Biogenic Amines in Packed Table Olives and Pickles

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ABSTRACT

The content of biogenic amines in different commercial preparations of table olives and other pickled foods was determined. Concentration of amines in packed table olives, capers, caperberries, and cucumbers was less than 60 mg of total biogenic amines per kg of fruit, and, therefore, these products represent no risk to human health. The highest concentrations of putrescine (50 mg/kg) and histamine (38 mg/kg) were found in untreated natural black olives and caperberries, respectively. Canned ripe olives were completely free of biogenic amines. Putrescine was found in all the samples of green olives and cucumbers but at levels lower than 18 mg/kg.

Biogenic amines in foods are generated as a result of endogenous amino acid carboxylase activity in raw material (19) or from the growth of decarboxylase-positive microorganisms under conditions favorable to enzymatic activity (11).

In Spain, table olives (Olea europaea L.) are produced in three main commercial presentations: Spanish-style green olives, untreated natural black olives, and ripe (black) olives.

The procedure for preparing the first of these products (Fig. 1) consists of treating the olives with dilute NaOH solutions, followed by one or two water washes to eliminate the excess of alkali; the olives are then stored in a NaCl solution (brine) in which they undergo a lactic fermentation process (2, 9, 10).

Biogenic amine content in industrial fermentation brines increased throughout the brining period, and concentration was always higher for putrescine than for cadaverine. After 12 months of brining, the presence of histamine, tyramine, and tryptamine was also detected (6).

The packing process for green Spanish-style olives requires prior dilution of fermentation brines to reduce combined acidity (buffer capacity) (9). Once the fruits are in the best possible conditions for packing, they are put through different operations, such as pitting or stuffing with anchovies (Engraulis encrasicholus), to obtain different commercial presentations (Fig. 1).

The processing of untreated natural black and ripe (black) olives includes an initial stage (0 to 18 months) of fermentation in brine (8–10). There is no information available on the content of biogenic amines in the preservation brine of natural black olives. The olives are then washed and packed in new brine for marketing (Fig. 2).

A previous study discovered that the preservation brine of ripe olives not only had less putrescine and cadaverine than Spanish-style olives but also that histamine and tyramine were totally absent (6). In the normal oxidation process, storage brines are discarded and fruits are darkened by successive treatments (1–3) with dilute NaOH (lye). During the intervals between lye treatments, olives are suspended in water bubbled with air (5). To prevent color deterioration, different iron salts are added to fix the color (7).

Finally, the olives are packed and sterilized, either whole or pitted (Fig. 2).

Capers and caperberries are the young flower buds and the fruits, respectively, of the Mediterranean shrub Cap-paris spinosa L. Commercial processing of capers includes pretreatment in high-salt brine before they are desalted and packed as finished product containing 6% salt and 1% acetic acid (1, 16).

The pickling of caperberries consists of a first step of immersion in water (4 to 7 days) during which a vigorous fermentation takes place. The fruits are then placed in a 10 to 12% NaCl (wt/vol) brine, where fermentation is completed. After a time, the salt concentration is raised to a level of around 15% (wt/vol) to guarantee bulk storage until the product is marketed (18). Vinegar, spices, and aromatic products are usually added to the packing brine.

There is no information available on the biogenic amine content of these two pickled products. Most cucumber (Cucumis sativus L.) fruits are pickled by fermentation, whereas the remainder are pasteurized (fresh-pack style) or pickled and stored refrigerated. Traditionally, cucumbers are fermented in 5 to 6% NaCl (wt/vol) brines to promote natural lactic fermentation and bulk stored after fermentation in brines up to 16 to 18% (3, 4). However, today many cucumbers are held at salt concentrations of 8 to 10%. Before processing and packaging, brined cucumbers are desalted with water to sensory acceptable levels (2 to 2.5%). Spices, essential oils, and vinegar are typically added to desalted pickles.

Commercial cucumbers packed in Spain contained only histamine (14). Concentration levels were highly variable (3 to 30 mg/kg of cucumbers).

The purpose of this study was to determine the bio-

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genic amine content of different commercial preparations of olives and other pickled foods, such as capers, caperberries, and cucumbers.

**MATERIALS AND METHODS**

**Samples.** All samples were purchased from the local market. They were 46 samples of Spanish-style green table olives (14 Manzanilla cv. stuffed with anchovies, 14 whole Manzanilla cv., 10 Gordial cv., and 8 Hojiblanca cv.), 8 samples of untreated natural black olives (Hojiblanca and Manzanilla cv.), 8 cans of pitted ripe olives (black) (Hojiblanca cv.), 8 bottles of capers, 9 bottles of caperberries, and 11 samples of cucumbers. Samples corresponding to the same type of product were from different factory batches. The cans and bottles were opened just before analysis.

**Extraction of biogenic amines from the flesh of fruits.** The procedure used was based on that described by Hornero-Méndez and Garrido-Fernández (12). Pitted olives and the other pickled foods were triturated. Whole olives were previously pitted. Ten grams of the homogenized paste and 200 ml of the internal standard solution (1 μg/ml of 1,8-diamineoctane) were extracted by shaking with 25 ml of 5% (wt/vol) trichloroacetic acid. The suspension was then filtered into a standard flask and the residue washed with 5% trichloroacetic acid solution to bring the volume up to 50 ml.

**Analytical method.** The simultaneous determinations of putrescine, cadaverine, tryptamine, β-phenylethylamine, spermine, spermidine, histamine, tyramine, and agmatine were made following the benzoyl derivative method of Hornero-Méndez and Garrido-Fernández (13) for table olive brines, with slight modifications made by García-García et al. (6). All determinations were made in duplicate using a Waters 717 plus autosampler and a Waters 996 photodiode array detector operated with Millennium 2010 software (Waters Inc., Milford, Mass.). The column, purchased from Hewlett-Packard (Avondale, Pa.), was packed with reversed-phase C18 Spherisorb ODS-2 (5-μm particle size, 12.5 by 0.4 cm).

**Statistical analysis of results.** Data were analyzed using the Statistica package software (20).

**RESULTS AND DISCUSSION**

**Spanish-style green olives.** In general, the content of biogenic amines in packed product was significantly lower than in fermented, stored green olives (6). There could be several causes for this decrease. The first decrease could be caused when combined acidity is corrected by replacing the fermentation brine with fresh brine (Fig. 1). Further brine dilution is produced during the conditioning operations (size grading, pitting, and stuffing, etc.) because these products require higher brine weight/fresh ratios, which are achieved by adding fresh brine. Finally, before packing, olives are washed again, and additional losses of these substances may occur. Thus, in packed green olives, the only biogenic amines found were those that were in higher concentrations in fermented olives: putrescine, cadaverine, and tyramine.

Putrescine was found in all the packed olive samples analyzed. Figure 3 shows its general distribution. Frequencies increase rapidly to reach the maximum for values of putrescine in the 6 to 8 mg/kg interval. Then there is a sharp decrease with low frequencies (except 12 to 14) to reach the upper limit of 18.2 mg/kg. The main distribution characteristics are as follows: average ($\bar{x}$) = 6.6 mg/kg; SD = 3.8 mg/kg, and range (R) = 1.3 to 18.2 mg/kg.

Cadaverine was found in all samples of Hojiblanca and
Manzanilla stuffed with anchovies. It was also present in most of the Manzanilla samples and was very rare (only two samples) in Gorda cv. The shape was quite different from that of putrescine, with maximum frequency for absence of the amine. Frequencies between 0 and 2 mg/kg were of similar length but decreased rapidly above 2 mg/kg. The main characteristics of the distribution were as follows: $\bar{x} = 1.1$ mg/kg; SD = 1.0; and R = 0.0 to 3.6. In general, there was a large amount of variability, about 100%, measured as relative standard deviation.

Tyramine (general distribution not shown) was only found in the Hojiblanca cv., with $\bar{x} = 0.5$ mg/kg and SD = 0.9 mg/kg. The maximum value was 2.6 mg/kg. This supports the fact that tyramine was produced in a higher proportion in the Hojiblanca cv. than in Manzanilla and Gorda cvs. (6).

The distribution of each biogenic amine among cultivars provided evidence of specific behavior patterns. No significant differences in the content of putrescine between cultivars could be established because of the wide range of values obtained. However, the average highest content was found in the Gorda cv. and the lowest in the Hojiblanca cv. (Fig. 4), similar to fermented, stored green olives (6).

Cadaverine, on the other hand, was higher in the Hojiblanca cv. and very low in the Gorda cv. (Fig. 4). The Manzanilla cv. was in an intermediate position. Relative positions were also similar to those obtained when olives had completed the fermentation process (6).

It must be emphasized that, qualitatively, the samples of green olives stuffed with anchovies had the same biogenic amines and similar concentrations as whole Manzanilla cv. packed olives. This indicates that the anchovies used to prepare the pastes were fresh, properly refrigerated anchovies or semipreserved anchovies in brine (17, 21) in very good conditions. Apparently, the raw material used by the Spanish industry to prepare anchovy-stuffed olives is of the highest quality. These products maintain the usual low proportion of biogenic amines in packed green olives.

**Untreated natural black olives.** In general, qualitatively, the biogenic amines found in these olives (Fig. 5) were the same as in Spanish-style green olives. However, the concentration of putrescine was significantly higher. In fact, the biogenic amine content in this packed product was similar to that found at the end of the storage period for ripe olives (6). Apparently, the similar fermentation-storage processes applied to these two types of olives generate similar amine production rates irrespective of the degree of maturity of the raw material.

The characteristics of the distribution of amines in this commercial presentation were as follows: putrescine: $\bar{x} = 18.6$, SD = 18.0, R = 1.5 to 50.2; cadaverine: $\bar{x} = 1.1$, SD = 1.1, R = 0.0 to 2.3; and tyramine: $\bar{x} = 0.5$, SD = 0.7, R = 0.2 to 2.1.

The most interesting amine was putrescine, the concentration of which was highest in all the packed olive

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**FIGURE 3.** Distribution of putrescine and cadaverine concentration in flesh in the 46 samples of packed Spanish-style green table olives.
products. Approximately half of the samples were free of cadaverine and tyramine. Variability was also large, with a relative standard deviation of about 100%. This dispersion was due to the variability that already existed in the original fermented product (6). The total content of biogenic amines in natural black olives was always far from levels (100 mg/kg) that could cause safety concerns (15).

**Ripe (black) olives.** No biogenic amines have been detected in any of the samples of this commercial presentation that were analyzed, although they were usually present in stored olives (6). Apparently, they were completely washed out during the oxidation process. The successive lye treatments and water washes (Fig. 2) applied during the oxidation process (5, 7) were enough to remove all these substances. Furthermore, the concentrations of biogenic amines in stored fruits were lower than in Spanish-style green olives (6).

**Capers and caperberries.** The biogenic amines found in packed capers were putrescine, spermine, histamine, and tyramine (Table 1). Histamine was at the highest level (\( \bar{x} = 8.2 \) mg/kg), with a range of 1.2 to 21.5. Tyramine concentration was very low and present in only two samples. Concentrations of the others were low (lower than 5 mg/kg).

In caperberries, cadaverine was also found in addition to the amines found in capers (Table 1). Cadaverine was present in most of the samples (except one), with a range of 0 to 9.5. As in packed olives, putrescine was always found at a range of 3.5 to 24.2. The other biogenic amines were absent in approximately 50% of the samples. However, high levels of histamine (27.5, 33.5, and 37.2 mg/kg)

### TABLE 1. Biogenic amines content (mg/kg) in capers, caperberries, and cucumbers in Spanish market

<table>
<thead>
<tr>
<th></th>
<th>Putrescine</th>
<th>Cadaverine</th>
<th>Spermine</th>
<th>Histamine</th>
<th>Tyramine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capers</td>
<td>2.3 (1.3)</td>
<td>ND</td>
<td>2.3 (2.4)</td>
<td>8.2 (6.7)</td>
<td>0.2 (0.6)</td>
</tr>
<tr>
<td>Caperberries</td>
<td>13.1 (8.5)</td>
<td>3.2 (3.1)</td>
<td>4.9 (4.4)</td>
<td>14.7 (17.2)</td>
<td>1.6 (2.6)</td>
</tr>
<tr>
<td>Cucumbers</td>
<td>4.5 (5.0)</td>
<td>ND</td>
<td>2.9 (4.2)</td>
<td>ND</td>
<td>0.7 (0.8)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are standard deviations. ND, not detected.
were detected in three samples, but these concentrations are lower than 50 mg/kg, which is the limit for safety concern (15).

Biogenic amines in this product may have been formed during the tumultuous fermentation of the caperberries in water alone during the initial days of treatment (18). The weak washings carried out before packing were hardly sufficient to eliminate amines produced at that stage. Therefore, the final product may contain a significant proportion of the original amines despite the dilution created by packing in fresh brine.

**Cucumbers**. Putrescine, spermine, and tyramine were the only biogenic amines found in packed fermented cucumbers (Table 1). In general, concentrations were low, and only putrescine was found in all the samples (R = 0.8 to 17.4). Spermine and tyramine were present in approximately 50% of the samples. These results do not coincide with reports by Inigo and Bravo-Abad, who identified histamine as the main biogenic amine produced during the fermentation of cucumbers (14).

There are no regulations in the world regarding the concentration of histamine and total biogenic amines in table olives or pickles, but several authors have indicated that a histamine content of less than 50 mg/kg or a total amine content of less than 100 mg/kg can be considered safe (11, 15). In general, concentrations found in packed table olives, capers, caperberries, and cucumbers are below these limits, and these products, therefore, represent no health risk.

Canned ripe olives were found to be completely free of biogenic amines, so they are not a point of concern for this commercial presentation.

The highest concentrations of biogenic amines have been found in caperberries and untreated natural black olives, with a couple of samples showing high amounts of histamine (37.2 mg/kg) and putrescine (50.2 mg/kg), respectively. Since these fermentations are spontaneous and not well controlled, such high levels may have been caused by contaminant microflora during the fermentation process because of the absence of good manufacturing practices. An improvement of sanitary conditions and the application of chemical and biological methods are recommended for an increased control of these fermentations.

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**REFERENCES**


