Effect of Ammonium Hydroxide on the Quality of Beef Burgers

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Abstract

The incidence of the different levels of ammonium hydroxide (% HA = 0.5, 1 and 1.5) was analyzed in the quality characteristics of the beef burger, a raw unprocessed fresh processed meat product, through the formulation that attributes parameters that allowed to evaluate some characteristics such as pH, water retention capacity (CRA), chromatic values and microbiology. 90/10 beef (low percentage of fat) was used as raw material, the results of this investigation showed that pH and CRA were significantly increased (p <0.05) during the first 3 days of treatment, the highest Incorporation of ammonium hydroxide (1.5% HA) decreased the CRA. The chromatic values for * increased significantly (p <0.05) during the entire period of storage in storage (4 ± 1°C) and acceptable microbial counts were obtained according to Colombian regulations. All these results showed the potential to use ammonium hydroxide solutions at 0.5% w/v to improve the quality characteristics of beef burgers.

Keywords: Ammonium hydroxide, beef, burger, microbiology, pH
1. Introduction

The meat sector in Colombia has recently entered into a very serious crisis, the commercial balance shows that 2016 was one of the worst years in figures, there was a fall of 8.4% compared to 2015, reaching 3,652,236 slaughtered cattle, it means 334 thousand animals less than the previous year, likewise the production of formal meat, derived from slaughter, decreased to reach 910 thousand tons in 2016 which means a deduction of 6.8%, all these data correspond to the fall in the annual per capita consumption in the year 2017 of 18.1% \[1\]. Despite this panorama, meat products such as hamburger meat retain their place of preference in Colombian consumption; However, the ambulatory sale of food in our country is considered a public health problem, becoming a critical risk factor for health, generated by insufficient hygiene conditions, lack of conservation measures, sometimes with limited access to potable water and sanitary services, thus increasing the possibilities of contamination and diseases transmitted by food (ETA), safety and good handling practices are an important element to improve the quality of meat products \[2\]. The meat used for the elaboration of hamburgers and other meat products is very susceptible to microbial spoilage, it must be kept in refrigerated or frozen conditions to stop the physicochemical damages, the enzymatic activity, the oxidation of the lipids in order to extend the useful life; However, fresh meat and meat products preserved in such conditions can develop a physical-chemical deterioration but at a reduced rate, this is due to the fact that during the preservation process this deterioration can not be completely interrupted since this phenomenon is directly related to the pH and lipid oxidation which have a great influence on the reduction of the palatability and texture of foods \[3\].

For the control of pH in meat processing, the use of ammonium hydroxide (NH4 +), also known as aqueous ammonia, has been reported as a mixture (equation 1) of ammonia (NH3) and water (H2O) in a molecular form and ionized \[4\]. It is generally recognized as safe (GRAS by its acronym in English Generally Recognized As Safe) by the FDA \[5\], when used according to good manufacturing practices (BPM), has other applications as a leavening and surface finishing agent in food OMRI \[6\] and colorant elimination and identification \[7, 8\].

\[
NH_3 + H_2O ⇌ NH_4^+ + OH^{-} \quad (1)
\]

In addition, its use is endorsed by other entities such as the Food and Agricultural Organization of the United Nations \[9\], is widely distributed, in food engineering, relevant fact because meat and its derivatives exhibit a tendency to rapid deterioration caused by its composition and the pH suitable for microbial growth, for all these must be treated with special care and greatly reduce the danger of contamination by undesirable pathogens such as salmonella and E. coli studies report the effectiveness of ammonium in these particular cases Borkowski et al.,
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[10], the Inspection and Food Safety Service. USDA [11] as responsible for the inspection of meat and poultry products to ensure the safety and health for human consumption accepted its use as a pH control agent in solving brine, although consumers may not know when the chemical is in their food. Therefore, in this work the objective was to analyze the incidence of different concentrations of ammonium hydroxide in the quality characteristics of the beef burger.

2. Methodology

2.1 Materials

Beef and pork fat were purchased in a refrigerator in the city of Cartagena-Bolivar, they were transferred to the Pilot Plants of Science and Meat Technology of the Food Engineering Program of the University of Cartagena, the corresponding conditioning and cleaning for the preparation of hamburgers. Ammonium hydroxide (NH3 aqueous ammonia, 25% solution) was obtained from a local chemical supplier, other ingredients such as sodium chloride, monosodium glutamate and other materials were supplied by a commercial establishment in the city.

2.2 Experimental design

The research process was based on a 4x4 factorial design [12] with ammonium hydroxide (HA) in distilled water solution in 4 levels: T1 = 0.5%, T2 = 1% and T3 = 1.5% p/v and the control treatment (0%) with three repetitions for each one. The response variables were evaluated: pH, water retention capacity (CRA), chromatic and microbiological values of the burgers at 3, 6 and 9 days of storage, the samples at 0 days were analyzed prior to packaging immediately after their elaboration.

2.3 Preparation of hamburgers

To establish the percentages in the elaboration of the meat product of the control formulation and the different treatments of the investigation (Table 1) the Colombian Technical Standard 1325 was consulted [13], the methodology was used (Figure 1) described previously by authors [14, 15], the beef was ground, then mixed with the other ingredients, the addition of sodium chloride allowed the extraction of proteins from the meat, the hydroxide of ammonium was used as established in the experimental design for T1, T2 and T3; the temperature at this point was kept below 15 °C to avoid protein denaturation, the mixture was homogenized and molds of the hamburgers were made by using petri dishes, finally the samples were placed in hermetic sealed bags and stored in refrigeration at 4 ± 1 °C until its corresponding analysis.
Table 1. Formulation for hamburgers in (%)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef 90/10</td>
<td>65</td>
</tr>
<tr>
<td>Pork Fat 25/75</td>
<td>16.88</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.84</td>
</tr>
<tr>
<td>Monosodium glutamate</td>
<td>0.04</td>
</tr>
<tr>
<td>Onion powder</td>
<td>0.42</td>
</tr>
<tr>
<td>Isolated soy</td>
<td>1.78</td>
</tr>
<tr>
<td>Smoke Grillin 10-73</td>
<td>0.04</td>
</tr>
<tr>
<td>Distilled water</td>
<td>15</td>
</tr>
</tbody>
</table>

**Total** *Distilled water solution with HA: T1=0.5%, T2=1% y T3= 1.5% p/v 100%*  

2.4 pH

The pH of the hamburgers was determined according to Figueroa [16] with modifications, a mixture of 10 g of sample was prepared with 100 ml of water for two minutes. The pH values of the resulting solution were measured using a MA920B / 1 electrode connected to a Milwaukee® digital pH meter (MW102, pH: ± 0.02, Hungary).

2.5 Water Retention Capacity (CRA)

20 g of the hamburger samples were taken from the treatments and the control, placed on Whatman 3® filter paper, previously weighed, then covered with Whatman 50® filter paper and centrifuged at 9,500 g for 20 min at a temperature of 4 °C. The water retained in the filter paper 3 was weighed, the results were expressed as the percentage of CRA [17].

2.6 Color

The color analysis of the surfaces of the burgers was determined using a Minolta CM-2600d spectrophotometer (Konica Minolta Sensing Americas, Inc.) determining the values L*, a* and b*: L* indicates luminosity (0 = black; 100 = white), a* (-) shade of green, a* (+) shade of red and b* (-) shade of blue, b* (+) shade of yellow; based on the CIELAB chromatic model [14, 16].

2.7 Microbiologic analysis

The preparation of the samples corresponds to the parameters of the established norms (NTC 5554 [18], controls to prevent contamination GTC 155 [19], tests and dilutions in meat products NTC 4491-2 [20]. Microbiological counts were performed in mesophilic aerobes NTC 4519 [21] in UFC), Salmonella spp. / 25 gr of sample NTC 4574 [22], coliforms and Escherichia Coli NTC 4458 in UFC [23] regulated in Colombian regulations NTC 1325 [13].
Figure 1. Flowchart of the preparation of hamburgers

2.8 Statistic analysis

The results were analyzed using STATGRAPHICS Centurión XVII®, an analysis of variance (ANOVA) was applied with a level of significance of 95% for samples of hamburgers treated with ammonium hydroxide and the control each in triplicate; the differences were considered significant (p <0.05); The method used to discriminate between means is Fisher's minimum significant difference procedure (LSD).

3. Results

The results of the physicochemical analysis for the pH in the treatments with ammonium hydroxide and control are observed in figure 2; the pH increased (p <0.05) in all samples of hamburgers T1, T2 and T3 up to values of 6.43, 8.40 and 9.32 with 0.5%, 1.0% and 1.5% p/v of HA respectively, in comparison with the control that had an Initial pH of 5.45; this can be explained by the pH of 10.62 of the chemical research agent HA; however, the storage time had no direct incidence in our study (p = 0.46). Some articles aimed at improving the tenderness of marinated buffalo meat for 48 hours with HA under similar conditions report a significant increase in pH, while decreasing the values of Warner-Bratzler [12]; Hamling et al., [24] who studied the effects of HA, salt and carbon monoxide solutions on aged meat, concluded that beef treated with hydroxide and ammonium salt improved the acceptance parameters but the oxygen increase in the modified atmosphere had a negative impact. The increase in pH has also been directly related to the concentration of ammonium hydroxide in meat and meat products [25, 26].
The water retention capacity (CRA) as well as the pH was influenced by the treatment with HA, in all the samples the CRA increased significantly (p < 0.05) in comparison with the control (Figure 3). The burgers with ammonium hydroxide in solutions of 0.5% and 1.0% w/v yielded the highest values (p < 0.05) of CRA, in contrast the formulations with 1.5% and the control presented the lowest values during the days of storage at 4 °C. The CRA is one of the most important quality parameters of the meat, it is directly linked to the perception of juiciness and weight loss of the product during later stages such as cooking and other technological processes [27]; when the concentration of HA was increased to 1.5%, the burgers showed a reduction in the CRA, this can be explained by the influence of the pH that determines the number of reactive groups of the proteins and their capacity to bind water [28].

**Figure 2.** Effect of ammonium hydroxide (HA) on the pH of burgers stored at 4°C during the days of treatment. LSD = 0.386

**Figure 3.** Effect of ammonium hydroxide (HA) on the water retention capacity (CRA) of hamburgers stored at 4 °C during the days of LSD treatment = 2.344.
The proteins lose their buffering capacity as the distance from the isoelectric point increases; the CRA presented different values during the days of storage, the treatment with 1.0% of HA obtained the highest percentages although these decreased after the 6th day of storage. CRA has been related to electrostatic repulsion between myofibrillar proteins, generating an expansion of the myofibrils and even a partial solubility of the myofilaments due to the repulsion between molecules [29]. The treatment with ammonium hydroxide in hamburger meat increased significantly (p <0.05) the CIE values for a* during days 3, 6 and 9 of storage at 4 °C compared to the control (Table 2) which indicates a reddish pigmentation characteristic of meat products; however, no significant differences were found in the measurement of surface color immediately after the processing of the hamburgers on day 0. In general, for the formulations with HA, a decrease in the luminosity values L* was observed during the storage period; in contrast, the values for b* increased significantly (p <0.05) in the three days after the initial measurement. Beef and pork products added with low concentrations of ammonium hydroxide exhibit significant changes in color intensity while allowing for a better shelf life [30]. Studies have been reported in beef steaks (Triceps brachii) improved with HA concluding that these were darker (low values of a*) and more reddish (high values of a*) than treatments without HA under modified atmosphere conditions high oxygen content during a period of 7 days at 4 °C [24]. These authors have also reported that the improved fillets almost always had less discoloration than their unimproved counterparts, the instrumental color measurements are related to those reported by Naveena et al., [12], indicating benefits to improve the reddish color in meat derivatives during storage under aerobic conditions.

Table 2. Effect of ammonium hydroxide on the color surface of burgers during storage days at 4 °C.

<table>
<thead>
<tr>
<th>L*</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>35.12 ± 0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.44 ± 0.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.22 ± 0.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.33 ± 0.53&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.5%</td>
<td>36.32 ± 0.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.22 ± 0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.45 ± 0.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.58 ± 0.54&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.0%</td>
<td>36.36 ± 0.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.28 ± 0.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.27 ± 0.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.23 ± 0.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.5%</td>
<td>37.48 ± 0.49&lt;sup&gt;c&lt;/sup&gt;</td>
<td>35.20 ± 0.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.05 ± 0.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.21 ± 0.52&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>a*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12.56 ± 0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.87 ± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.96 ± 0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.45 ± 0.45&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.5%</td>
<td>12.45 ± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.45 ± 0.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.89 ± 0.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.66 ± 0.43&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.0%</td>
<td>12.98 ± 0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.78 ± 0.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.72 ± 0.43&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.58 ± 0.44&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.5%</td>
<td>12.87 ± 0.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.96 ± 0.40&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.34 ± 0.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.21 ± 0.43&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>b*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>11.47 ± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.12 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.24 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.26 ± 0.42&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.5%</td>
<td>14.25 ± 0.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.22 ± 0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.75 ± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.33 ± 0.44&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.0%</td>
<td>13.63 ± 0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.10 ± 0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.23 ± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.66 ± 0.40&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.5%</td>
<td>13.23 ± 0.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.75 ± 0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.62 ± 0.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.08 ± 0.44&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
The microbiological analysis carried out on samples of hamburgers treated with HA and the control showed that the microbiological requirements established by NTC 1325 [13] were met, the count for E. coli in the samples did not exceed the maximum permissible index to identify a good quality level (m<10); In addition, salmonella was not detected in any case; these microorganisms correspond to a group of pathogens of special care in the food industry since they cause serious gastrointestinal diseases in humans, with meat, derived products and other foods as the main source of transmission, their presence represents an inadequate processing of the products [31].

**Figure 4.** Effect of ammonium hydroxide (HA) on the mesophilic aerobic counts of burgers during the days of treatment. Bars with different letters in the same storage period are significantly different (p <0.05). LSD = 0.42

**Figure 5.** Effect of ammonium hydroxide (HA) on the total coliform counts of burgers during the days of treatment. Bars with different letters in the same storage period are significantly different (p <0.05). LSD = 0.38

The count of mesophilic aerobes (Figure 4) was significantly reduced (p <0.05) in all the samples treated with HA, demonstrating its effectiveness as a microbial control
agent, these results agree with the reports investigated, where the sudden change of pH with the addition of HA reduces the growth and the amount of bacteria in the meat [12]. On the other hand, the effect of HA on the total coliform count (Figure 5) was considered significant (p <0.05) and its values are within those established by Colombian regulations, these microorganisms are considered an indicator of hygienic quality of food or an inadequate sanitary state, in quantities greater than those allowed, show poor handling and processing with flaws [16]. Other authors report the efficacy of the ammonium ion (NH4 +) with an antimicrobial effect [29].

4. Conclusions

Despite the controversy that has arisen from the use of ammonium hydroxide (HA) in food, it is important to highlight the benefits of this method; In our research, samples of beef burgers treated with HA significantly increased (p <0.05) the parameters evaluated such as pH, CRA and the count of microorganisms during the days of storage, likewise it was obtained that the chromatic values of a * (red) were higher when HA was used, in contrast L * values were less than 9 days of refrigerated storage when HA was used at the highest level. Therefore, the results of 0.5% HA are considered acceptable in the parameters studied compared to the control hamburger; Further research is needed to help establish the various quality characteristics of beef burger based on ammonium hydroxide.

References


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[22] NTC 4574. Norma Técnica Colombiana: Microbiología de Alimentos Y de


Received: April 16, 2018; Published: May 10, 2018