Changes in nitrogen fractions and free amino acids of Kourdas, a salted dried meat Moroccan product

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ABSTRACT
A microbial, physicochemical and proteolysis change during the ripening of the kourdas traditionally meat product was studied. Trials of kourdas making were carried out in the laboratory by the traditional procedure. Batches of 6 kg each of sheep fresh meat were purchased directly from the slaughterhouse. The meats were sliced, salted, spiced and coated in the rumen before exposed to the sun for drying. The batches were sampled at different times to follow up the microbiological, physico-chemical and proteolysis properties. Results indicated a considerable decrease in the moisture and proteins content (p < 0.05). NaCl content increased significantly during manufacture (p<0.05). The content of the different nitrogen fractions and of the free amino acids indicated that protein degradation during the manufacture of kourdas is only moderate. During ripening the total amine content increased significantly (P < 0.05).

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1. Introduction:
Traditional food is a significant element of the Moroccan cultural heritage. Their production and sale provide a decisive economic input to many regions [1-4]. But, small producers of traditional products may encounter technical and financial difficulties to comply with official food safety regulations [5]. Traditional meat product represents an important group among these products. They are characterized by handmade manufacturing usually in small-scale units, following spontaneous fermentation by their particular in-house flora, one of them is Kourdas [6]. It is a traditional meat product made in Morocco. kourdas is a product that is well appreciated in the areas where it is produced but, at present, there are some problems that prevent its diffusion to wider markets. Such problems, that include the heterogeneity of the product and the questionable quality of some units, are partly due to a lack of knowledge about the biochemical and microbiological phenomena that take place throughout the manufacturing process and that are responsible for the organoleptic quality of the final product [7-9].

The aim of the present study, which forms part of a wider study on both the microbiological and biochemical processes that occur during the manufacture of kourdas, was to obtain information on the biochemical changes that occur during the manufacturing process.

2. Materials and methods:
2.1. Ham preparation:
Meat preparation in laboratory: Batches of 6 kg each of fresh sheep meat were purchased from the slaughter house of Tangier (Morocco). These were allowed to mature for 24 h at ambient temperature (around 25°C) and cut into small pieces to facilitate the salt penetration. The pieces were salted and spiced by garlic, pepper, paprika, coriander and cumin in a 10-l plastic container and allowed to take for 24 h. The pieces of the spiced meat were coated in the rumen and they were
closed by intestine and exposed to sun by being hung on a string. All the determinations were performed within 20 days of drying.

2.2. Biochemical analysis:
Salt content was assessed according to the recommended standards as cited by Marra et al. (1999) [10]. The pH was measured using a pH meter Micro pH 2002 (Crisson Instruments, Barcelona, Spain) after mixing 10 g of the sample with 90 mL of distilled water for 2 min. Moisture, fat and protein (Kjeldahl N6.25) contents were assessed according to the recommended standards. Total non-protein nitrogen (NPN), a-aminoacidic nitrogen (NH₂-N) and total basic volatile nitrogen (TBVN) were also quantified following the methods cited above.

2.3. Amino acids:
The extraction of free amino acids was performed, as described by Alonso et al. (1994) [11]. The identification and quantification of amino acids were carried out by HPLC. The liquid chromatography equipment consisted of a Waters 2690 separation module (Waters, Milford, MA), a UV/Visible Waters 996 photodiode array detector, and a Millennium 2010 Waters computer program. The column used was a reversed phase C18 Ultrasphere 5-ODS, 4.6×250 mm from Beckman (San Ramón, CA). The temperature of the column was controlled to 50 ± 1°C with a column heater (Spectra Physics 8792). The wavelength of the detector was at 254 nm. Standards of the 22 different amino acids were supplied by Sigma Chemical Co. (St Louis, MO).

2.4. Statistical analysis:
All statistical analyses were performed using the Statistica 5.1 computer program for Windows (Statsoft Inc, Tulsa, OK, USA). Significant differences between different samples, between the two salting times and the olive oil and paprika effect were determined using two-way analysis of variance, with a confidence interval of 95% (P<0.05).

3. Results and discussion:
The moisture content decreased markedly in the product during the period of drying (Table 1). This pattern remained 20 days and the ultimate value reached was 20.32±1.81%. The decrease continued more slowly during the following days. This phenomenon is probably due to the free water driving into the product during the drying process. The moisture is the most important factor to monitor during the dehydrating process. Moisture must be reduced as quickly as possible to stop or to delay spoilage microorganisms in the product. Moreover, salting is usually accompanied by a reduction in microbes and may help in the preservation of foods using a combination of salting and drying.

A decrease in the protein content was observed during the manufacturing process, from an initial average value of 70.93±0.14/ of total solids to 60.74±0.26g of TS at the end of the post-salting stage. This decrease appears to be fundamentally due to the increase in the NaCl content during the salting and post-salting stages. The decrease in the protein content was less pronounced during the drying–ripening stage. Similar decreases in protein content throughout manufacturing have been also reported in raw-cured hams [11-12].

The fat content also decreased slightly from an initial average value of 26.94±0.77g/100 g of TS to 19.14±1.01g of TS at the end of the drying–ripening stage. As with the protein, this decrease appears to be due to the addition and distribution of salt in the pieces during the salting and post-salting stages, which would cause the fat contribution to the total solid content of the pieces to decrease substantially. Final protein and fat values similar to those obtained in the present study have been reported for hams [13-15]. The NaCl contents increased significantly (P < 0.05) after the salting and post-salting stages. The NaCl content of Kourass at the end of the manufacturing process (14.44±0.34% of TS) was higher than the values observed en goat ham ‘halal’ (5%) ham reported by Cherroud et al. (2014) [16], Iberian ham (9.29–11.4% of TS) reported in some works [17-18], Serrano ham [18], and Italian hams [19]. However other authors reported higher values (13–20% of TS) for the NaCl content in hams [20-22].

The pH decreased after salting to 4 days of ripening. Then its start increases significantly (p<0.05). An initial reduction of was due at the presence of bacteria lactic. This pH reduction can help to delay the growth of undesirable microorganisms during the first stage of processing. The pH value tended to increase during Kourass processing. This increase may be due to production of ammonia and amines as a result of proteolysis [23].

The changes in nitrogen fraction throughout the manufacture of Kourass are shown in Table 1. In the present study there was a significant (P < 0.05) increase in the total NPN throughout the process, and on the 20th day of the drying–ripening stage, this represented on average the 8.77% of the total nitrogen. These values obtained at the end of the manufacturing process basically coincide with those reported for ham by some authors [24-25]. However NPN values observed in this study was much higher than those obtained in others works [26-27]. Of the two NPN nitrogen fractions analyzed in the present study, the a-amino acid nitrogen underwent the greatest increase during manufacture, and at the end of the drying–
Concentrations of the free amino acids of the Kourdas analyzed are given in Table 2. The predominant amino acids in raw sheep meat were taurine, alanine and leucine-isoleucine mixture. Amino acids profile of unprocessed meat was defined by diet of the animal, while amino acid profile of the meat product is related to microbial and enzymatic proteolysis. In the final product, the most abundant free amino acids were alanine, taurine, serine, glutamine and leucine-isoleucine mixture. All free amino acids increased during ripening. Amino acids increased between 340.8±8.67 mg/100g of DM and 785.9±20.43 mg/100g of DM compared to concentrations in raw sheep (Table 2). The most rapid increase took place during the drying stages. A general increase, in levels of free amino acids during ripening has in various types of dry-cured hams [32-34]. Final concentration of free amino acids, suggest that this product undergoes moderate proteolysis, which could be due to the action of various microorganisms and not bound to enzymatic proteolysis because, it has been reported that, cathepsins B, D, H, and L remain active after 12-15 months of drying processing [35-36].

Table 3: changes in biogenic amine content (expressed as mg/kg) during the manufacturing process of kourdas (average values ± standard deviation).

<table>
<thead>
<tr>
<th>Days</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptamine</td>
<td>6.18±1.31a</td>
<td>8.70±0.52a</td>
<td>11.54±0.98b</td>
<td>14.75±0.82c</td>
<td>17.43±1.22d</td>
<td>20.56±0.87e</td>
<td>23.59±0.89f</td>
</tr>
<tr>
<td>Phenylethylamine</td>
<td>0.69±0.12d</td>
<td>1.11±0.51b</td>
<td>1.79±0.23b</td>
<td>2.91±0.54c</td>
<td>3.86±0.33d</td>
<td>4.59±0.42e</td>
<td>5.63±0.45f</td>
</tr>
<tr>
<td>Putrescine</td>
<td>0.21±0.18a</td>
<td>0.40±0.15b</td>
<td>0.77±0.19b</td>
<td>1.21±0.27c</td>
<td>1.78±0.42c</td>
<td>2.51±0.19d</td>
<td>2.58±0.22e</td>
</tr>
<tr>
<td>Cadaverine</td>
<td>0.43±0.25a</td>
<td>1.28±0.23b</td>
<td>1.87±0.43b</td>
<td>2.48±0.42c</td>
<td>3.52±0.19b</td>
<td>4.29±0.19c</td>
<td>5.65±0.10d</td>
</tr>
<tr>
<td>Histamine</td>
<td>ND</td>
<td>0.22±0.11a</td>
<td>0.26±0.07a</td>
<td>0.52±0.18b</td>
<td>0.61±0.15b</td>
<td>0.73±0.1a</td>
<td>0.90±0.06d</td>
</tr>
<tr>
<td>Agmatine</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.35±0.14a</td>
<td>0.39±0.17a</td>
<td>0.28±0.15a</td>
</tr>
<tr>
<td>Tyramine</td>
<td>8.23±1.62c</td>
<td>13.84±0.82b</td>
<td>16.80±1.04a</td>
<td>20.24±1.16a</td>
<td>23.83±1.07b</td>
<td>27.28±1.14c</td>
<td>30.54±0.97d</td>
</tr>
<tr>
<td>Spermidine</td>
<td>1.44±0.26a</td>
<td>2.07±0.31b</td>
<td>2.67±0.45a</td>
<td>3.54±0.27b</td>
<td>5.06±0.37a</td>
<td>6.00±0.91b</td>
<td>6.94±0.18a</td>
</tr>
<tr>
<td>Spermine</td>
<td>10.83±0.51a</td>
<td>15.95±1.16a</td>
<td>17.82±0.50b</td>
<td>19.88±1.22c</td>
<td>20.95±2.29a</td>
<td>23.15±0.60b</td>
<td>25.62±0.88b</td>
</tr>
<tr>
<td>Total</td>
<td>28.01±3.53a</td>
<td>43.56±5.33b</td>
<td>53.51±6.29a</td>
<td>65.53±7.34a</td>
<td>77.40±8.09a</td>
<td>89.50±9.15a</td>
<td>101.72±10.19a</td>
</tr>
</tbody>
</table>

a–g Values in the same row (corresponding to the same parameters) not followed by a common letter differ significantly (P < 0.05).

4. Conclusion:
In conclusion, conditions of meat transformation in Kourdas should be well-monitored during processing for the improvement of the organoleptic quality of the final product. The parameters measured in this study can be used to define a well-monitored process for the manufacture of a new meat product used in hot countries and for special dietary customs. This study gives a first survey of the different parameters to be controlled during the drying process, by which method the traditional procedure could be extended to a controlled high scale production of kourdas according to defined standards.
References:


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