reduced sugar			
G	50	31	3.73	65.3	0.937	0.418	15.1
H	55	40	3.60	60.6	0.924	0.336	31.7
I	45	55	3.31	43.6	0.898	0.391	42.8
J	55	42	3.56	58.8	0.920	0.427	55.1
K	55	48	3.47	50.3	0.921	0.447	75.3
L	50	44	3.68	57.3	0.942	0.127	85.8
M	50	40	3.58	59.3	0.941	0.357	108.6
N	50	45	3.58	53.6	0.928	0.335	119.5
O	-	55	3.46	39.5	0.868	0.375	127.4
P	50	40	3.63	58.4	0.937	0.291	137.9
Q	57	47	3.97	50.2	0.920	0.300	148.0
R	50	44	3.64	56.7	0.929	0.274	335.4

[a] Manufacturer-reported values.

Table 5. Content of Furosine (mg/100 g protein) and Other Parameters in the Fruit-Based Infant Foods

sample	pH	moisture (%)	a_w	protein (%)	furosine (mg/100 g protein)
1	3.98	72.0	0.970	0.574	44.0
2	4.01	80.5	0.977	0.488	44.2
3	3.77	81.6	0.980	0.542	46.1
4	3.89	83.8	0.981	0.344	47.0
5	3.71	84.5	0.978	0.426	53.0
6	4.25	81.2	0.982	0.786	56.5
7	3.93	74.1	0.971	0.651	56.8
8[a]	4.03	78.4	0.975	0.405	58.8
9	3.97	79.3	0.973	0.472	63.1
10	4.04	84.3	0.980	0.445	67.4
11	3.90	74.3	0.974	0.658	69.0
12[a]	4.20	73.7	0.970	0.726	75.1
13[a]	4.01	78.8	0.976	0.661	78.5
14	3.98	73.4	0.962	0.678	85.3
15[a]	3.84	79.2	0.976	0.248	88.7
16[a]	4.38	79.8	0.976	0.546	89.2
17[a]	4.05	81.8	0.976	0.914	95.2
18[a]	4.14	76.9	0.970	0.421	178.0

[a] Samples manufactured with juice from citrus fruits.

Levels of furosine in commercial samples of jams and fruit-based infant foods along with pH, moisture, a_w, and protein content, are presented in **Tables 3**−**5**. Jam samples showed pH values in the range 3.11−3.97, except two jam samples, one from banana and another one from fig, which had pH values around 4.4. As expected, samples with reduced sugar content showed the highest moisture and a_w values. Similar values of pH, moisture, a_w, and protein have been previously reported in jams from different types of fruit (*1*, *22*−*26*). In general terms, fruit-based infant foods (**Table 5**) presented higher values of pH, moisture, a_w, and protein than jams.

Furosine was detected in all studied samples. A wide variation in their furosine content was observed, which might be attributed to different processing conditions or composition. The formation of furosine in jams and fruit-based infant foods may take place

during the heat treatments to which products are submitted in the manufacture or during the storage before consumption, if the storage temperature is inappropriate.

Samples of jams with the lowest levels of furosine might have been treated under mild conditions, probably boiling under vacuum, whereas the highest furosine values may correspond to samples manufactured under severe conditions of heat treatment or stored at high temperature. Considering jam samples obtained from fruits other than peach (**Table 3**), furosine concentration ranged from 51.2 to 224.3 mg/100 g protein (average 109.7 mg/100 g protein) for reduced sugar jam and from 119.4 to 448.3 mg/100 g protein (average 215.1 mg/100 g protein) for jams with a content of sugar higher than 60%. To eliminate the possible variability due to the type of fruit, a study on jams made only from peach was carried out (**Table 4**). A similar trend was observed, with reduced-sugar jam samples having the lowest levels of furosine.

Although a high variability in the content of furosine was noticed, in general terms, reduced sugar jams presented lower amounts of furosine than samples of jams with more than 60% sugar. This may be, in part, due to the different availability of sugar responsible for Maillard reaction and to the higher a_w of the reduced sugar samples. Previous studies on Maillard reaction in model systems adjusted to different a_w have shown that a decrease in a_w from 0.90 to 0.60 resulted in increased browning rate, and Maillard reaction did not take place when moisture level was greater than 50% or a_w 0.95 (*27*). Because the Maillard reaction is favored at high pH (*28*), the considerable furosine content found in fig and banana jams may be in part attributed to their relatively high pHs. Differences in furosine content among samples with similar a_w and pH values may be due to the heat treatment conditions during manufacture. Lowest values may indicate mild processing conditions (low temperature under atmospheric pressure, sterilization under vacuum, etc.), whereas higher values are indicative of severe processing conditions.

Fruit-based infant foods (**Table 5**) showed furosine values in the range of 44.0 to 178.0 mg/100 g protein and most of the samples presented values of furosine lower than 80 mg/100 g protein. In general terms, these values were lower than those found in jams, probably due to a lower sugar content, less severe manufacturing conditions, and higher a_w of samples. Samples 8 and 12−18, prepared with added citrus juice, showed high amounts of furosine. Del Castillo et al. (*29*) demonstrated the presence of 2-furoylmethyl derivatives of amino acids in samples of orange juice elaborated from concentrate, and proposed these parameters as indicators of Maillard reaction during the manufacture and/or storage of orange juice concentrate.

CONCLUSIONS

The method described in this article is suitable for the determination of furosine in jams and fruit-based infant foods. Although more detailed studies are needed on the formation of furosine in these products, the present results point out the usefulness of this compound as one of the quality indicators in jams and fruit-based infant foods.

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