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Occurrence of Biogenic Amines and Polyamines in Spinach and Changes during Storage under Refrigeration

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Biogenic amines and polyamines were studied in 18 market samples of spinach. Histamine and spermidine were detected in relatively high amounts in all samples within the ranges of 9.5–69.7 and 15.6–53.0 mg/kg, respectively. Other biologically active amines were either detected at low levels or not found at all. Changes in amine content during storage at 6 °C were studied. The content of most of the amines remained constant during storage, with the exception of spermidine and histamine. Spermidine showed a clear decreasing trend, whereas histamine significantly increased in all trials, but decreased at the end of the storage in two of the trials. Trials showing a decrease in histamine content also showed the highest spermidine decrease and recorded the highest pH values. Microbial loads throughout storage were also followed, with *Pseudomonadaceae* and *Enterobacteriaceae* being the predominant bacterial groups. Trials with higher microbial loads in initial samples also showed the highest histamine content in these samples. Potential explanations for both the formation and the degradation of histamine during storage are discussed.

KEYWORDS: Histamine; biogenic amines; polyamines; spinach

INTRODUCTION

Biologically active amines include both biogenic amines (BAs) and polyamines (PAs). BAs are aliphatic, aromatic, or heterocyclic compounds of low molecular weight occurring in numerous foods as a result of metabolic processes in animals, plants, and microorganisms. Several toxicological problems resulting from the ingestion of food containing relatively high levels of BAs have been described (*1–3*). Histamine and tyramine have been the most studied biogenic amines due to their psychoactive and vasoactive properties, which can cause adverse effects, especially in individuals in which the usual detoxification systems for these amines [diamine oxidase (DAO) and monoamine oxidase (MAO)] are inhibited by genetic enzyme deficiency or by pharmacological blockage (*4, 5*). In high concentrations, histamine could cause direct effects in the human cardiovascular system (hypotension, palpitation), characteristic skin diseases (urticaria, edema, and localized inflammation), and gastrointestinal diseases (nausea, vomiting, diarrhea, stomach cramp) (*3*). Tyramine can trigger migraines caused by vasoactive mechanisms and it is also associated with hypertensive crises (*6, 7*).

BAs in food are mainly produced by decarboxylation of the precursor amino acids by specific microbial enzymes. However, the presence of PAs in food, as spermine and spermidine, results from a more complex biosynthesis and is not related to microbial activity. Also low amounts of the diamine putrescine, precursor of PAs, can be considered to be of physiological origin (*8*). PAs are essential for cell multiplication and can also protect against oxidative stress, similar to other food compounds with well-recognized antioxidant activity (*9, 10*).

BAs can be found in relatively large amounts in some fermented foods of plant origin, such as sauerkraut, soy sauces, or beer, basically as a consequence of microbial activity (*11, 12*). In addition, the widespread presence of PAs and some diamines in fresh plant foods have also been described. In fact, fruit and fruit juices are particularly rich in putrescine, while green vegetables are rich in spermidine (*13*). Generally, in plants spermine is present in lower amounts than spermidine, in contrast with foods of animal origin (*14*).

Fish, cheese, and red wine are foods commonly related to high histamine content (*15–17*). In 1983, Feldman (*18*) reported spinach as a food rich in histamine, with a mean value of 60 mg/kg. More recently Kalač et al. (*19*) indicated histamine content in cooked spinach from 2.1 to 9.8 mg/kg. In any case, current information about histamine content in this vegetable is very scarce. Simon-Sarkadi et al. (*20*) described a relationship between hygienic status and the presence of some BAs in fresh

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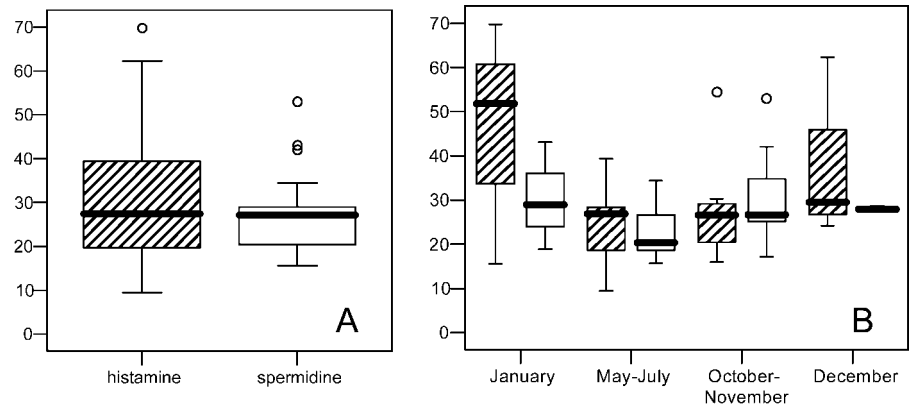

Figure 1. Boxplot, showing the minimum, lower quartil (Q1), median, upper quartil (Q3), maximum, and outliners, of histamine and spermidine contents (mg/kg) in fresh spinach from the market ($n = 18$) (**A**) and distribution throughout the year (**B**).

and packed vegetables such as Chinese cabbage, endive, iceberg lettuce, and radicchio. However, there is no information about the possible relationship between microbial load and histamine and other BAs in spinach. Simon-Sarkadi et al. (*20*) reported that in leafy vegetables stored at 5 °C *Enterobacteriaceae* represented 80% of the total microbial population, with their load directly related to putrescine content. Babic et al. (*21*) studied the microbial profile of spinach during storage and reported the highest increases for *Pseudomonadaceae* and *Enterobacteriaceae*. Some of the *Enterobacteriaceae* isolated by Babic et al. (*21*) in spinach, such as *Morganella morganii*, *Serratia marcescens*, or *Klebsiella pneumoniae*, have been extensively associated with histamine formation in foods of animal origin. Likewise, some *Pseudomonadaceae* such as *Acinetobacter* spp. and *Pseudomonas* spp. have also been related to histamine production (*22*). The goal of this work was to provide new data about the content of PAs and BAs, and histamine in particular, in fresh spinach. A further aim was to investigate whether there are any changes in amine content during refrigerated storage. In addition, this work aimed to monitor the counts of some specific microbial groups, mainly related to BA formation, to see whether any relationship existed.

MATERIALS AND METHODS

Sampling. Exploratory market study: 18 samples of fresh spinach (*Spinacea oleracea* L.) were bought in local retail markets in Barcelona at different seasons of the year. Storage trials: spinach samples (about 2 kg/trial) were acquired in Barcelona markets on 5 different occasions. After acquisition, samples were stored at 6 °C in a domestic refrigerator in a box lined with filter paper to absorb occurring condensing water. Leaves were taken out for sampling on days 0, 4, 8, and 12 for trial 1 (T1) and trial 2 (T2). An additional sampling point on day 15 was included in trial 3 (T3), trial 4 (T4), and trial 5 (T5). Samples of T1 and T2 were bought in November and December, respectively, and two replicates were taken for BA determination at each sampling point. Spinach of T3, T4, and T5 was bought from May to July and 6 replicates were taken at every sampling point for microbial counts, along with 3 replicates for measurements of pH, water content, and BAs. The appearance of spinach leaves was similar among samples corresponding to each sampling point in the first two trials, but not in the others. In order to find more representative values, 3 samples with different degrees of spoilage were taken for analysis in T3, T4, and T5. In the laboratory, samples were handled aseptically to avoid contamination.

Chemical Analysis. A Crison micro pH 2001 was used for pH measurement of the mixture of spinach (7 g) and deionized water (14 mL). Water content was determined by drying the sample (8 g of spinach) at 100–105 °C until constant weight was reached. BAs were extracted from 5–10 g of sample with 0.6 N perchloric acid and then determined as *ortho*-phthalaldehyde derivatives after separation by ion-pair high-performance liquid chromatography (*23*). Biogenic amine and polyamine standards, histamine dihydrochloride, tyramine free base, β-phenylethylamine hydrochloride, serotonin creatinine sulfate, tryptamine hydrochloride, octopamine free base, dopamine free base, cadaverine dihydrochloride, putrescine hydrochloride, agmatine sulfate, spermine tetrahydrochloride, and spermidine trihydrochloride were from Sigma (St.Louis, MO). All others reagents were analytical or HPLC grade, mostly from Merck (Darmstadt, Germany). The detection limits were below 0.07 mg/L for all amines except for spermine (0.4 mg/L). The precision of the method was satisfactory, with relative standard deviation for all amines lower than 8.3%, which were always acceptable according to Horwitz criterion (*23*).

Microbiological Analysis. For microbial analysis, 12–17 g of spinach leaves for each replicate was cut into small pieces and placed into a sterile Stomacher bag. The sterile diluent was 0.1% of Bacto Peptone (Difco, Detroit, MI) and 0.85% of NaCl (Merck, Darmstadt, Germany) in deionized water. Spinach was mixed with the sterile diluent in a proportion of 1:9. The mixture was homogenized using a Stomacher (Lab Blander, Seward, London, UK). Decimal dilutions were prepared and inoculated in the corresponding media: (a) aerobic microorganisms on plate count agar (PCA; Oxoid Unipath Ltd., Basingstoke, UK) incubated at 30 °C for 48 h for mesophilic, and at 6 °C for 5 days for psychrotrophic; (b) *Enterobacteriaceae* in Violet Red Bile Glucose Agar (VRBG; Oxoid), incubated with a double layer at 37 °C for 24 h; (c) *Pseudomonadaceae* on Pseudomonas Agar Base (Liofilchem, Roseto d.A. TE, Italy) with addition of Cetrimide (5 mg/500 mL), Fucidin (5 mg/500 mL), Cephaloridine (25 mg/500 mL) at 30 °C for 48 h; (d) *Enterococci* in Kanamycin Aesculin Azide Agar Base (KAA; Oxoid) at 37 °C for 24 h; (e) lactic acid bacteria (LAB) on Man, Rogosa, Sharpe Agar (MRS; Oxoid) at 30 °C for 72 h under anaerobic conditions; (f) *Micrococcaceae* on Mannitol Salt Agar (MSA; Oxoid) at 30 °C for 48 h; and (g) yeasts and molds on Yeast Glucose Chloramphenicol Agar base (Liofilchem) at 25 °C for 48 h.

Statistical Analysis. Differences between samples were compared by one-way ANOVA followed by a Scheffe's multiple comparison test. The statistical analysis was performed using SPSS software (v. 12.0).

RESULTS

Biologically Active Amine Contents. Histamine and spermidine were the major amines found in all fresh spinach samples. Mean contents of both amines were very similar, with values of 32.1 ± 17.5 and 28.9 ± 9.6 mg/kg, respectively. However, as it can be observed in **Figure 1A** the median value for histamine varied much more between samples than the median value for spermidine. Putrescine, spermine, and tyramine were also found in almost all samples; their mean values and standard deviations were 4.7 ± 2.4, 3.6 ± 1.8, and 2.0 ± 0.9 mg/kg, respectively. Cadaverine, agmatine, and β-phenylethylamine were only occasionally quantified and always in very low amounts. Octopamine, dopamine, serotonin, and tryptamine

Table 1. Content of Biogenic Amines and Polyamines (mg/kg of Fresh Weight) during Refrigerated Storage of Fresh Cut Spinach

day	tyramine	putrescine	histamine	spermidine	spermine
Trial 1					
0	0.9 (0.1)[a]	5.9 (0.1)	16.0 (0.2)	53.0 (1.4)	2.7 (0.1)
4	2.1 (0.0)	3.9 (0.4)	16.5 (0.6)	36.0 (0.7)	2.1 (0.2)
8	2.1 (0.1)	3.3 (0.8)	20.5 (4.1)	28.5 (2.9)	1.5 (0.2)
12	2.1 (0.1)	1.9 (0.2)	23.0 (0.4)	26.8 (0.1)	1.3 (0.0)
Trial 2					
0	2.9 (0.4)	3.1 (0.2)	19.7 (0.6)	42.1 (0.7)	n.q.[b]
4	1.1 (0.1)	2.5 (0.5)	28.8 (0.1)	28.0 (0.6)	n.q.
8	2.9 (0.6)	3.3 (0.0)	28.5 (0.1)	30.6 (0.7)	n.q.
12	4.4 (0.2)	4.5 (0.2)	29.1 (1.2)	26.1 (0.5)	n.q.
Trial 3					
0	1.9 (0.4)	5.3 (0.9)	9.4 (2.1)	26.7 (1.9)	3.8 (0.7)
4	1.5 (0.1)	0.9 (0.2)	13.6 (0.3)	24.3 (0.4)	2.6 (0.2)
8	1.8 (0.6)	3.0 (0.9)	29.8 (6.8)	24.2 (2.4)	1.6 (0.3)
12	4.9 (0.9)	5.7 (1.7)	67.5 (10.3)	39.3 (5.4)	4.4 (1.2)
15	2.6 (1.5)	7.7 (1.8)	83.8 (26.6)	41.6 (19.3)	3.9 (3.2)
Trial 4					
0	2.1 (0.2)	4.3 (0.1)	39.3 (1.0)	34.4 (0.4)	2.8 (0.1)
4	3.1 (0.5)	3.7 (0.7)	49.5 (1.7)	18.0 (1.3)	2.2 (0.4)
8	2.1 (0.7)	5.3 (0.3)	51.1 (23.6)	11.2 (5.5)	2.0 (0.9)
12	1.7 (0.4)	3.7 (0.4)	37.5 (9.8)	8.1 (0.7)	1.4 (0.2)
15	1.3 (0.2)	1.9 (0.3)	9.8 (2.3)	6.2 (1.1)	0.6 (0.6)
Trial 5					
0	2.4 (0.6)	2.2 (0.2)	26.9 (1.6)	15.6 (0.5)	1.2 (0.2)
4	3.5 (1.1)	5.3 (0.4)	30.9 (4.6)	6.6 (2.3)	0.0 (0.1)
8	1.3 (0.2)	5.9 (0.3)	26.0 (7.9)	8.1 (0.7)	0.4 (0.7)
12	0.4 (0.0)	6.5 (0.8)	1.1 (0.4)	6.1 (0.5)	n.q.
15	0.0 (0.1)	4.3 (0.9)	0.7 (0.7)	3.6 (0.5)	n.q.

[a] Mean (standard deviation). [b] Not quantifiable.

Table 2. Data for Water Content and pH Values of Fresh Cut Spinach during Refrigerated Storage

days of storage	water content %			pH		
	T3	T4	T5	T3	T4	T5
0	91.1 (0.3)[a]	93.2 (0.4)	92.9 (0.1)	5.8 (0.1)	6.2 (0.1)	6.1 (0.1)
4	91.1 (0.3)	93.3 (0.3)	93.1 (0.5)	6.1 (0.1)	6.9 (0.3)	8.2 (0.3)
8	89.1 (0.4)	92.9 (0.2)	93.5 (0.6)	6.4 (0.2)	8.6 (0.2)	9.0 (0.0)
12	86.6 (0.3)	92.0 (0.3)	92.3 (0.2)	6.9 (0.2)	9.0 0.1)	9.1 (0.1)
15	80.7 (1.5)	90.8 (1.2)	91.2 (0.8)	7.0 (0.5)	8.9 (0.1)	9.2 (0.0)

[a] Mean (standard deviation).

were not found in any sample. Differences in median amine content among samples acquired throughout the year were not found (**Figure 1B**).

During storage the most important changes in all 5 trials were observed in histamine and spermidine contents (**Table 1**). There was a general trend for histamine to increase and for spermidine to decrease. For the other amines, which were found at lower amounts than histamine and spermidine, a clear evolution pattern was not observed. As reported above for samples of exploratory market study, tyramine and putrescine were found in all samples, whereas cadaverine, agmatine, and β-phenylethylamine were only occasionally quantified and the other BAs were not found.

Water Content and pH Values. The values in **Table 2** show the evolution of water content and pH values of the spinach leaves in T3, T4, and T5. At the beginning the water values were similar, but differences were observed between T3 and T4/T5 over the course of the storage.

Differences in pH values between T3 and T4/T5 were also found. The initial pH of T3 (5.8) was only slightly lower than those of T4 (6.2) and T5 (6.1), but differences between trials increased during storage (**Table 2**).

Microbial Counts. Changes in microbial counts during storage of spinach are shown in **Table 3**. Total mesophilic and psychrotrophic aerobic counts in initial samples were clearly lower in T3 than in T4 and T5 ($p < 0.05$). However, the loads at the end of the storage were in all cases close to 10 log CFU/g. *Pseudomonadaceae* and *Enterobacteriaceae* were the predominant groups in initial samples and their counts increased during storage in T3 and T5 and remained constant in T4, in which the initial counts were the highest. Gram positive flora also increased during storage; at the end of storage, the counts were lower in T3 than in T4 and T5. Finally, yeasts and molds also increased, with counts being slightly higher in T3 than in T4and T5 at the end of the storage.

DISCUSSION

Biogenic Amines and Polyamines in Samples of Market Study. Histamine content in spinach leaves was the more noticeable fact of this study. This amine was found in all market samples and showed, on average, amounts similar to or higher than those sometimes found in foods, such as some sea fish, which are usually recognized as rich in histamine and frequently related to histamine intoxication (*3*, *24*). The risk of histamine adverse effects seems to be less important in spinach than in fish, because tyramine, putrescine, and cadaverine are lower in spinach than in unfresh fish. These amines have been reported as enhancers of histamine toxicity by competition for DAO enzymes at intestinal level.

The wide variability of histamine content in market samples could be explained by differences in endogenous levels and/or by different formation associated with loss of freshness. As it occurs with PAs, the presence of histamine in all the samples studied suggests that the histamine in spinach could be physiological. The possibility of histamine formation during storage, due to microbial activity, was the main reason for studying the changes in this and other BAs during refrigerated storage of spinach.

Regarding PAs, spermidine content was clearly higher than spermine content, as is usually reported for foods of plant origin (*13*). A high variability was also found in PA content but, in contrast to histamine and other BAs, their origin in foods is not usually related to loss of freshness.

Biogenic Amines and Polyamines during Storage Trials. In all cases, histamine and spermidine were the main amines found, but some differences between trials were observed. Over the course of the storage, a general decreasing trend was observed for spermidine, whereas in general an increase was observed for histamine, although there was not a uniform pattern for this last amine in all trials. In addition, the variability of histamine content between replicated samples was not very high in T1 and T2, in contrast to the high differences observed in T3, T4, and T5. The sampling strategy followed in these last 3 trials (samples with different spoilage degree) could, in part, explain the high variability found. Formation of histamine was observed in all trials, but was higher in T3, T4, and T5 than in T1 and T2. Two different evolution profiles during storage were observed for histamine. There was a continuous increase in T1, in T2, and in T3, whereas in T4 and T5, in which initial levels of histamine were higher than those in the other trials, there was an initial increase followed by a decrease after 8 or 4 days of storage, respectively, until it almost disappeared at the end of the storage in T5. It is also noteworthy that the general

Table 3. Changes in Microbial Counts (log CFU/g) during Refrigerated Storage of Fresh Cut Spinach

	Day 0	Day 4	Day 8	Day 12	Day 15
Trial 3					
mesophilic	6.40 (0.31)[a]	7.94 (0.06)	8.89 (0.09)	9.52 (0.11)	9.88 (0.18)
psychrotrophic	6.75 (0.18)	7.15 (0.06)	8.21 (0.26)	9.24 (0.31)	8.91 (0.29)
Enterobacteriaceae	5.59 (0.32)	6.66 (0.33)	7.00 (0.75)	7.40 (0.32)	7.47 (0.47)
Pseudomonadaceae	6.66 (0.31)	7.95 (0.16)	8.88 (0.19)	9.95 (0.32)	9.71 (0.08)
Enterococci	2.50 (0.46)	3.98 (0.42)			3.02 (0.75)
Micrococcaceae	4.55 (0.21)	4.84 (0.29)	5.90 (0.40)	7.63 (0.42)	7.52 (0.26)
lactic acid bacteria	2.86 (0.22)	3.78 (0.53)	3.85 (0.12)	4.47 (0.22)	6.20 (0.24)
yeasts and molds	5.70 (0.25)	7.13 (0.21)	7.91 (0.29)	8.71 (0.30)	9.17 (0.34)
Trial 4					
mesophilic	7.67 (0.07)	9.09 (0.13)	9.67 (0.20)	9.38 (0.27)	9.88 (0.10)
psychrotrophic	7.11 (0.19)	8.61 (0.16)	8.94 (0.09)	9.49 (0.06)	9.35 (0.05)
Enterobacteriaceae	7.29 (0.12)	7.72 (0.11)	7.83 (0.26)	7.05 (0.13)	7.52 (0.22)
Pseudomonadaceae	9.66 (0.01)	9.08 (0.11)	9.41 (0.34)	9.78 (0.44)	9.74 (0.13)
Enterococci	5.91 (0.13)	5.31 (0.09)	5.28 (0.08)	4.97 (0.44)	6.53 (0.27)
Micrococcaceae	7.07 (0.12)	7.46 (0.10)	7.60 (0.60)	8.33 (0.05)	8.32 (0.26)
lactic acid bacteria	5.34 (0.41)	4.53 (0.52)	6.76 (0.13)	6.20 (0.29)	6.93 (0.18)
yeasts and molds	6.76 (0.16)	8.36 (0.08)	8.48 (0.39)	8.53 (0.14)	8.26 (0.16)
Trial 5					
mesophilic	8.60 (0.15)	9.40 (0.25)	10.07 (0.22)	10.54 (0.19)	9.91 (0.08)
psychrotrophic	7.81 (0.65)	9.04 (0.26)	8.95 (0.18)	9.52 (0.12)	9.40 (0.07)
Enterobacteriaceae	6.94 (0.65)	8.04 (0.17)	8.05 (0.17)	7.99 (0.07)	8.04 (0.42)
Pseudomonadaceae	8.37 (0.04)	9.78 (0.20)	11.06 (0.52)	10.29 (0.29)	9.76 (0.11)
Enterococci	5.18 (0.25)	6.77 (0.27)	7.00 (0.30)	7.50 (0.12)	7.13 (0.23)
Micrococcaceae	6.75 (0.42)	7.44 (0.21)	8.14 (0.09)	6.72 (0.18)	8.33 (0.07)
lactic acid bacteria	4.71 (0.54)	7.51 (0.59)	7.89 (0.30)	8.63 (0.12)	8.55 (0.19)
yeasts and molds	6.73 (0.17)	8.06 (0.25)	8.47 (0.05)	8.18 (0.26)	8.01 (0.11)

[a] Mean (standard deviation).

decreasing trend observed for spermidine in all trials was especially important in T4 and T5, where histamine clearly decreased.

Decreases in BA and PA content after long storage periods have been previously reported for other foods (*25*, *26*). Two hypotheses have been formulated regarding the decrease of these compounds: (a) microorganisms could use them as a nutritional source (*27*); (b) degradation by amino oxidase microbial enzymes released to the media such as polyamine-oxidase (PAO) or diamine-oxidase (DAO) (*28*, *29*). However, the possibility of a simple chemical degradation in an alkaline pH medium should be also considered. Indeed, trials in which there was a decrease in amine content recorded the highest pH values (>8). In concordance with the chemical degradation of amines at high pH values, alkalinization has been reported as a procedure for obtaining free amine samples to be used as blank for reliability studies of a method for BA and PA determination in beers (*30*).

The difference in the histamine evolution pattern between trials could be linked to the date of sample acquisition. Samples of T4 and T5 bought in summer showed a faster and higher spoilage than those of T1 and T2 acquired in winter. This was evident not only in the amine evolution but also in the external appearance of spinach leaves. Spinach leaves from summer lose their original texture and become dark and glossy faster than spinach bought in winter. Photographs at each sampling point were taken to compare external appearance between trials.

Microbial Counts. The microbial loads increased during storage in agreement with previous reports for spinach (*21*) and other leafy vegetables (*20*). But counts in initial samples of this study were higher than those found in other foods of plant origin (*31*, *32*).

The higher microbial counts in initial samples of T4 and T5, in comparison with those in T3, could be explained by differences in post-harvest environmental conditions. Samples of these trials were bought in May, June, and July, when the average minimum and maximum temperatures were 13–20, 17–25, and 21–30 °C for T3, T4, and T5, respectively (data from the Catalan Met Office). The high temperatures in the post-harvest period for T4 and T5 could favor microbial growth.

Bacteria evolution during refrigerated storage of spinach did not reproduce the histamine evolution profile. In fact, never there was a decrease in microbial counts. However, differences in general microbial loads, in particular *Pseudomonadaceae* and *Enterobacteriaceae*, in initial samples of T3 versus T4 or T5 could explain the differences in amine contents. So, T4 and T5 with the highest initial microbial counts showed the highest contents of histamine at the start and the lowest at the end of storage.

The high pH values allowed the growth of gram-negative bacteria during the spinach storage, and these microorganisms could be responsible for histamine formation. Babic et al. (*21*) associated the growth of *Enterobacteriaceae* and *Pseudomonadaceae* with spoilage of spinach under refrigeration and several authors have demonstrated the histidine decarboxylase activity of both *Enterobacteriaceae* (*33–37*) and *Pseudomonadaceae* (*38*, *39*).

The relatively high pH value of fresh spinach is not optimum for histidine decarboxylase activity to take place, but it is within the range in which this enzyme can act. Rodtong et al. (*37*) reported that histidine decarboxylase activity for *Morganella morgani*, *Proteus vulgaris*, and *Enterobacter aerogenes* remains active at pH 7.

Among others, the accumulation of histamine in spinach could be influenced by the content of glucides and proteins. Spinach has a glucidic content of around 0.5–0.7 g/100 g, which is very low in comparison with other vegetables (*40*). Low availability of sugars can hinder the development of lactic bacteria and the production of acid metabolites, allowing spoilage to be dominated by gram-negative microorganisms (*40*) such as *Enterobacteriaceae* and *Pseudomonadaceae*. Changes in appearance of spinach leaves have been linked to *Pseudomonadaceae* growth, microorganisms with recognized pectolytic, proteolytic,

and lipolytic enzymatic activities (*21*). Likewise, *Enterobacteriaceae* have enzymes for digesting vegetable tissues (*41*). Furthermore, spinach contains up to 3 g/100 g of proteins (*42*), a fairly high value in comparison with other leafy vegetables such as lettuce, endive, and chicory. The hydrolysis of proteins yields basic compounds responsible for pH increase (*40*) and also free amino acids available for biogenic amine formation. Histidine content in spinach is 53 mg/100 g (*42*), a higher value than that found in other vegetables in which the low amounts of the precursor amino acid could be a limitative factor for histamine formation.

Together with the possible low activity of histidine decarboxylase and the potential chemical decomposition at high pH values, bacterial amine oxidase activity could also explain the decrease in histamine content in T4 and T5. The presence of histamine oxidase has been reported in several microbial groups (*29*). This enzymatic activity seems to be strongly dependent on the pH, and Janz (*43*) reported that histamine oxidase activity from *Pseudomonas aeruginosa* increases at alkaline pH. Thus, the high pH values found in T4 and T5, in which histamine decreased, could favor amino oxidase activity.

In summary, spinach storage at 6 °C leads to the growth of microorganisms which could be involved in the production of histamine. The high pH values found during storage seem to favor the growth of *Enterobacteriaceae* and *Pseudomonadaceae*, which are usually recognized as histamine formers in other foods. However, the high pH values could also be behind the decrease in histamine at the end of the storage in some trials.

Nevertheless, additional studies of the specific histidine decarboxylation ability of the microflora isolated during spinach storage are necessary to find out the specific role of each microorganism and in particular to confirm the capacity of the gram-negative bacteria to form histamine. Likewise, other studies would be required to offer a better explanation of the decrease in histamine in spoiled samples.

Abbreviations Used. BAs, biogenic amines; DAO, diamine-oxidase; MAO, monoamine-oxidase; PAs, polyamines; PAO, polyamine-oxidase, T1, trial1; T2, trial 2; T3, trial 3; T4, trial4; T5, trial5.

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